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**NOTICE OF ENTITLEMENT**

We, Institut für Genbiologische Forschung Berlin GmbH, the applicant/Nominated person in respect of Application No. 45003/93 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person would, on the grant of a patent for the invention to the inventors, be entitled to have the patent assigned to the Nominated Person.

The Nominated person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person made the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

The Nominated Person is the depositor of a deposit with an International Depository Authority as listed hereafter:

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a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

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**DNA SEQUENCES ENCODING OLIGOSACCHARIDE TRANSPORTER**

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(57) Claim

1. An isolated DNA sequence which contains the coding region of an oligosaccharide transporter, characterised in that the information contained in the nucleotide sequence allows, by sequence integration in a plant genome, the formation of RNA, and with this RNA, a new oligosaccharide transport activity can be introduced in the plant cells or an endogenous oligosaccharide transporter activity can be expressed, whereby the formation and transport of storage materials is changed.

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(54) Title: DNA SEQUENCES ENCODING OLIGOSACCHARIDE TRANSPORTER

(57) Abstract

There are described DNA sequences, that contain the coding region of an oligosaccharide transporter, whose introduction in a plant genome modifies the formation and transfer of storage materials in transgenic plants, plasmids, bacteria and plants containing these DNA sequences, a process for the preparation and transformation of yeast strains, that makes possible the identification of the DNA sequences of the plant oligosaccharide transporter of the invention, as well as the use of DNA sequences of the invention.

# DNA SEQUENCES ENCODING OLIGOSACCHARIDE TRANSPORTER.

## Field of the invention

The present invention relates to DNA sequences, that contain the coding region of an oligosaccharide transporter, whose introduction in a plant genome modifies the formation and transfer of storage materials in transgenic plants, plasmids, bacteria and plants containing these DNA sequences, a process for the preparation and transformation of yeast strains, that makes possible the identification of the DNA sequences of the plant oligosaccharide transporter of the invention, as well as the use of DNA sequences of the invention.

The most important transport metabolite for stored energy in many plants, for example potatoes, is sucrose. In other species, other oligosaccharides can serve this role. In japanese artichokes for example it is stachyose.

The central position of the oligosaccharide transport in the energy content of the plant has already been shown in transgenic plants, in which by expression of an invertase, the sucrose is split into the monosaccharides, leading to considerable changes in its habit (EP 442 592). Because of the significance of sucrose in the formation of storage materials, numerous experiments have been carried out into investigating the biosynthesis or the metabolism of disaccharides. From DE 42 13 444, it is known that the improvement of the storage properties of the harvested parts can be achieved in transgenic potatoes, in which

through expression of an apoplastic invertase, the transfer of energy rich compounds to the heterotrophic parts of growing shoots is inhibited.

In spite of much effort, the mechanism for distributing storage materials, such as oligosaccharides in plants has not been clarified and, in order to influence it, it is not yet known, how the sucrose formed in the leaves following photosynthesis, reaches the transport channels of the phloem of the plant and how it is taken up from the storage organs, e.g. the tubers of potato plants or seeds. On isolated plasma membranes of cells of leaf tissue of sugar beet (*Beta vulgaris*) it has been demonstrated that the transport of sucrose through the membrane can be induced by providing an artificial pH gradient and can be intensified by providing an electrochemical potential (Lemoine & Delrot (1989) FEBS letters 249: 129-133). The membrane passage of sucrose follows a Michaelis-Menten kinetic, in which the $k_m$ value of the sucrose transport is around 1 mM (Slone & Buckhout, 1991, Planta 183: 484-589). This form of kinetic indicates the involvement of transporter protein. Experiments on plasma membranes of sugar beet, *Ricinus communis* and *Cyclamen persicum* has shown that the sucrose transport is concerned with a co-transport of protons (Buckhout,1989, Planta 178: 393-399; Williams et al., 1990, Planta 182: 540-545; Grimm et al., 1990, Planta 182: 480-485). The stoichiometry of the co-transport is 1:1 (Bush, 1990, Plant Physiol 93: 1590-1596). Mechanisms have also been proposed however, for transport of the sucrose through the plasmodium of the plant cells (Robards & Lucas, 1990, Ann Rev Plant Physiol 41: 369-419). In spite of the knowledge of the existence of an active transport system, that allows the cells to deliver sucrose to the transport channels, a protein with these kind of properties is not yet known. In

N-ethylmaleinimide staining of sugar beet plasma membrane in the presence and absence of sucrose Gallet et al. (1989, Biochem Biophys Acta 978: 56-64) obtained information that a protein of size 42 kDa can interact with sucrose. Antibodies against a fraction of plasma membrane protein of this size range can inhibit the sucrose transport through plasma membranes (Lemoine et al., 1989, Bichem Biophys Acta 978: 65-71). In contrast, information has been obtained (Ripp et al., 1988, Plant Physiol 88: 1435-1445) by the photoaffinity marking of soyabean protein with the sucrose analogue, desoxyazido-hydroxybenzamidosucrose, on the participation of a 62 kDa protein in the transport of sucrose through membranes. An amino acid sequence of a sucrose transporter is not known.

There are now described DNA sequences which contain the coding region of an oligosaccharide transporter, and whose information contained in the nucleotide sequence allows, by sequence integration in a plant genome, the formation of RNA, by which a new oligosaccharide transport activity can be introduced in the plant cells or an endogenous oligosaccharide transporter activity can be expressed. By the term oligosaccharides transporter is for example to the understood a sucrose transporter from plants such as spinach or potatoes.

The identification of the coding region of the oligosaccharide transporter is carried out by a process which allows the isolation of plant DNA sequences which code the transporter molecules by means of expression in specific mutants of yeast *Saccharomyces cerevisiae*. For this, suitable yeast mutants have to be provided which cannot take up a substance for which the coding region of a transporter molecule has to be isolated from a plant gene library. A process is already known for

complementation of a potassium transport deficiency in yeast mutants (Anderson et al., 1992, Proc Natl Acad Sci USA 89: 3736-3740). In this, yeast cDNA sequences for plant mRNA are expressed in yeast mutants by use of expression vectors. Those yeasts which can now take up the substances to be transported into the cells, contain the coding region for a transporter molecule in the expression vector. The known process is however not useful for identification of the coding region for a sucrose transporter, since yeasts contain no endogenous sucrose transporter which could be switched off through mutation. Yeasts code a cleaving invertase, which cleaves extracellular sucrose so that hexoses can be taken up from the cells via a hexose transporter.

For the preparation and transformation of yeast strains which serve to identify a plant oligosaccharide transporter:

a) first, a yeast strain with a defective suc2 gene, which cannot be cleaved by invertase, is prepared by homologous recombination and then from this,

b) by transformation with a gene for a sucrose synthase activity from plant cells, a yeast strain which can cleave intracellular sucrose is extracted, and

c) by transformation of this strain with a plant cDNA library in an expression vector for yeast cells, the DNA sequence, which codes for a plant oligosaccharide transporter, is identified.

The yeast strains obtained from this process, for identification of plant oligosaccharide transporters are for example the yeast strains YSH 2.64-1A-SUSY (DSM 7106)

and YSH 2.64-1A-INV (DSM 7105).

With the yeast strain YSH 2.64-1A-SUSY, a DNA sequence for a plant sucrose transporter is identified which has the following sequences.

**Sucrose-Transporter from spinach (Seq. ID No.1):**

```
AAAAACACAC ACCCAAAAAA AAAACACTAC GACTATTTCA AAAAAAACAT TGTTACTAGA   60

AATCTTATT  ATG GCA GGA AGA AAT ATA AAA AAT GGT GAA AAT AAC          105
           Met Ala Gly Arg Asn Ile Lys Asn Gly Glu Asn Asn
           1               5                  10

AAG ATT GCG GGT TCT TCT CTT CAC TTA GAG AAG AAC CCA ACA ACT         150
Lys Ile Ala Gly Ser Ser Leu His Leu Glu Lys Asn Pro Thr Thr
       15                  20                  25

CCC CCC GAG GCC GAG GCT ACC TTA AAG AAG CTC GGC CTC GTG GCT         195
Pro Pro Glu Ala Glu Ala Thr Leu Lys Lys Leu Gly Leu Val Ala
       30                  35                  40

TCA GTA GCG GCC GGG GTT CAG TTC GGG TGG GCT TTA CAG CTC TCC         240
Ser Val Ala Ala Gly Val Gln Phe Gly Trp Ala Leu Gln Leu Ser
       45                  50                  55

CTA CTG ACC CCG TAC GTC CAA CTA CTG GGC ATT CCC CAC ACT TGG         285
Leu Leu Thr Pro Tyr Val Gln Leu Leu Gly Ile Pro His Thr Trp
       60                  65                  70

GCC GCC TAC ATC TGG TTG TGC GGC CCA ATC TCG GGG ATG ATT GTC         330
Ala Ala Tyr Ile Trp Leu Cys Gly Pro Ile Ser Gly Met Ile Val
       75                  80                  85
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CAG CCA TTG GTC GGG TAC TAT AGT GAC CGG TGC ACC TCC CGC TTC      375
Gln Pro Leu Val Gly Tyr Tyr Ser Asp Arg Cys Thr Ser Arg Phe
        90                  95                  100

GGC CGA CGT CGC CCC TTC ATT GCA GCA GGG GCG GCT CTA GTG GCC      420
Gly Arg Arg Arg Pro Phe Ile Ala Ala Gly Ala Ala Leu Val Ala
        105                 110                 115

GTA GCG GTG GGG CTA ATC GGA TTC GCC GCC GAT ATC GGC GCA GCG      465
Val Ala Val Gly Leu Ile Gly Phe Ala Ala Asp Ile Gly Ala Ala
        120                 125                 130

TCG GGT GAT CCA ACG GGA AAC GTG GCA AAA CCC CGG GCC ATC GCG      510
Ser Gly Asp Pro Thr Gly Asn Val Ala Lys Pro Arg Ala Ile Ala
        135                 140                 145

GTG TTT GTG GTC GGG TTT TGG ATC CTC GAC GTG GCT AAC AAC ACC      555
Val Phe Val Val Gly Phe Trp Ile Leu Asp Val Ala Asn Asn Thr
        150                 155                 160

CTG CAA GGC CCA TGC AGG GCG TTG TTA GCA GAC ATG GCC GCC GGG      600
Leu Gln Gly Pro Cys Arg Ala Leu Leu Ala Asp Met Ala Ala Gly
        165                 170                 175

TCG CAA ACC AAA ACC CGG TAC GCT AAC GCC TTC TTC TCC TTC TTC      645
Ser Gln Thr Lys Thr Arg Tyr Ala Asn Ala Phe Phe Ser Phe Phe
        180                 185                 190

ATG GCG TTA GGA AAC ATC GGA GGG TAC GCC GCC GGT TCA TAC AGC      690
Met Ala Leu Gly Asn Ile Gly Gly Tyr Ala Ala Gly Ser Tyr Ser
        195                 200                 205

CGC CTC TAC ACG GTG TTC CCC TTT ACC AAA ACC GCC GCC TGC GAC      735
Arg Leu Tyr Thr Val Phe Pro Phe Thr Lys Thr Ala Ala Cys Asp
        210                 215                 220
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GTC TAC TGC GCC AAT CTA AAA TCC TGC TTC TTC ATC TCC ATC ACA      780
Val Tyr Cys Ala Asn Leu Lys Ser Cys Phe Phe Ile Ser Ile Thr
        225                 230                 235

CTC CTA ATC GTC CTC ACA ATC CTA GCA CTT TCC GTC GTA AAA GAG      825
Leu Leu Ile Val Leu Thr Ile Leu Ala Leu Ser Val Val Lys Glu
        240                 245                 250

CGT CAA ATC ACA ATC GAC GAA ATC CAA GAA GAA GAA GAC TTA AAA      870
Arg Gln Ile Thr Ile Asp Glu Ile Gln Glu Glu Glu Asp Leu Lys
        255                 260                 265

AAC AGA AAC AAT AGC AGC GGT TGT GCA AGA CTA CCG TTC TTC GGA      915
Asn Arg Asn Asn Ser Ser Gly Cys Ala Arg Leu Pro Phe Phe Gly
        270                 275                 280

CAA TTA ATA GGC GCT CTC AAA GAT CTA CCA AAA CCA ATG CTA ATC      960
Gln Leu Ile Gly Ala Leu Lys Asp Leu Pro Lys Pro Met Leu Ile
        285                 290                 295

CTA TTA CTA GTA ACA GCC CTA AAT TGG ATC GCA TGG TTT CCA TTC     1005
Leu Leu Leu Val Thr Ala Leu Asn Trp Ile Ala Trp Phe Pro Phe
        300                 305                 310

TTG TTG TTC GAT ACT GAT TGG ATG GGT AAA GAA GTG TAC GGT GGT     1050
Leu Leu Phe Asp Thr Asp Trp Met Gly Lys Glu Val Tyr Gly Gly
        315                 320                 325

ACA GTC GGA GAA GGT AAA TTG TAC GAC CAA GGA GTT CAT GCC GGT     1095
Thr Val Gly Glu Gly Lys Leu Tyr Asp Gln Gly Val His Ala Gly
        330                 335                 340

GCC TTA GGT CTG ATG ATT AAC TCC GTT GTC TTA GGT GTT ATG TCG     1140
Ala Leu Gly Leu Met Ile Asn Ser Val Val Leu Gly Val Met Ser
        345                 350                 355
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TTG AGT ATT GAA GGT TTG GCT CGT ATG GTA GGC GGT GCT AAA AGG      1185
Leu Ser Ile Glu Gly Leu Ala Arg Met Val Gly Gly Ala Lys Arg
      360                 365                 370

TTA TGG GGA ATT GTC AAT ATT ATT CTT GCT GTT TGT TTA GCT ATG      1230
Leu Trp Gly Ile Val Asn Ile Ile Leu Ala Val Cys Leu Ala Met
      375                 380                 385

ACG GTG TTA GTT ACT AAG TCC GCC GAA CAC TTC CGT GAT AGC CAC      1275
Thr Val Leu Val Thr Lys Ser Ala Glu His Phe Arg Asp Ser His
      390                 395                 400

CAT ATT ATG GGC TCC GCC GTC CCT CCG CCG CCG CCT GCT GGT GTT      1320
His Ile Met Gly Ser Ala Val Pro Pro Pro Pro Pro Ala Gly Val
      405                 410                 415

AAG GGT GGC GCT TTG GCT ATC TTT GCC GTT CTT GGT ATC CCT CTT      1365
Lys Gly Gly Ala Leu Ala Ile Phe Ala Val Leu Gly Ile Pro Leu
      420                 425                 430

GCG ATC ACT TTC AGT ATT CCT TTG GCC TTG GCG TCA ATC TTT TCA      1410
Ala Ile Thr Phe Ser Ile Pro Phe Ala Leu Ala Ser Ile Phe Ser
      435                 440                 445

GCA TCT TCC GGT TCA GGA CAA GGT CTT TCT CTA GGA GTT CTC AAC      1455
Ala Ser Ser Gly Ser Gly Gln Gly Leu Ser Leu Gly Val Leu Asn
      450                 455                 460

CTC GCC ATC GTT GTA CCC CAG ATG TTT GTG TCG GTA ACA AGT GGG      1500
Leu Ala Ile Val Val Pro Gln Met Phe Val Ser Val Thr Ser Gly
      465                 470                 475

CCA TGG GAT GCA ATG TTT GGT GGA GGA AAT TTG CCA GCA TTC GTG      1545
Pro Trp Asp Ala Met Phe Gly Gly Gly Asn Leu Pro Ala Phe Val
      480                 485                 490
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GTG GGA GCT GTA GCA GCA ACA GCC AGT GCA GTT CTT TCA TTT ACA 1590
Val Gly Ala Val Ala Ala Thr Ala Ser Ala Val Leu Ser Phe Thr
495 500 505

TTG TTG CCT TCT CCA CCC CCT GAA GCT AAA ATT GGT GGG TCC ATG 1635
Leu Leu Pro Ser Pro Pro Pro Glu Ala Lys Ile Gly Gly Ser Met
510 515 520

GGT GGT CAT TAAGAAATTT AATACTACTC CGTACAATTT AAACCCAAAT 1684
Gly Gly His
525

TAAAAATGAA AATGAAAATT TTTAACCCAT GTTCGTTACG TTGTAATTAG 1734

AGAGAAAAAT GATATATTGA ACGAAGCCGT TAATTTATGC TCCGTTCATC 1784

TTGTAATTCT TTTTCTCTCT GCTTTTTTTT TTTTTTTTTA ACGCGACGTG 1834

TTTTTGAGAT AAGGAAGGGC TAGATCGAGG ATGGGGGAAT TGGCAAGAAA 1884

TTGCTCGGGT ATAAATATTT ATCCCTCTTT GTAATTTTCA GTAACATTTA 1934

ATAGCCAGAA ATCAAAAAGT CAAGAAAAAT CGAAA 1969

**Sucrose Transporter from potato (Seq-ID No. 2):**

AAAA 4

ATG GAG AAT GGT ACA AAA AGA GAA GGT TTA GGG AAA CTT ACA GTT 49
Met Glu Asn Gly Thr Lys Arg Glu Gly Leu Gly Lys Leu Thr Val
5 10 15

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TCA TCT TCT CTA CAA GTT GAA CAG CCT TTA GCA CCA TCA AAG CTA     94
Ser Ser Ser Leu Gln Val Glu Gln Pro Leu Ala Pro Ser Lys Leu
                20                  25                  30

TGG AAA ATT ATA GTT GTA GCT TCC ATA GCT GCT GGT GTT CAA TTT    139
Trp Lys Ile Ile Val Val Ala Ser Ile Ala Ala Gly Val Gln Phe
                35                  40                  45

GGT TGG GCT CTT CAG CTC TCT TTG CTT ACA CCT TAT GTT CAA TTG    184
Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln Leu
                50                  55                  60

CTC GGA ATT CCT CAT AAA TTT GCC TCT TTT ATT TGG CTT TGT GGA    229
Leu Gly Ile Pro His Lys Phe Ala Ser Phe Ile Trp Leu Cys Gly
                65                  70                  75

CCG ATT TCT GGT ATG ATT GTT CAG CCA GTT GTC GGC TAC TAC AGT    274
Pro Ile Ser Gly Met Ile Val Gln Pro Val Val Gly Tyr Tyr Ser
                80                  85                  90

GAT AAT TGC TCC TCC CGT TTC GGT CGC CGC CGG CCA TTC ATT GCC    319
Asp Asn Cys Ser Ser Arg Phe Gly Arg Arg Arg Pro Phe Ile Ala
                95                  100                 105

GCC GGA GCT GCA CTT GTT ATG ATT GCG GTT TTC CTC ATC GGA TTC    364
Ala Gly Ala Ala Leu Val Met Ile Ala Val Phe Leu Ile Gly Phe
                110                 115                 120

GCC GCC GAC CTT GGT CAC GCC TCC GGT GAC ACT CTC GGA AAA GGA    409
Ala Ala Asp Leu Gly His Ala Ser Gly Asp Thr Leu Gly Lys Gly
                125                 130                 135

TTT AAG CCA CGT GCC ATT GCC GTT TTC GTC GTC GGC TTT TGG ATC    454
Phe Lys Pro Arg Ala Ile Ala Val Phe Val Val Gly Phe Trp Ile
                140                 145                 150
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CTT GAT GTT GCT AAC AAC ATG TTA CAG GGC CCA TGC AGA GCA CTA   499
Leu Asp Val Ala Asn Asn Met Leu Gln Gly Pro Cys Arg Ala Leu
                155                 160                 165

CTG GCT GAT CTC TCC GGC GGA AAA TCC GGC AGG ATG AGA ACA GCA   544
Leu Ala Asp Leu Ser Gly Gly Lys Ser Gly Arg Met Arg Thr Ala
                170                 175                 180

AAT GCT TTT TTC TCA TTC TTC ATG GCC GTC GGA AAC ATT CTG GGG   589
Asn Ala Phe Phe Ser Phe Phe Met Ala Val Gly Asn Ile Leu Gly
                185                 190                 195

TAC GCC GCC GGT TCA TAT TCT CAC CTC TTT AAA GTA TTC CCC TTC   634
Tyr Ala Ala Gly Ser Tyr Ser His Leu Phe Lys Val Phe Pro Phe
                200                 205                 210

TCA AAA ACC AAA GCC TGC GAC ATG TAC TGC GCA AAT CTG AAG AGT   679
Ser Lys Thr Lys Ala Cys Asp Met Tyr Cys Ala Asn Leu Lys Ser
                215                 220                 225

TGT TTC TTC ATC GCT ATA TTC CTT TTA CTC AGC TTA ACA ACC ATA   724
Cys Phe Phe Ile Ala Ile Phe Leu Leu Leu Ser Leu Thr Thr Ile
                230                 235                 240

GCC TTA ACC TTA GTC CGG GAA AAC GAG CTC CCG GAG AAA GAC GAG   769
Ala Leu Thr Leu Val Arg Glu Asn Glu Leu Pro Glu Lys Asp Glu
                245                 250                 255

CAA GAA ATC GAC GAG AAA TTA GCC GGC GCC GGA AAA TCG AAA GTA   814
Gln Glu Ile Asp Glu Lys Leu Ala Gly Ala Gly Lys Ser Lys Val
                260                 265                 270

CCG TTT TTC GGT GAA ATT TTT GGG GCT TTG AAA GAA TTA CCT CGA   859
Pro Phe Phe Gly Glu Ile Phe Gly Ala Leu Lys Glu Leu Pro Arg
                275                 280                 285
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CCG ATG TGG ATT CTT CTA TTA GTA ACC TGT TTG AAC TGG ATC GCG   904
Pro Met Trp Ile Leu Leu Leu Val Thr Cys Leu Asn Trp Ile Ala
                290                 300                 305

TGG TTT CCC TTT TTC TTA TAC GAT ACA GAT TGG ATG GCT AAG GAG   949
Trp Phe Pro Phe Phe Leu Tyr Asp Thr Asp Trp Met Ala Lys Glu
                310                 315                 320

GTT TTC GGT GGA CAA GTC GGT GAT GCG AGG TTG TAC GAT TTG GGT   994
Val Phe Gly Gly Gln Val Gly Asp Ala Arg Leu Tyr Asp Leu Gly
                325                 330                 335

GTA CGC GCT GGT GCA ATG GGA TTA CTG TTG CAA TCT GTG GTT CTA  1039
Val Arg Ala Gly Ala Met Gly Leu Leu Leu Gln Ser Val Val Leu
                340                 345                 350

GGG TTT ATG TCA CTT GGG GTT GAA TTC TTA GGG AAG AAG ATT GGT  1084
Gly Phe Met Ser Leu Gly Val Glu Phe Leu Gly Lys Lys Ile Gly
                355                 360                 370

GGT GCT AAG AGG TTA TGG GGA ATT TTG AAC TTT GTT TTG GCT ATT  1129
Gly Ala Lys Arg Leu Trp Gly Ile Leu Asn Phe Val Leu Ala Ile
                375                 380                 385

TGC TTG GCT ATG ACC ATT TTG GTC ACC AAA ATG GCC GAG AAA TCT  1174
Cys Leu Ala Met Thr Ile Leu Val Thr Lys Met Ala Glu Lys Ser
                390                 395                 400
CGC CAG CAC GAC CCC GCC GGC ACA CTT ATG GGG CCG ACG CCT GGT  1219
Arg Gln His Asp Pro Ala Gly Thr Leu Met Gly Pro Thr Pro Gly
                405                 410                 415

GTT AAA ATC GGT GCC TTG CTT CTC TTT GCC GCC CTT GGT ATT CCT  1264
Val Lys Ile Gly Ala Leu Leu Leu Phe Ala Ala Leu Gly Ile Pro
                420                 425                 430
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CTT GCG GCA ACT TTT AGT ATT CCA TTT GCT TTG GCA TCT ATA TTT  1309
Leu Ala Ala Thr Phe Ser Ile Pro Phe Ala Leu Ala Ser Ile Phe
                435                 440                 445

TCT AGT AAT CGT GGT TCA GGA CAA GGT TTG TCA CTA GGA GTG CTC  1354
Ser Ser Asn Arg Gly Ser Gly Gln Gly Leu Ser Leu Gly Val Leu
                450                 455                 460

AAT CTT GCA ATT GTT GTA CCA CAG ATG TTG GTG TCA CTA GTA GGA  1399
Asn Leu Ala Ile Val Val Pro Gln Met Leu Val Ser Leu Val Gly
                465                 470                 475

GGG CCA TGG GAT GAT TTG TTT GGA GGA GGA AAC TTG CCT GGA TTT  1444
Gly Pro Trp Asp Asp Leu Phe Gly Gly Gly Asn Leu Pro Gly Phe
                480                 485                 490

GTA GTT GGA GCA GTT GCA GCT GCC GCG AGC GCT GTT TTA GCA CTC  1489
Val Val Gly Ala Val Ala Ala Ala Ala Ser Ala Val Leu Ala Leu
                495                 500                 505

ACA ATG TTG CCA TCT CCA CCT GCT GAT GCT AAG CCA GCA GTC GCC  1534
Thr Met Leu Pro Ser Pro Pro Ala Asp Ala Lys Pro Ala Val Ala
                510                 515                 520

ATG GGG CTT TCC ATT AAA TAATTACAAA AGAAGGAGAA GAACAACTTT    1582
Met Gly Leu Ser Ile Lys
                525

TTTTTAATAT TAGTACTTCT CTTTTGTAAA CTTTTTTTAT TTTAGAAAAC      1632

AAACATAACA TGGAGGCTAT CTTTACAAGT GGCATGTCCA TGTATCTTCC      1682

TTTTTTCATA AAGCTCTTTA GTGGAAGAAG AATTAGAGGA AGTTTCCTTT      1732

TAATTTCTTC CAAACAAATG GGGTATGTGT AGTTGTTTTC A               1773
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The identified DNA sequences can be introduced into plasmids and thereby be combined with steering elements for expression in eukaryotic cells (see Example 5). These steering elements are on the one handed transcription promoters, and on the other hand transcription terminators. With the DNA sequences of the invention contained on the plasmids, eukaryotic cells can be transformed, with the aim of expression of a translatable mRNA which makes possible the synthesis of a sucrose transporter in the cells or with the aim of expression of a non-translatable RNA, which prevents synthesis of an endogenous sucrose transporter in the cells. By expression of an RNA corresponding to the inventive sequences of the oligosaccharide transporter, a modification of the plant carbohydrate metabolism is possible, which can be of significance in that an improvement in the delivery of storage substances in the harvested parts results in an increase in yield of agricultural plants. The possibility of forcing the take-up of storage materials in individual organs allows the modification of the whole plant by which the growth of individual tissues, for example leaves, is slowed down, whilst the growth of the harvested parts is increased. For this, one can imagine a lengthening of the vegetative phase of crops, which leads to an increased formation of storage substances.

Processes for the genetic modification of dicotyledonous and monocotyledonous plants are already known, (see for example Gasser, C.S., Fraley, R.T., 1989, Science 244: 1293-1299; Potrykus, 1991, Ann Rev Plant Mol Biol Plant Physiol 42: 205-225). For expression in plants - the coding sequences must be coupled with the transcriptional regulatory elements. Such elements called promoters, are known (EP 375091).

Further, the coding regions must be provided with transcription termination signals with which they can be correctly transcribed. Such elements are also described (see Gielen et al., 1989, EMBO J 8: 23-29). The transcriptional start region can be both native and/or homologous as well as foreign and/or heterologous to the host plant. If desired, termination regions are interchangeable with one another. The DNA sequence of the transcription starting and termination regions can be prepared synthetically or obtained naturally, or obtained from a mixture of synthetic and natural DNA constituents. For introduction of foreign genes in higher plants a large number of cloning vectors are available that include a replication signal for *E. coli* and a marker which allows a selection of the transformed cells. Examples of such vectors are pBR 322, pUC-Series, M13 mp-Series, pACYC 184 etc. Depending on the method of introduction of the desired gene in the plants, other DNA sequences may be suitable. Should the Ti- or Ri-plasmid be used, e.g. for the transformation of the plant cell, then at least the right boundary, often however both the right and left boundary of the Ti- and Ri-Plasmid T-DNA, is attached, as a flanking region, to the gene being introduced. The use of T-DNA for the transformation of plants cells has been intensively researched and is well described in EP 120 516; Hoekama, In: The Binary Plant Vector System, Offset-drukkerij Kanters B.V. Alblasserdam, (1985), Chapter V; Fraley, et al., Crit. Rev. Plant Sci., 4:1-46 and An et al. (1985) EMBO J. 4: 277-287. Once the introduced DNA is integrated in the genome, it is as a rule stable there and remains also in the offspring of the original transformed cells. It normally contains a selection marker, which induces resistance in the transformed plant cells against a biocide or antibiotic such as kanamycin, G 418, bleomycin, hygromycin or

phosphinotricin etc. The individual marker employed should therefore allow the selection of transformed cells from cells, which lack the introduced DNA.

For the introduction of DNA into a plant host cell, besides transformation using *Agrobacteria*, there are many other techniques available. These techniques include the fusion of protoplasts, microinjection of DNA and electroporation, as well as ballistic methods and virus infection. From the transformed plant material, whole plants can be regenerated in a suitable medium, which contains antibiotics or biocides for the selection. The resulting plants can then be tested for the presence of introduced DNA. No special demands are placed on the plasmids in injection and electroporation. Simple plasmids, such as e.g. pUC-derivatives can be used. Should however whole plants be regenerated from such transformed cells the presence of a selectable marker gene is necessary. The transformed cells grow within the plants in the usual manner (see also McCormick et al.(1986) Plant Cell Reports 5: 81-84). These plants can be grown normally and crossed with plants, that possess the same transformed genes or different. The resulting hybrid individuals have the corresponding phenotypical properties.

The DNA sequences of the invention can also be introduced in plasmids and thereby combined with steering elements for an expression in prokaryotic cells. The formation of a translatable RNA sequence of a eukaryotic sucrose transporter from bacteria leads, in spite of the considerable differences in the membrane structures of prokaryotes and eukaryotes, leads in addition surprisingly, to prokaryotes now being able to take up sucrose. This makes possible the production of technically interesting bacterial strains, which could be grown on the

relatively cheap substrate sucrose (see Example 5). For example, the production of polyhydroxybutyric acid in the bacteria *Alkaligenes eutrophus* is described (Steinbüchel & Schubert, 1989, Arch Microbiol 153: 101-104). The bacterium only uses however a very limited selection of substrates. The expression of a gene for a sucrose transporter, amongst others, in *Alkaligenes eutrophus* would therefore be of great interest.

The DNA sequences of the invention can also be introduced in plasmids which allow mutagenesis or a sequence modification through recombination of DNA sequences in prokaryotic or eukaryotic systems. In this way the specificity of the sucrose transporter can be modified.

By using standard processes (see Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2. Edn., Cold Spring Harbor Laboratory Press, NY, USA). Base exchanges can be carried out or natural or synthetic sequences can be added. For binding DNA fragments with one another adaptors or linkers can be introduced on the fragments. Further, manipulations can be carried which prepare suitable restriction cleavage sides or remove the excess DNA or restriction cleavage sites. Where insertions, deletions or substitutions such as for example transitions and transversions are to be carried, *in vitro* mutagenesis, primer repair, restrictions or ligations can be used. For methods of analysis, in general a sequence analysis, restriction analysis and other biochemical molecular biological methods can be used. After each manipulation, the DNA sequence, used, can be cleaved and bound with another DNA sequence. Each plasmid sequence can be cloned in the same or different plasmids.

Derivatives or parts of the DNA sequences and plasmids of

the invention can also be used for the transformation of prokaryotic and eukaryotic cells. Further, the DNA sequences of the invention can be used according to standard processes for the isolation of similar sequences on the genome of plants of various species, which also code for sucrose or other oligosaccharide transporter molecules. With these sequences, constructs for the transformation of plant cells can be prepared which modify the transport process in transgenic plants.

In order to specify related DNA sequences, gene libraries must first be prepared, which are representative for the content in genes of a plant type or for the expression of genes in a plant type. The former are genomic libraries, whilst the latter are cDNA libraries. From these, related sequences can be isolated using the DNA sequences of the invention as probes. Once the related gene has been identified and isolated, a determination of the sequence and an analysis of the properties of the proteins coded from this sequence is possible.

In order to understand the examples forming the basis of this invention all the processes necessary for these tests and which are known per se will first of all be listed:

1. Cloning process

For cloning, there was used the phage Lambda ZAP II, as well as the vector pBluescriptSK (Short et al., 1988, Nucl Acids Res 16: 7583-7600).

For the transformation of yeasts, the vectors YIplac 128 and YEplac 112 (Gietz & Sugino, 1988, Gene 74: 527-534) were used.

For the plant transformation the gene constructs in the

binary vector pBinAR (Höfgen & Willmitzer, 1990, Plant Sci 66: 221-230) were cloned.

2. Bacterial and yeast strains

For the pBbluescriptSK vector as well as for PBinAR constructs, the *E. coli* strain XL1blue (Bullock et al., 1987, Biotechniques, 5, 376- 378) was used.

As starting strain for the production of yeast strain YSH 2.64-1A-susy of the invention, the strain YSH 2.64-1A (Gozalbo & Hohmann, 1990, Curr Genet 17: 77-79) was used.

The transformation of the plasmid in potato plant was carried out using *Agrobacterium tumefaciens* strain LBA4404 (Bevan (1984) Nucl. Acids Res 12: 8711-8720).

3. Transformation of *Agrobacterium tumefaciens*

The transfer of the DNA in the *Agrobacteria* was carried out by direct transformation by the method of Höfgen & Willmitzer (1988, Nucleic Acids Res 16: 9877). The plasmid DNA of the transformed *Agrobacterium* was isolated in accordance with the method of Birnboim and Doly (1979) (Nucl Acids Res 7: 1513-1523) and was analysed by gel electrophoresis after suitable restriction cleavage.

4. Plant transformation

Ten small leaves, wounded with a scalpel, of a sterile potato culture were placed in 10 ml of MS medium with 2% sucrose containing 30-50 µl of an *Agrobacterium tumefaciens* overnight culture grown under selection. After 3-5 minutes gentle shaking, the leaves were laid out on MS medium of 1.6% glucose, 2 mg/l of zeatin ribose, 0.02 mg/l of naphthylacetic acid, 0.02 mg/l of gibberellic acid, 500 mg/l of claforan, 50 mg/l of kanamycin and 0.8% bacto agar. After incubation for one week at 25°C and 3000 lux,

the claforan concentration in the medium was reduced by half.

Deposits

The following plasmids and yeast strains were deposited at the Deutschen Sammlung von Mikroorganismen (DSM) in Braunschweig, Germany on the 12.06.1992 (deposit number):

| | | |
|---|---|---|
| Plasmid | pSK-S21 | (DSM 7115) |
| Yeast strain | YSH 2.64-1A-SUSY | (DSM 7106) |
| Yeast strain | YSH 2.64-1A-INV | (DSM 7105) |

Description of the Figures

Fig. 1
Time pattern of the uptake of sucrose in yeast cells. "pmol/mg cells" = amount of the $^{14}C$-labelled sucrose in pmol, taken up in relation to the moisture weight of the yeast cells mg; "112" = starting strain (without transporter); "+Glucose" = pre-incubation of the cells in a medium of 10mM glucose; "-Glucose" = no pre-incubation of the cells; "2.5µMCCCP" = coincubation with the inhibitor, carbonyl cyanide m-chlorophenylhydrazone (CCCP) in a concentration of 2.5µM; "500µMPCMBS" = coincubation with the inhibitor p-chloromercuribenzenesulfonic acid (PCMBS) in a concentration of 500µM; "10µM Antimycin" = coincubation with the inhibitor antimycin in a concentration of 10µM.

Fig. 2
Cloning of the plasmid pMA5-INV. Shows the insertion of 2.4 kb size HindIII Fragment from pSEYC 306-1 in pMA5-10

Fig. 3
shows the plasmid pBinAR-S21.
In this::

| | |
|---|---|
| CaMV 35S promoter: | promoter of the gene for the 35S RNA of the cauliflower mosaic virus |
| A: | coding region of the sucrose transporter in spinach, orientation in the reading direction |
| OCS: | terminator of the octopine synthase gene from *Agrobacterium tumefaciens* |
| SmaI, NotI, BamHI: | Cleavage positions of restriction enzymes |

Fig. 4:
shows the plasmid pBinAR-P62-anti.

In this::

| | |
|---|---|
| CaMV 35S promoter: | promoter of the gene for the 35S RNA of the cauliflower mosaic virus |
| OCS: | terminator of the octopine synthase gene from *Agrobacterium tumefaciens* |
| SmaI, SacI, BamHI, XbaI: | Cleavage positions of restriction enzymes |

Fig. 5:
Content of various carbohydrates in leaves of BinAR-P62-anti transformands.

In this

| | |
|---|---|
| fru: | fructose |
| suc: | sucrose |
| sta: | starch |
| control: | untransformed starting plants |
| sp-5 to sp-43: | transformands with individual numbers |

Fig. 6:
Efflux of carbohydrates from petioles of BinAR-P62-anti transformands
wt: Wild type
sp-5 to sp-43: transformands with individual numbers

The following examples describe the preparation of the yeast strains, the identification as well as the function and use of a plant sucrose transporter.

Example 1
Preparation of the yeast strains YSH 2.64-1A-SUSY and YSH 2.64-1A-INV

The yeast strain YSH 2.64-1A has the features suc2-, mal0, leu2, trp1 (Gozalbo & Hohmann, 1990, Curr Genet 17: 77-79). In order to introduce a sucrose cleaving enzymatic activity in this strain, it was transformed with the integrative plasmid YIplac128A2-SUSY, that contains the coding region of the sucrose synthase from potato as a fusion to the promoter of the gene for alcohol dehydrogenase from yeast. The plasmid YIplac 128A2-SUSY was prepared as follows. The plasmid YIplac128 (Gietz & Sugino, 1988, Gene 74: 527-534) was shortened by cleavage

with PstI and EcoRI, degradation and/or filling in of overhanging single strands and ligation in the region of the polylinker. In the remaining SphI cleavage position, a 728 bp cassette was inserted with the promoter of alcohol dehydrogenase from the plasmid pVT102U (Vernet et al., 1987, Gene 52: 225-233). In this way YIplac128A2 was obtained. The coding region of the sucrose synthase was amplified by polymerase chain reaction with the oligonucleotides

SUSY1 (GAGAGAGGATCCTGCAATGGCTGAACGTGTTTTGACTCGTG)

and

SUSY2 (GAGAGAGGATCCTTCATTCACTCAGCAGCCAATGGAACAGCT)

to a lambda clone of sucrose synthase from potato (Salanoubat & Belliard, 1987, Gene 60: 47-56). From the product, the coding region was prepared as BamHI fragment and inserted in the BamHI cleavage sited of the polylinker of the cassette. The yeast strain YSH 2.64-1A was transformed with the so prepared plasmid YIplac128A2-SUSY. Since the plasmid does not carry the 2$\mu$ region, it cannot be autonomically replicated in yeast. Therefore such transformands only acquire leucine auxotrophy, which at least partially chromosomally integrate the plasmid-DNA.

Leucine autotroph colonies were analysed for expression of the sucrose synthase gene. For this cells of a 5 ml liquid culture were decomposed by addition of glass pearls with vigorous shaking, and then, after centrifuging, total protein from the supernatant was added for an enzyme activity measurement. The activity of the expressed sucrose synthase contains 25mU/mg protein.

In a similar manner an invertase activity was introduced in the yeast strain YSH 2.64-1A, in which by help of the plasmid YIplac128A1-INV, a gene for a cytosolic, non-cleavable invertase was chromosomally integrated in the

yeast genome. YIplac128A1-INV contains instead of the coding region for sucrose synthase, an invertase gene, which lacks the signal sequence for export of the gene product. The precursor of the plasmid is the plasmid YIplac128A1, which differs from YIplac128A2 in the orientation of the polylinker in the cassette with the promoter of the alcohol dehydrogenase genet. The cassette for this plasmid derives from the plasmid pVT100U (Vernet et al., 1987, Gene 52: 225-233). The coding region of the invertase was obtained on the DNA of the suc2 gene by polymerase chain reaction with the oligonucleotides INV3 (GAGCTGCAGATGGCAAACGAAACTAGCGATAGACCTTTGGTCACA) and INV4 (GAGACTAGTTTATAACCTCTATTTTACTTCCCTTACTTGGAA). The coding region was ligated as PstI/SpeI fragment in the linearised vector YIplac128A1den using PstI and XbaI. A test of the enzymatic activity of the invertase activity expressed in the yeast cells, resulted in an enzyme activity of 68mU/mg total protein from yeast cells.

Example 2

Cloning of the cDNA plant sucrose transporter

From polyadenylated RNA from leaf tissue of growing spinach and potato plants, a library of the cDNA in the phage Lambda ZAP II library was prepared. From 500,000 Pfu, the phage DNA was prepared and purified using a caesium chloride sarcosyl gradient. After cleaving the phage DNA with the enzyme NotI, insertions from the size regions above and below 1.5kbp were prepared on a 1% agarose gel and ligated in the NotI cleavage sites of the expressions vector YEplac112A1NE. The vector is a derivative of the vector YEplac 112 (Gozalbo & Hohmann, 1990, Curr Genet 17: 77-79), with which, as described in Example 1, the polylinker was exchanged with a cassette

with the promoter of the alcohol dehydrogenase gene. The polylinker of the cassette was again removed by cleavage with the enzymes PstI and XbaI and replaced by a double stranded oligonucleotide that introduces a NotI and EcoRI cleavage site
(Sequence: GATCCGCGGCCGCCCGGAATTCTCTAGACTGCA).
Approximately 90,000 clones for the size region below 1.5kbp and approximately 40,000 clones for the size region above 1.5kbp were obtained by transformation in *E. coli*. From these, plasmid DNA was prepared. 2µg DNA was transformed fourteen times in the yeast strain YSH2.64-1A suc-.susy. Transformands were grown on a medium containing 2% glucose, and five to ten thousand of each were washed off with agar plate liquid medium and plated out on sucrose containing medium. Those colonies, which could be grown faster were further analysed. The insertion in the vector YEplac112A1NE of transformands S21 or P62 (YEplac112A1NE-S21) were sequenced. The sequences are given above

Example 3
Analysis of sucrose metabolising yeast transformands

The yeast transformand YEplac112A1NE-S21, corresponding to that obtained in Example 2, was grown in liquid medium until the culture had reached the logarithmic phase. After centrifuging the culture, the cells were subjected to a pre-incubation for 5 minutes in a glucose containing medium and then taken up in a medium containing $^{14}C$-labelled sucrose. The uptake of the labelled sucrose was measured by the process described by Cirillo (1989, Meth Enzymol 174: 617-622). The uptake of the labelled sucrose without preincubation with glucose was compared with that with co-incubation with the inhibitors

carbonyl cyanide m-chlorophenylhydrazone (CCCP), p-chloromercuribenzenesulfonic acid (PCMBS) and antimycin. The time pattern is shown in Fig. 1. The calculated reduction of the sucrose uptake by the inhibitors is shown in table I. A competition experiment with various sugars as competitor for the labelled sucrose, from which the specificity of the transporter can be read off, is shown in table II.

Analogous measurements were carried out with the yeast strain YEplac112A1NE-P62. These gave similar results.

Example 4

Transformation of bacterial strains with DNA sequences for expression of a sucrose transporter activity

In order to be able to metabolise taken-up sucrose, bacterial cells are needed which have an enzymatic activity for cleavage of the monosaccharide. To introduce such an activity, bacteria were transformed by the plasmid pMA5-INV and tested for invertase activity. The plasmid pMA5-INV was prepared as follows. The plasmid pMA5-10 (Stanssens et al., 1989, Nucl Acids Res 17: 4441-4454) was linearised at the HindIII cleavage site of the polylinker. The 2.4kb HindIII fragment of the plasmid pSEYC306-1 (Taussig & Carlson, 1983, Nucl Acids Res 11: 1943-1954) was cloned in the HindIII cleavage site. The corresponding cutout of the plasmid is shown in Fig. 2. The enzymatic activity of the invertase in bacteria cells, transformed with the plasmid pMA5-INV was determined in a gel electrophoresis activity test, in known manner. The possibility of the formation of a functional sucrose transporter though expression of a plant cDNA in bacteria cells was tested by transformation of *E. coli* with the plasmid pSK-S21. The plasmid is described Example 3. After

transformation of bacteria cells with pSK-S21, tests for sucrose uptake were carried out.

Example 5

Transformation of plants with a construct for over-expression of the coding region of the sucrose transporter

From the vectors YEplac 112A1NE-S21 and YEplac 112A1NE-P62, which contain, as inserts, the cDNA for the sucrose transporter from spinach and/or potato (see Examples 2 and 3), the inserts after NotI cleavage were isolated and ligated in the NotI cleavage site of the plasmids pBluescript-SK (pSK-S21 and/or pSK-P62). For pSK-S21, the insert was prepared as a SacI/XbaI fragment and cloned, after filling in the overhanging single strand-DNA, in the "sense" orientation in pBinAR (Höfgen & Willmitzer, 1990, Plant Sci 66: 221-230) which was previously cleaved with the enzymes SmaI and XbaI. The resulting plasmid pBinAR-S21 (see Fig. 3) can be inserted for transformation of plants in order to over-express the sucrose transporter. For pSK-P62, the insert was isolated as a 1.7 kbp NotI fragment and cloned in an "antisense" orientation in the SmaI cleavage site of the binary vector pBinAR, resulting in pBinAR-P62-anti (see Fig 4). This plasmid is suitable for transformation of plants with the aim of "antisense" inhibition of the expression of the sucrose transporter.

Transformation *Agrobakteria* were then used for infection of leaf segments of tobacco and potato.

Ten independently obtained transformands, in which the presence of the intact, non-rearranged chimeric gene was demonstrated by Southern blot analysis, changes on sucrose, hexose and starch content were respectively

tested.

Transformands, which contain the T-DNA from pBinAR-P62-anti, showed a strongly increased concentration of starches, hexoses and sucrose in the leaves (see Fig 5). The efflux of carbohydrate from the petioles taken from the plants is greatly reduced in aqueous medium (see Fig. 6). From these data, the significance of the sucrose transporter for the transport away of the photoassimilate from the photosynthetically active organs is clearly seen. Since an inhibition of the activity of the transporter limits the transport away of carbohydrates, this results from a lowering of the transfer of photoassimilates to storage organs in the case of an over-expression for example using the plasmid pBinAR-S21. Tobacco plants, in which the T-DNA from pBinAR-S21 has been integrated, show further a reduced apical dominance, i.e. they show a bushy growth. Such a phenotypical change is for example very desirable in tomato plants. The plasmid pBinAR-S21 with the DNA sequence (Seq. ID No. 1) of the invention is therefore suited for the modification of plants with the purpose of improving important breeding characteristics such as bushy growth.

Table I

| Inhibitor | sucrose transport* (%) |
|---|---|
| Control | 100 |
| 0.5μM CCCP | 65 |
| 2.5μM CCCP | 21 |
| 25μM PCMBS | 73 |
| 100μM PCMBS | 21 |
| 25μM 2,4-DNP | 61 |
| 100μM 2,4-DNP | 9 |
| 1mM sodium arsenate | 34 |
| 10μM antimycin A | 59 |
| 1m cAMP | 102 |

CCCP = carbonyl cyanide m-chlorophenylhydrazone
PCMBS = p-chloromercuribenzenesulfonic acid
2,4-DNP = 2,4-dinitrophenol

* = in relation to Y Eplac 112 A1NE-S21 (control)

Table II

| Competitor | sucrose transport* (%) |
|---|---|
| Control | 100 |
| 2mM sucrose | 28 |
| 2mM maltose | 58 |
| 10mM maltose | 37 |
| 2mM phenylglucoside | 7 |
| 2mM phloridzin | 16 |
| 2mM lactose | 91 |
| 10mM palatinose | 102 |
| 10mM trehalose | 103 |

* = in relation to YEplac 112 A1NE - S21 (control)

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated DNA sequence which contains the coding region of an oligosaccharide transporter, characterised in that the information contained in the nucleotide sequence allows, by sequence integration in a plant genome, the formation of RNA, and with this RNA, a new oligosaccharide transport activity can be introduced in the plant cells or an endogenous oligosaccharide transporter activity can be expressed, whereby the formation and transport of storage materials is changed.

2. An isolated DNA sequence according to claim 1, characterised in that, it contains the following nucleotide sequence (SEQ ID No. 1):

```
AAAAACACAC ACCCAAAAAA AAAACACTAC GACTATTTCA AAAAAAACAT TGTTACTAGA   60

AATCTTATT  ATG GCA GGA AGA AAT ATA AAA AAT GGT GAA AAT AAC          105
           Met Ala Gly Arg Asn Ile Lys Asn Gly Glu Asn Asn
           1               5                  10

AAG ATT GCG GGT TCT TCT CTT CAC TTA GAG AAG AAC CCA ACA ACT         150
Lys Ile Ala Gly Ser Ser Leu His Leu Glu Lys Asn Pro Thr Thr
        15                  20                  25

CCC CCC GAG GCC GAG GCT ACC TTA AAG AAG CTC GGC CTC GTG GCT         195
Pro Pro Glu Ala Glu Ala Thr Leu Lys Lys Leu Gly Leu Val Ala
        30                  35                  40

TCA GTA GCG GCC GGG GTT CAG TTC GGG TGG GCT TTA CAG CTC TCC         240
Ser Val Ala Ala Gly Val Gln Phe Gly Trp Ala Leu Gln Leu Ser
        45                  50                  55
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CTA CTG ACC CCG TAC GTC CAA CTA CTG GGC ATT CCC CAC ACT TGG        285
Leu Leu Thr Pro Tyr Val Gln Leu Leu Gly Ile Pro His Thr Trp
        60                  65                  70

GCC GCC TAC ATC TGG TTG TGC GGC CCA ATC TCG GGG ATG ATT GTC        330
Ala Ala Tyr Ile Trp Leu Cys Gly Pro Ile Ser Gly Met Ile Val
        75                  80                  85

CAG CCA TTG GTC GGG TAC TAT AGT GAC CGG TGC ACC TCC CGC TTC        375
Gln Pro Leu Val Gly Tyr Tyr Ser Asp Arg Cys Thr Ser Arg Phe
        90                  95                  100

GGC CGA CGT CGC CCC TTC ATT GCA GCA GGG GCG GCT CTA GTG GCC        420
Gly Arg Arg Arg Pro Phe Ile Ala Ala Gly Ala Ala Leu Val Ala
        105                 110                 115

GTA GCG GTG GGG CTA ATC GGA TTC GCC GCC GAT ATC GGC GCA GCG        465
Val Ala Val Gly Leu Ile Gly Phe Ala Ala Asp Ile Gly Ala Ala
        120                 125                 130

TCG GGT GAT CCA ACG GGA AAC GTG GCA AAA CCC CGG GCC ATC GCG        510
Ser Gly Asp Pro Thr Gly Asn Val Ala Lys Pro Arg Ala Ile Ala
        135                 140                 145

GTG TTT GTG GTC GGG TTT TGG ATC CTC GAC GTG GCT AAC AAC ACC        555
Val Phe Val Val Gly Phe Trp Ile Leu Asp Val Ala Asn Asn Thr
        150                 155                 160

CTG CAA GGC CCA TGC AGG GCG TTG TTA GCA GAC ATG GCC GCC GGG        600
Leu Gln Gly Pro Cys Arg Ala Leu Leu Ala Asp Met Ala Ala Gly
        165                 170                 175
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TCG CAA ACC AAA ACC CGG TAC GCT AAC GCC TTC TTC TCC TTC TTC      645
Ser Gln Thr Lys Thr Arg Tyr Ala Asn Ala Phe Phe Ser Phe Phe
        180                 185                 190

ATG GCG TTA GGA AAC ATC GGA GGG TAC GCC GCC GGT TCA TAC AGC      690
Met Ala Leu Gly Asn Ile Gly Gly Tyr Ala Ala Gly Ser Tyr Ser
        195                 200                 205

CGC CTC TAC ACG GTG TTC CCC TTT ACC AAA ACC GCC GCC TGC GAC      735
Arg Leu Tyr Thr Val Phe Pro Phe Thr Lys Thr Ala Ala Cys Asp
        210                 215                 220
GTC TAC TGC GCC AAT CTA AAA TCC TGC TTC TTC ATC TCC ATC ACA      780
Val Tyr Cys Ala Asn Leu Lys Ser Cys Phe Phe Ile Ser Ile Thr
        225                 230                 235

CTC CTA ATC GTC CTC ACA ATC CTA GCA CTT TCC GTC GTA AAA GAG      825
Leu Leu Ile Val Leu Thr Ile Leu Ala Leu Ser Val Val Lys Glu
        240                 245                 250

CGT CAA ATC ACA ATC GAC GAA ATC CAA GAA GAA GAA GAC TTA AAA      870
Arg Gln Ile Thr Ile Asp Glu Ile Gln Glu Glu Glu Asp Leu Lys
        255                 260                 265

AAC AGA AAC AAT AGC AGC GGT TGT GCA AGA CTA CCG TTC TTC GGA      915
Asn Arg Asn Asn Ser Ser Gly Cys Ala Arg Leu Pro Phe Phe Gly
        270                 275                 280

CAA TTA ATA GGC GCT CTC AAA GAT CTA CCA AAA CCA ATG CTA ATC      960
Gln Leu Ile Gly Ala Leu Lys Asp Leu Pro Lys Pro Met Leu Ile
        285                 290                 295

CTA TTA CTA GTA ACA GCC CTA AAT TGG ATC GCA TGG TTT CCA TTC     1005
Leu Leu Leu Val Thr Ala Leu Asn Trp Ile Ala Trp Phe Pro Phe
        300                 305                 310
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TTG TTG TTC GAT ACT GAT TGG ATG GGT AAA GAA GTG TAC GGT GGT      1050
Leu Leu Phe Asp Thr Asp Trp Met Gly Lys Glu Val Tyr Gly Gly
        315                 320                 325

ACA GTC GGA GAA GGT AAA TTG TAC GAC CAA GGA GTT CAT GCC GGT      1095
Thr Val Gly Glu Gly Lys Leu Tyr Asp Gln Gly Val His Ala Gly
        330                 335                 340

GCC TTA GGT CTG ATG ATT AAC TCC GTT GTC TTA GGT GTT ATG TCG      1140
Ala Leu Gly Leu Met Ile Asn Ser Val Val Leu Gly Val Met Ser
        345                 350                 355
TTG AGT ATT GAA GGT TTG GCT CGT ATG GTA GGC GGT GCT AAA AGG      1185
Leu Ser Ile Glu Gly Leu Ala Arg Met Val Gly Gly Ala Lys Arg
      360                 365                 370

TTA TGG GGA ATT GTC AAT ATT ATT CTT GCT GTT TGT TTA GCT ATG      1230
Leu Trp Gly Ile Val Asn Ile Ile Leu Ala Val Cys Leu Ala Met
        375                 380                 385

ACG GTG TTA GTT ACT AAG TCC GCC GAA CAC TTC CGT GAT AGC CAC      1275
Thr Val Leu Val Thr Lys Ser Ala Glu His Phe Arg Asp Ser His
        390                 395                 400

CAT ATT ATG GGC TCC GCC GTC CCT CCG CCG CCG CCT GCT GGT GTT      1320
His Ile Met Gly Ser Ala Val Pro Pro Pro Pro Pro Ala Gly Val
        405                 410                 415

AAG GGT GGC GCT TTG GCT ATC TTT GCC GTT CTT GGT ATC CCT CTT      1365
Lys Gly Gly Ala Leu Ala Ile Phe Ala Val Leu Gly Ile Pro Leu
        420                 425                 430

GCG ATC ACT TTC AGT ATT CCT TTG GCC TTG GCG TCA ATC TTT TCA      1410
Ala Ile Thr Phe Ser Ile Pro Phe Ala Leu Ala Ser Ile Phe Ser
        435                 440                 445
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GCA TCT TCC GGT TCA GGA CAA GGT CTT TCT CTA GGA GTT CTC AAC 1455
Ala Ser Ser Gly Ser Gly Gln Gly Leu Ser Leu Gly Val Leu Asn
450 455 460

CTC GCC ATC GTT GTA CCC CAG ATG TTT GTG TCG GTA ACA AGT GGG 1500
Leu Ala Ile Val Val Pro Gln Met Phe Val Ser Val Thr Ser Gly
465 470 475

CCA TGG GAT GCA ATG TTT GGT GGA GGA AAT TTG CCA GCA TTC GTG 1545
Pro Trp Asp Ala Met Phe Gly Gly Gly Asn Leu Pro Ala Phe Val
480 485 490

GTG GGA GCT GTA GCA GCA ACA GCC AGT GCA GTT CTT TCA TTT ACA 1590
Val Gly Ala Val Ala Ala Thr Ala Ser Ala Val Leu Ser Phe Thr
495 500 505

TTG TTG CCT TCT CCA CCC CCT GAA GCT AAA ATT GGT GGG TCC ATG 1635
Leu Leu Pro Ser Pro Pro Pro Glu Ala Lys Ile Gly Gly Ser Met
510 515 520

GGT GGT CAT TAAGAAATTT AATACTACTC CGTACAATTT AAACCCAAAT 1684
Gly Gly His
525

TAAAAATGAA AATGAAAATT TTTAACCCAT GTTCGTTACG TTGTAATTAG 1734

AGAGAAAAAT GATATATTGA ACGAAGCCGT TAATTTATGC TCCGTTCATC 1784

TTGTAATTCT TTTTCTCTCT GCTTTTTTTT TTTTTTTTTA ACGCGACGTG 1834

TTTTTGAGAT AAGGAAGGGC TAGATCGAGG ATGGGGGAAT TGGCAAGAAA 1884

TTGCTCGGGT ATAAATATTT ATCCCTCTTT GTAATTTTCA GTAACATTTA 1934

ATAGCCAGAA ATCAAAAAGT CAAGAAAAAT CGAAA 1969

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3. An isolated DNA sequence according to claim 1, characterised in that, it contains the following nucleotide sequence (SEQ ID No. 2):

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                                                    AAAA      4

ATG GAG AAT GGT ACA AAA AGA GAA GGT TTA GGG AAA CTT ACA GTT    49
Met Glu Asn Gly Thr Lys Arg Glu Gly Leu Gly Lys Leu Thr Val
                  5                  10                  15

TCA TCT TCT CTA CAA GTT GAA CAG CCT TTA GCA CCA TCA AAG CTA    94
Ser Ser Ser Leu Gln Val Glu Gln Pro Leu Ala Pro Ser Lys Leu
                 20                  25                  30

TGG AAA ATT ATA GTT GTA GCT TCC ATA GCT GCT GGT GTT CAA TTT   139
Trp Lys Ile Ile Val Val Ala Ser Ile Ala Ala Gly Val Gln Phe
                 35                  40                  45

GGT TGG GCT CTT CAG CTC TCT TTG CTT ACA CCT TAT GTT CAA TTG   184
Gly Trp Ala Leu Gln Leu Ser Leu Leu Thr Pro Tyr Val Gln Leu
                 50                  55                  60

CTC GGA ATT CCT CAT AAA TTT GCC TCT TTT ATT TGG CTT TGT GGA   229
Leu Gly Ile Pro His Lys Phe Ala Ser Phe Ile Trp Leu Cys Gly
                 65                  70                  75

CCG ATT TCT GGT ATG ATT GTT CAG CCA GTT GTC GGC TAC TAC AGT   274
Pro Ile Ser Gly Met Ile Val Gln Pro Val Val Gly Tyr Tyr Ser
                 80                  85                  90

GAT AAT TGC TCC TCC CGT TTC GGT CGC CGC CGG CCA TTC ATT GCC   319
Asp Asn Cys Ser Ser Arg Phe Gly Arg Arg Arg Pro Phe Ile Ala
                 95                 100                 105
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GCC GGA GCT GCA CTT GTT ATG ATT GCG GTT TTC CTC ATC GGA TTC   364
Ala Gly Ala Ala Leu Val Met Ile Ala Val Phe Leu Ile Gly Phe
                110                 115                 120

GCC GCC GAC CTT GGT CAC GCC TCC GGT GAC ACT CTC GGA AAA GGA   409
Ala Ala Asp Leu Gly His Ala Ser Gly Asp Thr Leu Gly Lys Gly
                125                 130                 135

TTT AAG CCA CGT GCC ATT GCC GTT TTC GTC GTC GGC TTT TGG ATC   454
Phe Lys Pro Arg Ala Ile Ala Val Phe Val Val Gly Phe Trp Ile
                140                 145                 150

CTT GAT GTT GCT AAC AAC ATG TTA CAG GGC CCA TGC AGA GCA CTA   499
Leu Asp Val Ala Asn Asn Met Leu Gln Gly Pro Cys Arg Ala Leu
                155                 160                 165

CTG GCT GAT CTC TCC GGC GGA AAA TCC GGC AGG ATG AGA ACA GCA   544
Leu Ala Asp Leu Ser Gly Gly Lys Ser Gly Arg Met Arg Thr Ala
                170                 175                 180

AAT GCT TTT TTC TCA TTC TTC ATG GCC GTC GGA AAC ATT CTG GGG   589
Asn Ala Phe Phe Ser Phe Phe Met Ala Val Gly Asn Ile Leu Gly
                185                 190                 195

TAC GCC GCC GGT TCA TAT TCT CAC CTC TTT AAA GTA TTC CCC TTC   634
Tyr Ala Ala Gly Ser Tyr Ser His Leu Phe Lys Val Phe Pro Phe
                200                 205                 210

TCA AAA ACC AAA GCC TGC GAC ATG TAC TGC GCA AAT CTG AAG AGT   679
Ser Lys Thr Lys Ala Cys Asp Met Tyr Cys Ala Asn Leu Lys Ser
                215                 220                 225

TGT TTC TTC ATC GCT ATA TTC CTT TTA CTC AGC TTA ACA ACC ATA   724
Cys Phe Phe Ile Ala Ile Phe Leu Leu Leu Ser Leu Thr Thr Ile
                230                 235                 240
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GCC TTA ACC TTA GTC CGG GAA AAC GAG CTC CCG GAG AAA GAC GAG 769
Ala Leu Thr Leu Val Arg Glu Asn Glu Leu Pro Glu Lys Asp Glu
245 250 255

CAA GAA ATC GAC GAG AAA TTA GCC GGC GCC GGA AAA TCG AAA GTA 814
Gln Glu Ile Asp Glu Lys Leu Ala Gly Ala Gly Lys Ser Lys Val
260 265 270

CCG TTT TTC GGT GAA ATT TTT GGG GCT TTG AAA GAA TTA CCT CGA 859
Pro Phe Phe Gly Glu Ile Phe Gly Ala Leu Lys Glu Leu Pro Arg
275 280 285

CCG ATG TGG ATT CTT CTA TTA GTA ACC TGT TTG AAC TGG ATC GCG 904
Pro Met Trp Ile Leu Leu Leu Val Thr Cys Leu Asn Trp Ile Ala
290 300 305

TGG TTT CCC TTT TTC TTA TAC GAT ACA GAT TGG ATG GCT AAG GAG 949
Trp Phe Pro Phe Phe Leu Tyr Asp Thr Asp Trp Met Ala Lys Glu
310 315 320

GTT TTC GGT GGA CAA GTC GGT GAT GCG AGG TTG TAC GAT TTG GGT 994
Val Phe Gly Gly Gln Val Gly Asp Ala Arg Leu Tyr Asp Leu Gly
325 330 335

GTA CGC GCT GGT GCA ATG GGA TTA CTG TTG CAA TCT GTG GTT CTA 1039
Val Arg Ala Gly Ala Met Gly Leu Leu Leu Gln Ser Val Val Leu
340 345 350

GGG TTT ATG TCA CTT GGG GTT GAA TTC TTA GGG AAG AAG ATT GGT 1084
Gly Phe Met Ser Leu Gly Val Glu Phe Leu Gly Lys Lys Ile Gly
355 360 370

GGT GCT AAG AGG TTA TGG GGA ATT TTG AAC TTT GTT TTG GCT ATT 1129
Gly Ala Lys Arg Leu Trp Gly Ile Leu Asn Phe Val Leu Ala Ile
375 380 385

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TGC TTG GCT ATG ACC ATT TTG GTC ACC AAA ATG GCC GAG AAA TCT  1174
Cys Leu Ala Met Thr Ile Leu Val Thr Lys Met Ala Glu Lys Ser
                390                 395                 400

CGC CAG CAC GAC CCC GCC GGC ACA CTT ATG GGG CCG ACG CCT GGT  1219
Arg Gln His Asp Pro Ala Gly Thr Leu Met Gly Pro Thr Pro Gly
                405                 410                 415

GTT AAA ATC GGT GCC TTG CTT CTC TTT GCC GCC CTT GGT ATT CCT  1264
Val Lys Ile Gly Ala Leu Leu Leu Phe Ala Ala Leu Gly Ile Pro
                420                 425                 430

CTT GCG GCA ACT TTT AGT ATT CCA TTT GCT TTG GCA TCT ATA TTT  1309
Leu Ala Ala Thr Phe Ser Ile Pro Phe Ala Leu Ala Ser Ile Phe
                435                 440                 445

TCT AGT AAT CGT GGT TCA GGA CAA GGT TTG TCA CTA GGA GTG CTC  1354
Ser Ser Asn Arg Gly Ser Gly Gln Gly Leu Ser Leu Gly Val Leu
                450                 455                 460

AAT CTT GCA ATT GTT GTA CCA CAG ATG TTG GTG TCA CTA GTA GGA  1399
Asn Leu Ala Ile Val Val Pro Gln Met Leu Val Ser Leu Val Gly
                465                 470                 475

GGG CCA TGG GAT GAT TTG TTT GGA GGA GGA AAC TTG CCT GGA TTT  1444
Gly Pro Trp Asp Asp Leu Phe Gly Gly Gly Asn Leu Pro Gly Phe
                480                 485                 490

GTA GTT GGA GCA GTT GCA GCT GCC GCG AGC GCT GTT TTA GCA CTC  1489
Val Val Gly Ala Val Ala Ala Ala Ala Ser Ala Val Leu Ala Leu
                495                 500                 505

ACA ATG TTG CCA TCT CCA CCT GCT GAT GCT AAG CCA GCA GTC GCC  1534
Thr Met Leu Pro Ser Pro Pro Ala Asp Ala Lys Pro Ala Val Ala
                510                 515                 520
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ATG GGG CTT TCC ATT AAA TAATTACAAA AGAAGGAGAA GAACAACTTT      1582
Met Gly Leu Ser Ile Lys
                525

TTTTTAATAT TAGTACTTCT CTTTTGTAAA CTTTTTTTAT TTTAGAAAAC      1632

AAACATAACA TGGAGGCTAT CTTTACAAGT GGCATGTCCA TGTATCTTCC      1682

TTTTTTCATA AAGCTCTTTA GTGGAAGAAG AATTAGAGGA AGTTTCCTTT      1732

TAATTTCTTC CAAACAAATG GGGTATGTGT AGTTGTTTTC A               1773
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4. A plasmid, characterised in that it contains a DNA sequence according to any one of claims 1 to 3.

5. Plasmid pSK-S21 (DSM 7115).

6. A transformed prokaryotic or eukaryotic cell containing an isolated DNA sequence according to any one of claims 1 to 5 or a derivative or part thereof.

7. A transformed plant containing the isolated DNA sequence according to any one of claims 1 to 3.

8. The transformed cell according to claim 6, further characterised as a bacterial cell.

9. The transformed eukaryotic or prokaryotic cell according to claim 6 or claim 8, wherein the isolated DNA sequence is operably linked in the sense orientation to a steering element which is capable of functioning in said cell to regulate the expression of said DNA sequence in said cell.

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10. A method of expressing an oligosaccharide transporter in a prokaryotic or eukaryotic cell, said method comprising growing the cell according to claim 9 for a time and under conditions sufficient to enable expression of the isolated DNA sequence to occur.

11. The method according to claim 10 wherein the oligosaccharide transporter is a sucrose transporter.

12. The method according to claim 10 or 11 wherein the cell is a bacterial cell.

13. The method according to claim 12 wherein the bacterial cell is capable of taking up sucrose.

14. A method of modifying the specificity of an oligosaccharide transporter, said method comprising mutating the isolated DNA sequence according to any one of claims 1 to 3 through recombination in a prokaryotic or eukaryotic system such that the mutated DNA sequence encodes a functional oligosaccharide transporter with altered specificity for oligosaccharides or transports a different oligosaccharide compared to the unmutated oligosaccharide transporter.

15. A modified oligosaccharide transporter produced essentially according to the method of claim 14.

16. A method of hindering the expression of an endogenous sucrose transporter in a cell, said method comprising expressing a genetic construct therein which comprises an isolated DNA sequence according to any one of claims 1 to 3 or a derivative or part thereof, placed operably in connection with a suitable steering element such that expression of said DNA sequence produces a non-translatable mRNA which is capable of hindering the synthesis of the endogenous sucrose transporter.

17. A method of detecting or isolating a nucleic acid molecule which encodes or is

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complementary to a nucleic acid molecule which encodes a plant oligosaccharide transporter, said method comprising contacting said nucleic acid molecule with a DNA sequence according to any one of claims 1 to 3 for a time and under conditions sufficient for hybridisation to occur.

18. A method of identifying a plant oligosaccharide transporter comprising the steps of:

(i) producing a first yeast strain which is defective in the suc2 gene and cannot secrete invertase;

(ii) transforming said first yeast strain with an isolated DNA sequence which encodes sucrose synthase such that a second yeast strain which is capable of cleaving intracellular sucrose is produced;

(iii) transforming said second yeast strain with a plant cDNA sequence placed operably in connection with a yeast-expressible promoter in a yeast vector to produce a third strain; and

(iv) assaying said third yeast strain for its ability to grow on media containing sucrose.

19. An isolated yeast strain comprising a defective suc2 gene and transformed with an isolated DNA sequence which encodes a sucrose synthase gene such that said yeast is unable to secrete invertase but is able to cleave intracellular sucrose.

20. An isolated yeast strain designated YSH 2.64-1A-SUSY (DSM 7106).

21. An isolated yeast strain designated YSH 2.64-1A-INV (DSM 7105).

22. A method of producing a transformed plant which over-expresses a sucrose transporter, said method comprising transforming a plant host cell with the binary plasmid pBin-AR-S21 derived from plasmid pSK-S21 (DSM 7115).

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23. A method according to claim 16 wherein the genetic construct comprises the isolated DNA sequence in the antisense orientation.

24. The method according to claim 23 wherein the genetic construct is plasmid pBinAr-P62-anti set forth in Figure 4.

25. A method of modifying plant carbohydrate metabolism, said method comprising transforming a plant cell with an isolated DNA sequence according to any one of claims 1 to 3 or a derivative or part thereof and regenerating a whole plant therefrom.

26. The method according to claim 25 wherein the DNA sequence is placed operably in connection with a plant-expressible steering element.

Dated this 31ST day of MAY, 1996

INSTITUT FÜR GENBIOLOGISCHE FORSCHUNG BERLIN GMBH

by DAVIES COLLISON CAVE
Patent Attorneys for the Applicant

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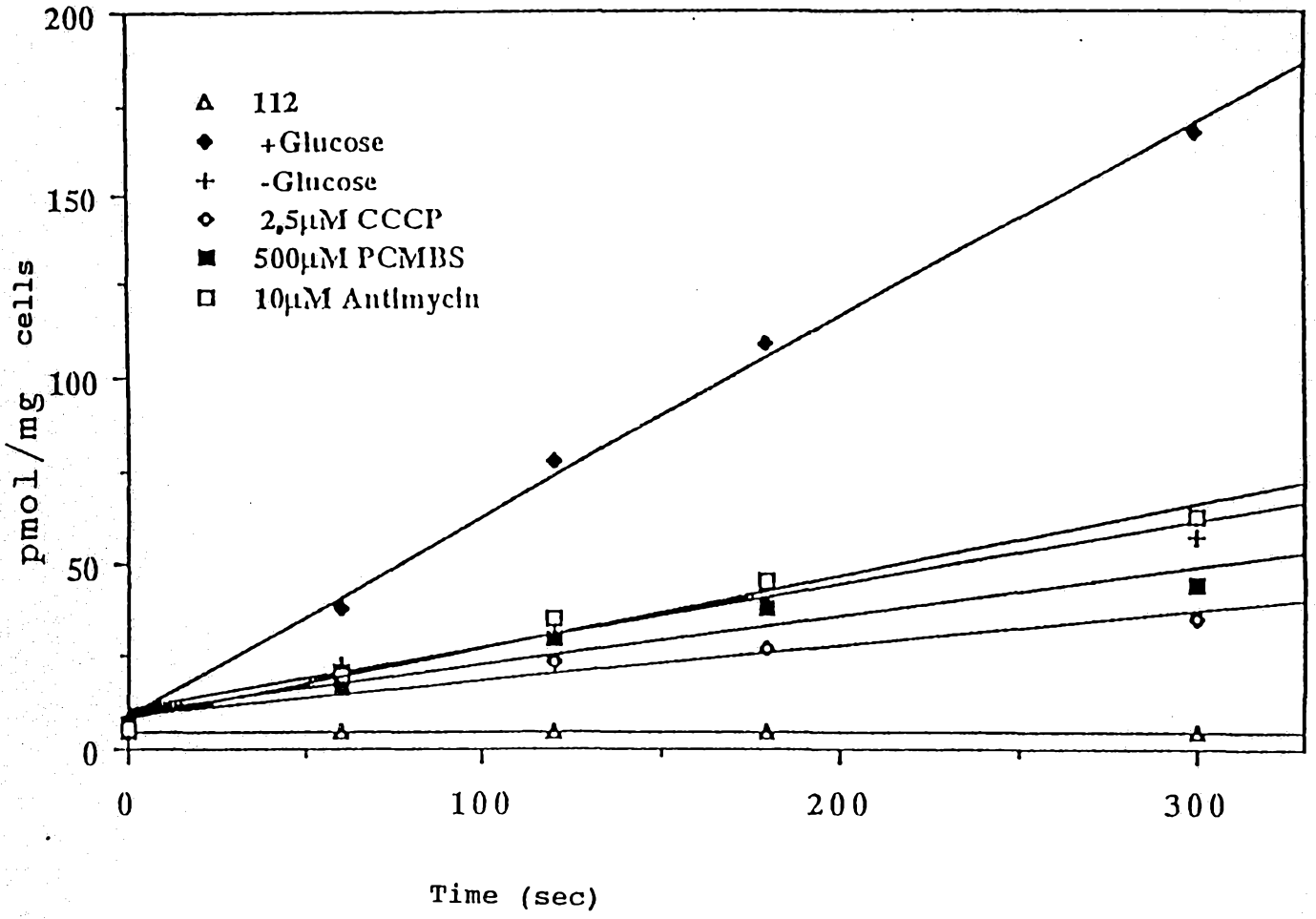
200
150
100
50
0
pmol/mg cells
112
+Glucose
-Glucose
2,5µM CCCP
500µM PCMBS
10µM Antimycin
0
100
200
300
Time (sec)

Fig. 1

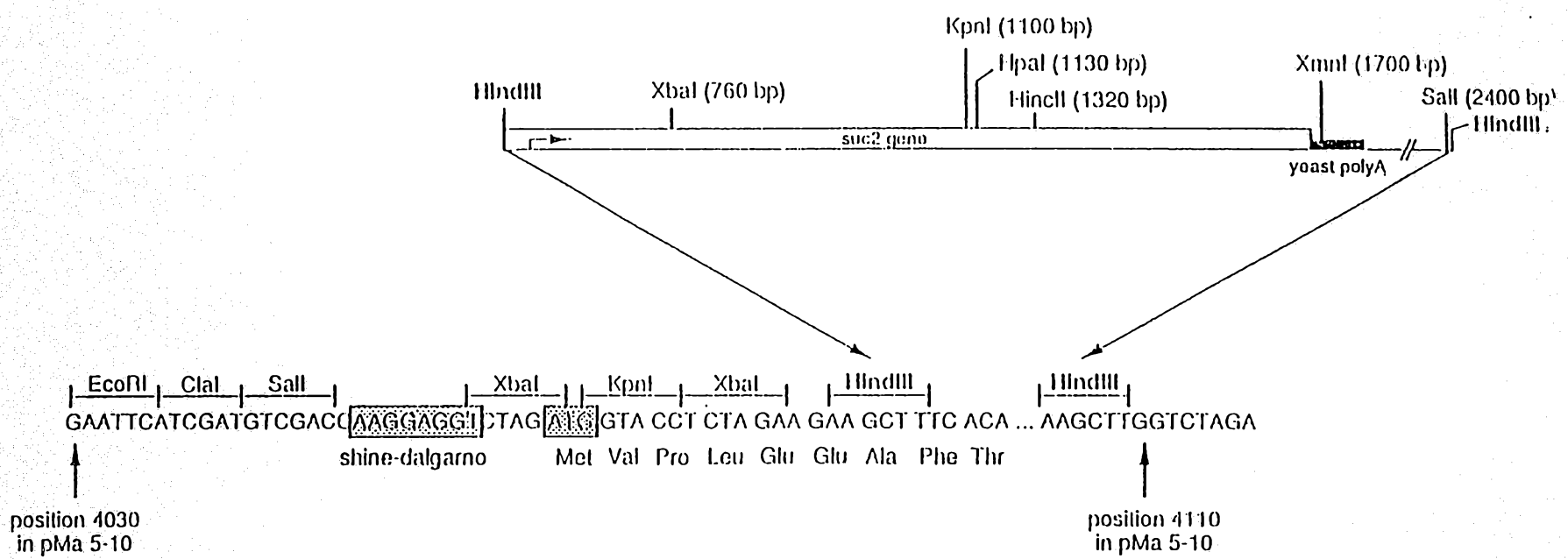
KpnI (1100 bp)
HpaI (1130 bp)
HindIII
XbaI (760 bp)
HincII (1320 bp)
XmnI (1700 bp)
SalI (2400 bp)
HindIII
suc2 gene
yeast polyA
EcoRI
ClaI
SalI
XbaI
KpnI
XbaI
HindIII
HindIII
GAATTCATCGATGTCGACC AAGGAGG T CTAG ATG GTA CCT CTA GAA GAA GCT TTC ACA ... AAGCTTGGTCTAGA
shine-dalgarno
Met Val Pro Leu Glu Glu Ala Phe Thr
position 4030
in pMa 5-10
position 4110
in pMa 5-10

Fig. 2

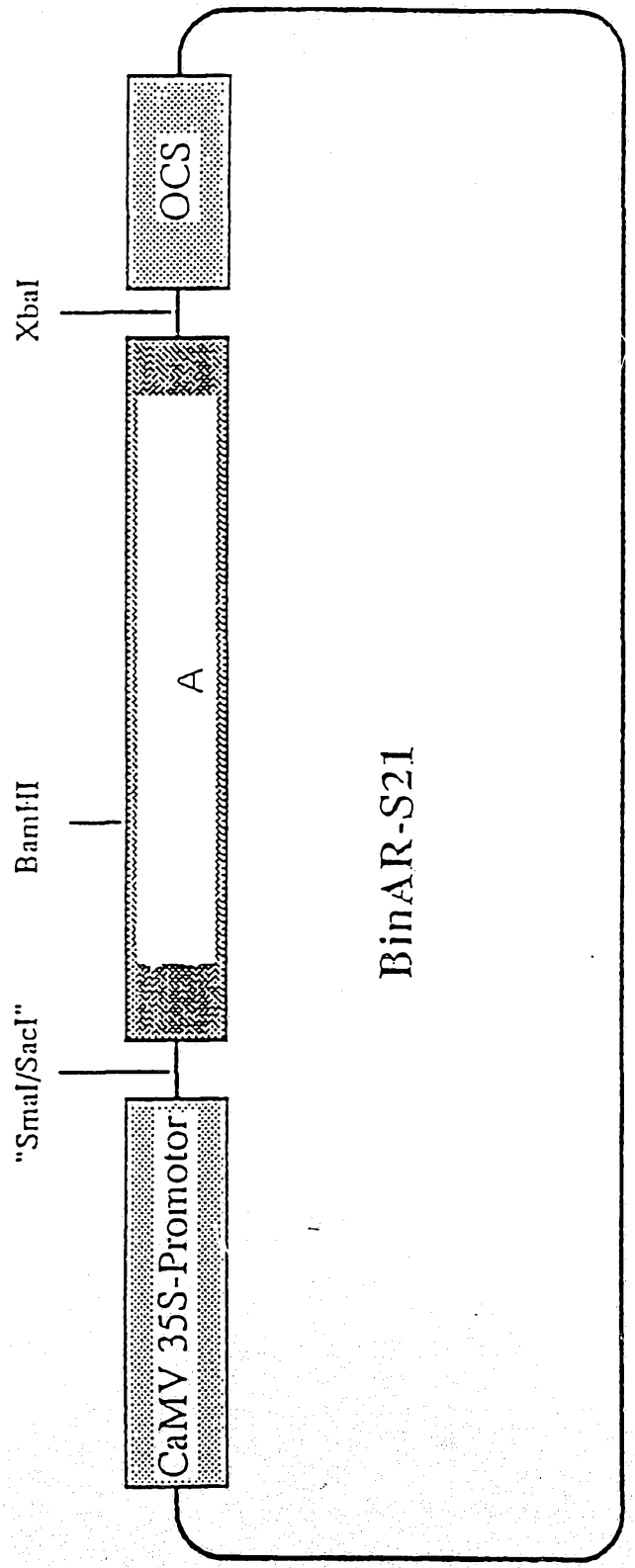


Fig. 3

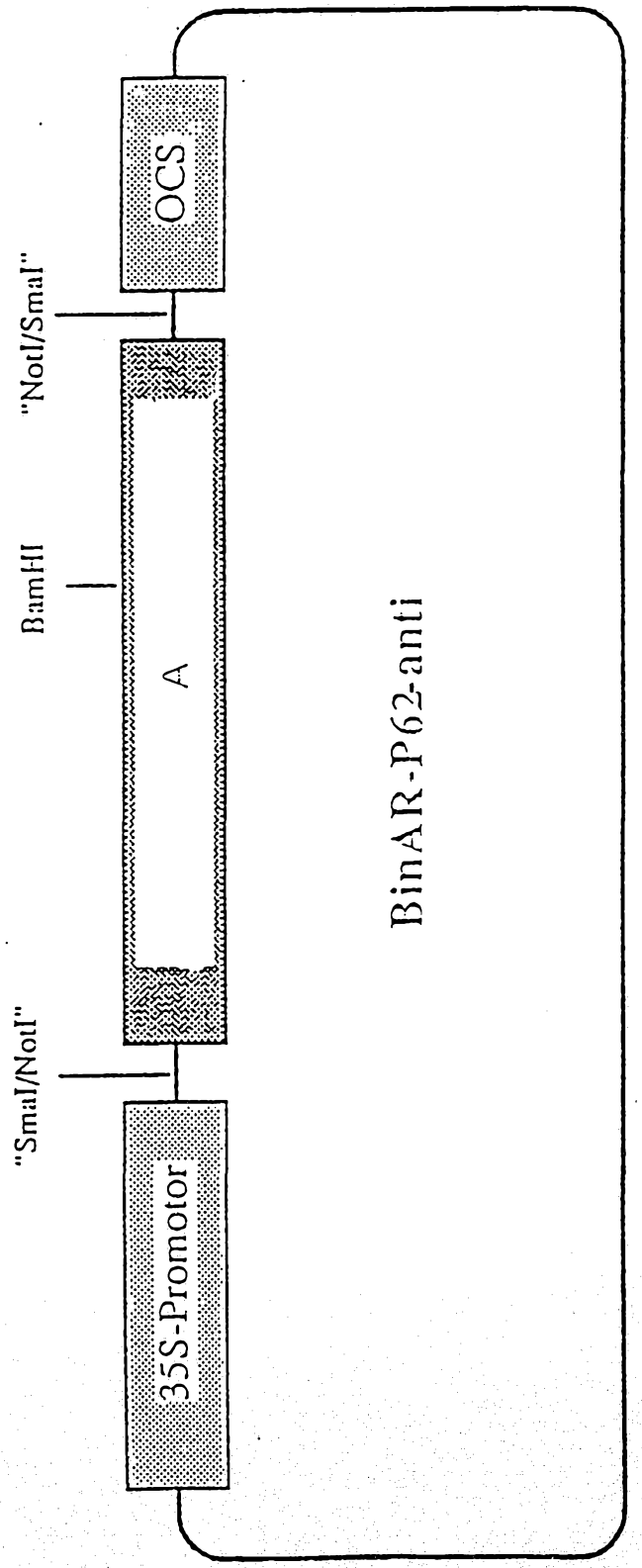


Fig. 4

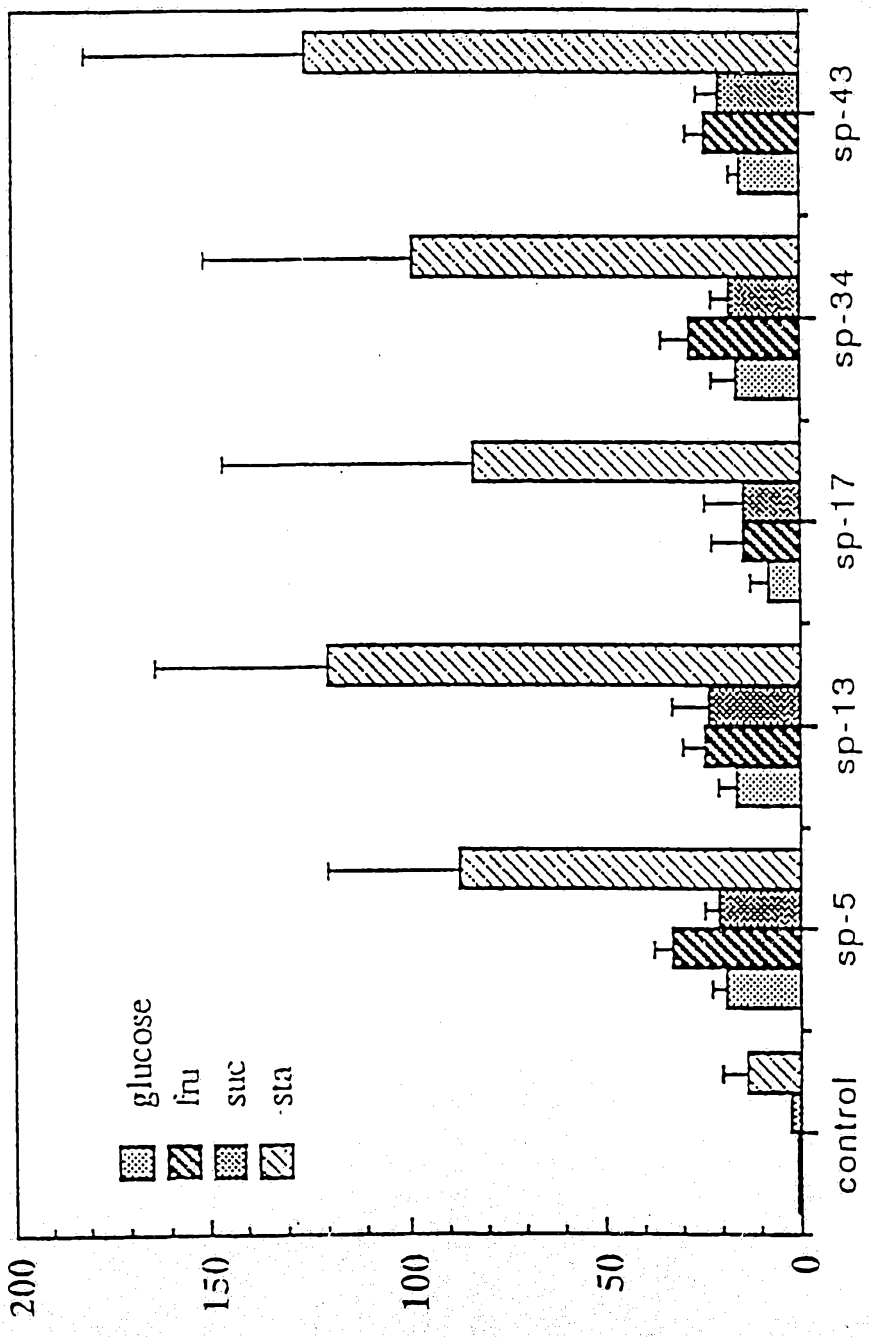
carbohydrate content [mmol/m2]
200
150
100
50
0
glucose
fru
suc
sta
control
sp-5
sp-13
sp-17
sp-34
sp-43

Fig. 5

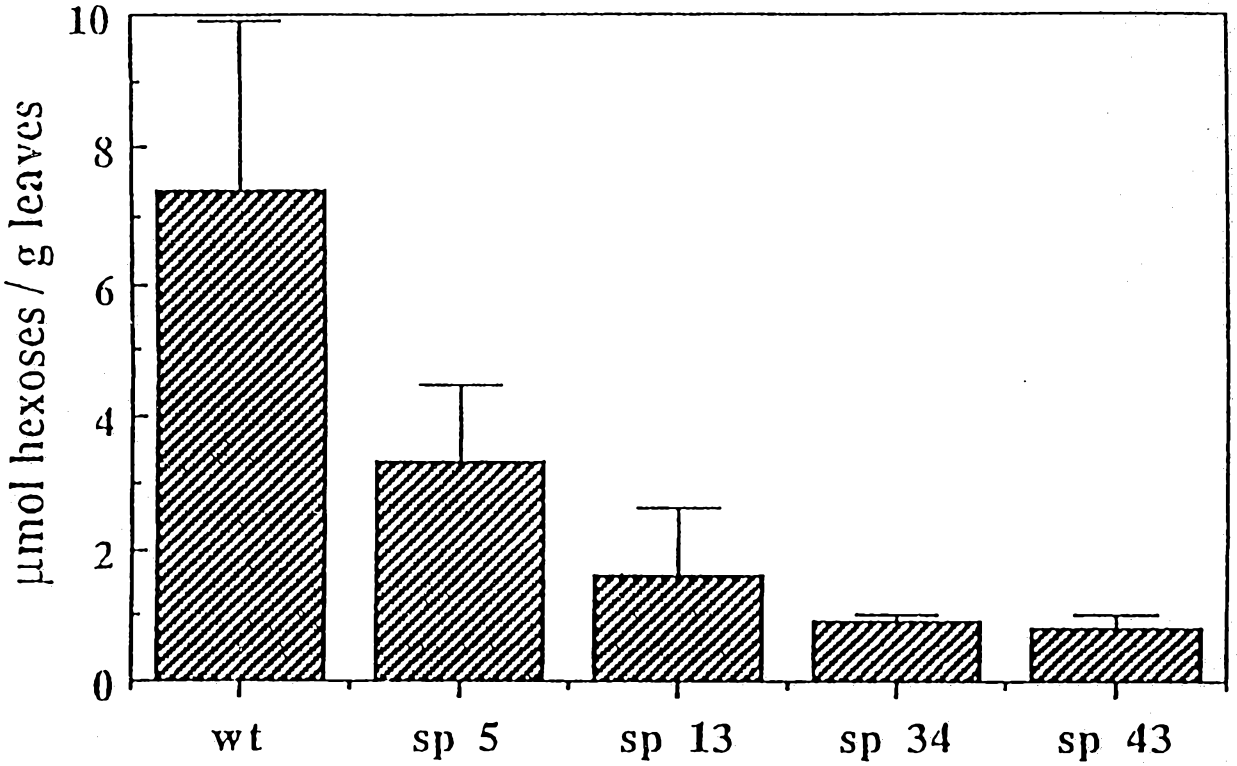


Fig. 6

# INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01604

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/29; C12N15/82; C12N15/81; C12N1/21
A01H5/00

## II. FIELDS SEARCHED

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
|---|---|
| Int.Cl. 5 | C12N ; A01H |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

| Category ° | Citation of Document,[11] with indication, where appropriate, of the relevant passages[12] | Relevant to Claim No.[13] |
|---|---|---|
| X | PLANT PHYSIOLOGY. vol. 89, no. 4, April 1989, ROCKVILLE, MD, USA. page 155 RIPP, K.G., ET AL 'cDNA cloning and the deduced amino acid sequence of a plasma membrane protein implicated in sucrose transport' see abstract 928 --- | 1 |
| X | PLANT PHYSIOLOGY. vol. 99, no. 1, May 1992, ROCKVILLE, MD, USA. page 84 OVERVOORDE, P.J., ET AL. 'Biochemical and molecular characterization of the soybean membrane 62kD sucrose binding protein and its possible role in sucrose transport' see abstract 502 --- -/-- | 1 |

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 06 OCTOBER 1993 | 14 -10- 1993 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | MADDOX A.D. |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|---|---|---|
| Y | BIOCHIM. BIOPHYS. ACTA<br>vol. 1103, no. 2, 1992,<br>pages 259 - 267<br>LI, Z-S., ET AL. 'The sucrose carrier of the plant plasmalemma: III. Partial purification and reconstitution of active sucrose transport in liposomes'<br>see the whole document | 1,4,6-8,<br>12 |
| Y | EMBO JOURNAL<br>vol. 9, no. 10, October 1990, EYNSHAM, OXFORD GB<br>pages 3045 - 3050<br>SAUER, N., ET AL. 'Primary structure, genomic organization and heterologous expression of a glucose transporter from Arabidopsis thaliana'<br>see page 3045, right column, paragraph 2 | 1,4,6-8,<br>12 |
| P,X | EMBO JOURNAL<br>vol. 11, no. 13, December 1992, EYNSHAM, OXFORD GB<br>pages 4705 - 4713<br>RIESMEIER, J.W., ET AL. 'Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast'<br>see the whole document | 1,2,4-6,<br>15-19 |
| P,X | THE PLANT CELL<br>vol. 5, no. 8, August 1993,<br>pages 823 - 830<br>RAIKHEL, N.V., ET AL. 'The wide world of plant molecular genetics'<br>see page 825, column 2<br>& NATO ADVANCED STUDY INSTITUTE COURSE, MAY 10-19, 1993. | 1,4,6-8,<br>13 |
| A | PLANT PHYSIOLOGY.<br>vol. 99, no. 1, May 1992, ROCKVILLE, MD, USA.<br>page 9<br>KOSSMAN, J., ET AL. 'Functional analysis of the plastidic fructose-1,6-biphosphatase and the triose phosphate translocator from potato'<br>see abstract 51 | 21 |

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|---|---|---|
| A | EMBO JOURNAL<br>vol. 9, no. 10, 1990, EYNSHAM, OXFORD GB<br>pages 3033 - 3044<br>VON SCHAEWEN, A., ET AL. 'Expression of a yeast-derived invertase in the cell wall of tobacco and Arabidopsis plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants'<br>see the whole document | 6-13 |
| A | PLANT PHYSIOLOGY.<br>vol. 95, 1991, ROCKVILLE, MD, USA.<br>pages 420 - 425<br>DICKINSON, C.D., ET AL. 'Slow-growth phenotype of transgenic tomato expressing apoplastic invertase'<br>see the whole document | 6-13 |
| A | GENE<br>vol. 95, 1990, AMSTERDAM NL<br>pages 17 - 23<br>BLATCH, G.L., ET AL. 'Nucleotide sequence and analysis of the Vibrio alginolyticus sucrose uptake-encoding region'<br>see the whole document | 9 |
| A | RECENT ADVANCES IN PHLOEM TRANSPORT AND ASSIMILATE COMPARTMENTATION . FOURTH INTERNATIONAL CONFERENCE , AUGUST 19-24, 1990. 1991,<br>pages 154 - 166<br>DELROT, S., ET AL. 'Use of plasma membrane vesicles from sugar beet leaves for the study of sucrose transport and of sucrose transporters'<br>see page 155, paragraph 3 | 1-22 |