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(54) Title: NUCLEIC ACID MOLECULES DERIVED FROM PLANTS WHICH CODE FOR ENZYMES WHICH ARE INVOLVED IN THE SYNTHESIS OF STARCH

(54) Bezeichnung: NUKLEINSÄUREMOLEKÜLE AUS PFLANZEN CODIEREND ENZYME, DIE AN DER STÄRKESYN-
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(57) Abstract: The invention relates to nucleic acid molecules which code for enzymes which are involved in the synthesis of starch in plants. Said enzymes are novel isoforms of starch synthetase. Vectors for the production of transgenic plant cells and plants which synthesize modified starch are also disclosed. A method for the production of said transgenic plant cells and plants is additionally disclosed as well as a method for the production of modified starch.

(57) Zusammenfassung: Es werden Nukleinsäuremoleküle beschrieben, die Enzyme codieren, die an der Stärkesynthese in Pflanzen beteiligt sind. Bei diesen Enzymen handelt es sich um eine neue Isoform der Stärkesynthase. Weiterhin werden Vektoren zur Herstellung transgener Pflanzenzellen und Pflanzen beschrieben, die eine modifizierte Stärke synthetisieren. Ferner werden Verfahren zur Herstellung dieser transgenen Pflanzenzellen und Pflanzen beschrieben sowie Verfahren zur Herstellung modifizierter Stärken.



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Description

Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

The present invention relates to nucleic acid molecules which encode an enzyme which participates in starch synthesis in plants. This enzyme is a new isoform of starch synthase.

The invention furthermore relates to vectors and to host cells transformed with the described nucleic acid molecules or vectors, in particular plant cells and plants which can be regenerated from these.

Furthermore, methods for the generation of transgenic plant cells and plants are described which, due to the introduction of DNA molecules encoding a starch synthase, synthesize a starch whose properties are altered. The present invention also relates to the starch which can be obtained from the plant cells and plants according to the invention, and to processes for the production of this starch.

Bearing in mind the recently increasing importance of plant constituents as renewable raw materials, it is one of the tasks of biotechnology research to attempt to adapt these plant raw materials to the demands of the processing industry. Thus, to make possible the use of renewable raw materials in as many fields of application as possible, it is necessary to make available a great variety of materials.

Not only oils, fats and proteins, but also polysaccharides, constitute important renewable raw materials from plants. Apart from cellulose, the starch which is one of the most important storage materials in higher plants plays a central role. In this context, maize is one of the most interesting plants, since it is the most important crop plant worldwide for starch production.

The polysaccharide starch is a polymer of chemically uniform base units, the glucose molecules. However, it is a highly complex mixture of different forms of molecules which differ with regard to their degree of polymerization and the occurrence of branchings in the glucose chains. Starch is therefore no uniform raw material. In particular, we differentiate between amylose starch, an essentially unbranched polymer of α -1,4-glycosidically linked glucose molecules, and amylopectin starch, which, in turn, constitutes a complex mixture of differently branched glucose chains. The branchings are generated by the occurrence of additional α -1,6-glycosidic linkages. In typical plants used for starch production such as, for example, maize or potatoes, the starch synthesized consists of approx. 20% - 25% of amylose starch and approx. 75% - 80% of amylopectin starch.

To allow as broad an application of starch as possible, it appears desirable to provide plants which are capable of synthesizing modified starch which is particularly suitable for various purposes. One possibility of providing such plants is – besides plant-breeding measures – the targeted genetic alteration of the starch metabolism of the starch-producing plants by recombinant methods. However, a prerequisite for this is the identification and characterization of the enzymes which participate in starch synthesis and/or starch modification, and the isolation of the relevant DNA molecules which encode these enzymes.

The biochemical synthetic pathways which lead to the synthesis of starch are essentially known. In plant cells, starch synthesis takes place in the plastids. In photosynthetically active tissues, these are the chloroplasts, in photosynthetically inactive, starch-storing tissues, the amyloplasts.

The most important enzymes which participate in starch synthesis are the starch synthases and the branching enzymes. Amongst the starch synthases, various isoforms have been described, all of which catalyze a polymerization reaction by transferring a glucosyl residue from ADP-glucose to α -1,4-glucans. Branching enzymes catalyze the introduction of α -1,6-branchings into linear α -1,4-glucans.

Two classes of starch synthases can be distinguished: the granule-bound starch synthases (GBSS) and the soluble starch synthases (SS). However, this distinction is not clear-cut in each individual case, since some of the starch synthases are

present both in granule-bound form and in soluble form (Denyer et al., *Plant J.* 4 (1993), 191-198; Mu et al., *Plant J.* 6 (1994), 151-159).

Besides the class of the granule-bound starch synthases, GBSSI, at least three different isoforms have so far been described in maize plants within the class of the soluble starch synthases, based on cDNA and amino acid sequence comparisons.

Isoform I of starch synthase (SSI) includes genes, which, in maize, encode an approx. 76 kDa protein zSSI (Mu et al., *Plant J.* 6, (1994), 151-159) and which have as yet only been described for monocotyledonous plants such as, for example, for rice (Baba et al., *Plant Physiol.* 103, (1993), 565-573), are expressed mainly in the endosperm. As a rule, these proteins are stimulated by citrate and are independent of so-called primer molecules.

In contrast, isoform II starch synthases (= SSII) are, as a rule, dependent on primer molecules and show the highest sequence homology with the SSII Isoforms – some of which used to be termed GBSSII – from pea (Dry et al., *Plant J.* 2, (1992), 193-202) and potato (Edwards et al., *Plant J.* 8, (1995), 283-294).

When considering the maize SSII, a distinction must be made between the genes, or cDNAs, which are termed zSSIIa and zSSIIb in the literature (Harn et al., *Plant Mol. Biol.* 37, (1998), 639-649; Imparl-Radosevich, *Arch. Biochem. Biophys.* 362, (1999), 131-138), and the so-called SSII protein, an approx. 180 kDa protein (molecular weight determined by means of gel filtration (Mu et al., *Plant J.* 6, (1994), 151-159)) from maize endosperm, whose name is based on earlier biochemical studies (Boyer and Preiss, *Plant Physiol.* 67, (1981), 1141-1145; Mu et al., *Plant J.* 6, (1994), 151-159). The question of which gene actually corresponds to this 180 kDa protein is currently not conclusively answered (Imparl-Radosevich, *Arch. Biochem. Biophys.* 362, (1999), 131-138). Cao et al. (*Plant Physiol.* 120, (1999), 205-215) propose the so-called du1 gene as the gene which corresponds to the 180 kDa protein.

The third class of starch synthase genes which has been described to date, termed SSIII, encode, in potatoes, an 139 kDa protein (Abel et al., *Plant J.* 10, (1996), 981-991; Marshall et al., *Plant Cell* 8, (1996), 1121-1135), which amount to 80% of the total starch synthase activity in potato tubers. Since certain sequence regions of the C-terminus are highly conserved in comparison with the potato SSIII amino acid sequence, it was proposed to rename the maize gene originally termed du1 gene

"zSSIII" (Cao et al., Plant Physiol. 120, (1999), 205-215), the prefix "z" denoting the organism of origin *Zea mays*.

The detailed function in starch synthesis has as yet only been determined for the isoform GBSS I. Plants in which this enzyme activity is greatly or fully reduced synthesize an amylase-free "waxy" starch (Shure et al., Cell 35 (1983), 225-233; Visser et al., Mol. Gen. Genet. 225 (1991), 289-296; WO9211376A1), so that an important role in amylose starch synthesis is attributed to this enzyme. This phenomenon is likewise observed in cells of the green algae *Chlamydomonas reinhardtii* (Delrue et al., J. Bacteriol. 174 (1992), 3612-3620). In *Chlamydomonas*, it was additionally possible to demonstrate that GBSS I not only participates in amylose synthesis, but also affects amylopectin synthesis. Mutants which have no GBSSI activity lack a particular fraction of the amylopectin usually synthesized, which contains longer-chain glucans.

The functions of the isoforms of the soluble starch synthases remain unclear, it is assumed that the soluble starch synthases together with branching enzymes participate in amylopectin synthesis (see, for example, Ponstein et al., Plant Physiol. 92 (1990), 234-241) and that they play an important role in the regulation of the starch synthesis rate.

Besides maize, soluble starch synthases were also identified in a series of other plant species. For example, soluble starch synthases have been isolated until homogeneous from pea (Denyer and Smith, Planta 186 (1992), 609-617) and potato (Edwards et al., Plant J. 8 (1995), 283-294). It emerged in these cases that the isoform of the soluble starch synthase which is identified as SS II is identical with the granule-bound starch synthase GBSS II (Denyer et al., Plant J. 4 (1993), 191-198; Edwards et al., Plant J. 8 (1995), 283-294). The presence of a plurality of SS isoforms has been described for some other plant species with the aid of chromatographic methods, for example in barley (Tyynelä and Schulman, Physiologia Plantarum 89 (1993) 835-841; Kreis, Planta 148 (1980), 412-416) and wheat (Rijven, Plant Physiol. 81 (1986), 448-453). DNA sequences which encode these proteins have also been described (see, for example, GenBank Acc. No. U48227; Vrinten et al., Mol. Gen. Genet. 261 (3), (1999), 463-471).

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

To provide further options of altering any starch-storing plants in such a way that they synthesize a modified starch, it is necessary to identify DNA sequences in each case which encode further isoforms of the starch synthases.

One embodiment of the present invention provides nucleic acid molecules which encode enzymes which participate in starch biosynthesis and with the aid of which it is possible to generate recombinant plants which exhibit an increased or reduced activity of these enzymes, thus resulting in altered chemical and/or physical properties of the starch synthesized in these plants, which is therefore better suited to general and/or specific purposes.

The present invention therefore relates to nucleic acid molecules which encode proteins with the bioactivity of starch synthase or a bioactive fragment of such a protein, such molecules preferably encoding proteins with the amino acid sequence indicated under SEQ ID No. 2.

In particular, the invention relates to nucleic acid molecules which comprise the nucleotide sequence indicated under SEQ ID No. 1, or part thereof, preferably molecules which encompass the coding region indicated in SEQ ID No. 1, or corresponding ribonucleotide sequences.

The invention also relates to nucleic acid molecules which have a sequence which is complementary to all or part of the sequence shown in SEQ ID No. 1.

Subject matter of the invention are also nucleic acid molecules which encode a starch synthase or a bioactive fragment thereof and/or whose sequence deviates from the nucleotide sequences of the above-described molecule due

to the degeneracy of the genetic code.

The present invention furthermore relates to nucleic acid molecules which
encode a starch synthase or a bioactive fragment thereof and which hybridize
5 with one of the above-described molecules.

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The nucleic acid molecules according to the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. RNA molecules can be, for example, mRNA or antisense-RNA molecules.

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The present invention therefore also relates to nucleotide sequences of introns which are part of the genomic sequences which correspond to the cDNA sequences indicated under SEQ ID No. 1. Suitable intron sequences can be isolated and identified, for example, using the nucleic acid molecules indicated

10 under SEQ ID No. 1, for example, by screening a genomic DNA library.

In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or

15 "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

The term "hybridization" means for the purposes of the present invention hybridization under conventional hybridization conditions, preferably under stringent conditions as they are described, for example, by Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. "Hybridization" especially preferably means that hybridization takes place under the following conditions:

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Hybridization buffer: 2 x SSC; 10 x Denhardt solution (Ficoll 400 + PEG + BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na² HPO⁴ ; 250 µg/ml herring sperm DNA; 50 µg/ml tRNA; or

30 0.25 M sodium phosphate buffer pH 7.2;
1 mM EDTA
7% SDS

hybridization temperature T + 65° to 68° C

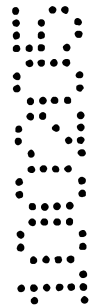
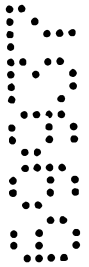
washing buffer: 0.2 x SSC; 0.1% SDS

35

washing temperature T= 40 to 68° C.

Nucleic acid molecules which hybridize with the nucleic acid molecules

according to the invention can be derived, in principle, from any organism (i.e. prokaryotes or eukaryotes, in particular from bacteria, fungi, algae, plants or animal organisms) which has such molecules. They are preferably derived from monocotyledonous or dicotyledonous plants, in particular from useful
5 plants, especially preferably from starch-storing plants, in particular from maize.



Nucleic acid molecules which hybridize with the molecules according to the invention can be isolated, for example, from genomic or from cDNA libraries of various organisms. Alternatively, they can be generated by recombinant methods or synthesized chemically.

Such nucleic acid molecules can be identified and isolated from plants or other organisms using the molecules according to the invention or parts of these molecules or the reverse complements of these molecules, for example by means of hybridization following standard methods (see, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Hybridization probes which can be used are, for example, nucleic acid molecules which exactly or essentially have the nucleotide sequence indicated under Seq ID No. 1 or parts of this sequence. The fragments used as hybridization probe can also be synthetic fragments which were prepared with the aid of the customary synthetic techniques and whose sequence essentially is identically with that of a nucleic acid molecule according to the invention. Once genes which hybridize with the nucleic acid sequences according to the invention have been identified and isolated, the sequence must be determined and the properties of the proteins encoded by this sequence analyzed.

The invention furthermore relates to plasmid IR 65/87, which was deposited with the Deutsche Sammlung für Mikroorganismen, Braunschweig, Germany, on August 5, 1999 under the number DSM 12970, and to the nucleic acid molecule contained in the insertion of plasmid IR 65/87, which encodes a protein with the enzymatic activity of a starch synthase. In addition, the present invention relates to fragments of the nucleic acid molecule contained in the insertion of plasmid IR 65/87, preferably to fragments which encompass the encoding region or part thereof. Furthermore, the present invention also relates to nucleic acid molecules which hybridize with the nucleic acid molecule contained in the insertion of plasmid IR 65/87, and to nucleic acid molecules which have a sequence which is complementary to all or part of the insertion of the nucleic acid molecule contained in plasmid IR 65/87. In addition, the

present invention relates to nucleic acid molecules whose nucleotide sequence deviates in comparison with the nucleic acid molecules of the insertion of plasmid IR 65/87, due to the degeneracy of the genetic code.

The present invention also relates to fragments and allelic variants of the above-described nucleic acid molecules according to the invention .

In this context, fragments are parts of the nucleic acid molecules according to the invention which encode a protein according to the invention or parts of this protein and which are, as a rule, oligo- or polynucleotides composed of about 25 to 150, preferably of at least 150, especially preferably at least 500, in particular at least 1000 and especially preferably of at least 3500 nucleotides of the nucleic acid molecules according to the invention .

The term "fragment" in the present context means a part of the nucleic acid molecules according to the invention which encodes a part of the protein according to the invention and is functionally active. Furthermore, the fragment may also encode an antisense-mRNA or be contained in a molecule which mediates a cosuppression effect or an in-vivo mutagenesis effect. "Functionally active" means in the present context that the bioactivity of the protein encoded by the nucleic acid molecule according to the invention is either increased or reduced in a plant cell according to the invention.

The allelic variants can be not only naturally occurring variants, but also synthesized variants or variants which have been generated by recombinant DNA technology.

The invention also relates to derivatives of the above-described nucleic acid molecules according to the invention . The term "derivative" means in this context that the sequences of these molecules differ from the sequences of the above-described nucleic acid molecules with regard to one or more positions and have a high degree of homology to these sequences, in particular the coding region of the nucleotide sequence indicated under SEQ ID No. 1. Homology in this context means a sequence identity of at least 50%, in particular an identity of at least 70%,

preferably over 85% and especially preferably over 95%. The deviations with regard to the above-described nucleic acid molecules may have been generated by deletion, substitution, insertion or recombination.

"Homology" for the purposes of the present invention means that a functional and/or structural equivalence exists between the nucleic acid molecules in question and the proteins encoded by them. The nucleic acid molecules which are homologous to the above-described molecules and which constitute derivatives of these molecules are, as a rule, variations of these molecules which constitute modifications which exert the same biological function. They may be naturally occurring variations, for example sequences from other organisms, or mutations, it being possible for these mutations to have occurred naturally or to have been introduced by directed mutagenesis.

The proteins encoded by the various variants (fragments, derivatives, allelic variants) of the nucleic acid molecules according to the invention share certain characteristics with the amino acid sequence defined under Seq ID No. 2. These may include, for example, enzyme activity, molecular weight, immunological reactivity, conformation etc., and physical properties such as, for example, the migration behavior in gel electrophoresis, the chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability, pH optimum, temperature optimum, etc.

Important characteristics of a starch synthase are: i) its localization in the stroma of the plastids of plant cells; ii) its capability of synthesizing linear α -1,4-linked polyglucans using ADP-glucose as substrate. This activity can be determined as described by Denyer and Smith (Planta 186 (1992), 609-617).

The nucleic acid molecules according to the invention can originate from a pro- or eukaryotic organism which expresses the genes described, preferably from plants, in particular from starch-synthesizing or starch-storing plants. These can be both monocotyledonous and dicotyledonous plants. Especially preferred in this context are, for example, cereal species (such as barley, rye, oats, wheat etc.), maize, rice, peas, cassava, potatoes and the like.

The proteins encoded by the nucleic acid molecules according to the invention are an isoform of a plant starch synthase which has so far not been identified and characterized. These proteins have the enzymatic activity of a starch synthase and show significant homology to the potato SSIII (Marshall et al., Plant Cell 8, (1996), 1121-1135) and the maize isoform termed zSSIII (du1) (Cao et al., Plant Physiol. 120, (1999), 205-215) in the region of amino acids 740 to 1170 of the amino acid sequence indicated under SEQ ID No. 2. The proteins encoded by the nucleic acid molecules according to the invention differ from the potato SSIII and the maize zSSIII significantly by their N-terminus. Furthermore, the calculated isoelectric point of the protein indicated under SEQ ID No. 2 differs significantly from the calculated isoelectric points for the potato SSIII and the maize zSSIII. Moreover, the protein indicated under SEQ ID No. 2 has a clearly reduced calculated molecular weight of approx. 132 kDa in comparison with the zSSIII (calculated molecular weight approx. 188 kDa). In contrast to zSSIII, expression of the genes of the isoform according to the invention is greater in young leaves than in the endosperm.

In a further embodiment, the present invention therefore relates to the above-described nucleic acid molecules according to the invention which encode proteins with the bioactivity of a starch synthase, such molecules preferably encoding for proteins which have a homology of at least 50%, preferably of at least 65%, in particular of at least 80% and especially preferably of at least 95% with the amino acid sequence indicated under SEQ ID No. 2 in the N-terminal region. The term "N-terminus" means in this context the amino acids 1 to 150, preferably the amino acids 1 to 300, and especially preferably the amino acids 1 to 480 of the amino acid sequence indicated under SEQ ID No. 2.

In a further embodiment of the invention, the nucleic acid molecules according to the invention encode proteins with the bioactivity of a starch synthase, such molecules encoding proteins which have a calculated isoelectric point $pI = 6.95 \text{ pH} \pm 1.00 \text{ pH}$, preferably $pI = 6.95 \text{ pH} \pm 0.75 \text{ pH}$, especially preferably $pI = 6.95 \text{ pH} \pm 0.50 \text{ pH}$.

In a further embodiment, the nucleic acid molecules according to the invention encode proteins with the bioactivity of a starch synthase which have at least one deletion in at least one of the eight sequence motifs which are characteristic of starch synthases and which have been described by Cao et al. (Plant Physiol. 120, (1999), 205-215). The deleted motif is preferably that termed sequence motif VII by Cao et al. (Plant Physiol. 120, (1999), 205-215). In a further embodiment, the nucleic acid molecules according to the invention therefore encode proteins with the bioactivity of a starch synthase which have at least one deletion in one or more of the sequence motifs VII, selected from the group consisting of

SHTIYAASDLFIIPSIFEPCGLTQMIAMRYGS (Seq ID No. 3);
 SHLIYAGADFILVPSIFEPCGLTQLTAMRYGS (Seq ID No. 4);
 SHLIYAGSDFILVPSIFEPCGLTQLVAMRYGT (Seq ID No. 5);
 AHQMMAGADVLAVTSRFEPGLIQLQGMRYGT (Seq ID No. 6);
 AHQMMAGADVLAVTSRFEPGLIQLQGMRYGT (Seq ID No. 7);
 AHMITAGADFMLIPSRFEPGLIQLHAMRYGT (Seq ID No. 8);
 AHMITAGADFMLVPSRFEPGLIQLHAMRYGT (Seq ID No. 9);
 AHLIMAGADVLAVPSRFEPGLIQLQGMRYGT (Seq ID No. 10);
 AHKIIAGADFIVIPSRFEPGLVQLHAMPYGT (Seq ID No. 11);
 AHHIMAGADLLAVTSRFEPGLIQLQGMRYGT (Seq ID No. 12);
 AHHIMAGADVLAVTSRFEPGLIQLQGMRYGT (Seq ID No. 13);
 SHRITAGCDILLMPSRFEPGLNQLYAMQYGT (Seq ID No. 14);
 AHRITAGSDILLMPSRFEPGLNQLYAMSYGT (Seq ID No. 15);
 SHRITAGCDILLMPSRFEPGLNQLYAMRYGT (Seq ID No. 16);
 SHRITAGADILLMPSRFEPGLNQLYAMKYGT (Seq ID No. 17);
 AHRITAGADIALMPSRFEPGLNQLYAMAYGT (Seq ID No. 18);
 SHRITAGCDILLMPSRFEPGLNQLYAMQYGT (Seq ID No. 19);
 SHRITAGCDILLMPSRFEPGLNQLYAMQYGT (Seq ID No. 20);
 AHRITAGADVLVMPSPRFEPGLNQLYAMAYGT (Seq ID No. 21);
 AHRITAGADILLMPSRFEPGLNQLYAMAYGT (Seq ID No. 22);
 ARKLYASSDFILMPSYFEPGLTQMIGMRYGC (Seq ID No. 23);
 AHQIYAGSDMFLMPSKFEPGLTQLYALRYGC (Seq ID No. 24);
 AHQIYAGADFLIPSLFEPGLSQMIALRYGT (Seq ID No. 25);

AHQIYAGADLFLIPSLFEPCGLGQLIALQYGA (Seq ID No. 26);
SHRIMGGADVILVPSRFEPCLTQLYGSKYGT (Seq ID No. 27);
SHLMVAGGDVILVPSRFEPCLTQLYGLQYGT (Seq ID No. 28) and
AHLIYGAADIIVPSNYEPCGLTQMIGLRYGA (Seq ID No. 29) (cf. sequence motif VII as defined by Cao et al., Plant Physiol. 120, (1999), p. 207, table 1).

Methods for identifying said sequence motifs are known to the skilled person and may be based on amino acid sequence comparisons with the characteristic sequence motifs VII described by Cao et al (see above).

In a further preferred embodiment of the invention, the nucleic acid molecules according to the invention encode proteins with the function of a starch synthase which has a deletion of at least 2 amino acids, especially preferably of at least 5 amino acids, in particular of at least 10 amino acids and especially preferably of at least 20 amino acids in one or more of the motifs as shown in Seq ID No. 3 to 29.

In a further especially preferred embodiment of the invention, said sequence motif VII of the proteins encoded by the above-described nucleic acid molecules according to the invention has the following amino acid sequence: (1)SH---AMRYG-(11), it being possible for positions 3, 4, 5 and 11 of this motif to be occupied by any amino acid. In Seq ID No. 2, sequence motif VII starts at amino acid 1067 (= S) and ends at amino acid 1077 (= S).

In position 3, there is especially preferably an amino acid selected from the group consisting of T, L, M, Q or R. In position 4, there is preferably an amino acid selected from the group consisting of I, L or M. In position 5, there is preferably an amino acid selected from the group consisting of I, Y, M, T or V. In position 11, there is preferably an amino acid selected from the group consisting of S, T, C or A.

The present invention also relates to the use of the above-described nucleic acid molecules according to the invention for screening nucleic acid libraries, in particular cDNA and genomic libraries and/or as hybridization probe. In addition, the present invention also relates to the use of the above-described nucleic acid molecules according to the invention for generating transgenic plant cells or transgenic plants.

Furthermore, the invention relates to vectors, in particular plasmids, cosmids, viruses, bacteriophages and other vectors commonly used in genetic engineering which contain the above-described nucleic acid molecules according to the invention.

In a preferred embodiment, the nucleic acid molecules contained in the vectors are linked to regulatory elements which ensure the transcription and synthesis of a translatable RNA in prokaryotic or eukaryotic cells or in the cell-free system.

Regulatory elements for expressing the nucleic acid molecules according to the invention in microorganisms (for example *E. coli*, *S. cerevisiae*) have been described widely in the literature. Promoters which permit a particularly strong expression of the gene downstream are, for example, the T7 promoter (Studier et al., Methods in Enzymology 185, (1990), 60-89), lacuv5, trp, trp-lacUV5 (DeBoer et al., in: Rodriguez and Chamberlin (Eds.), Promoters, Structure and Function; Praeger, New York, (1982), 462-481; DeBoer et al, Proc. Natl. Acad. Sci. USA (1983), 21-25), lp1, rac (Boros et al., Gene 42 (1986), 97-100). As a rule, protein quantities reach their maximum from the middle towards the end of the logarithmic phase of the growth cycle of the microorganisms. Thus, inducible promoters are preferably used for synthesizing proteins. These frequently lead to higher protein yields than constitutive promoters. The use of strong constitutive promoters frequently leads to the loss of energy for other essential cell functions, due to the constant transcription and translation of a cloned gene, thus slowing down cell growth (Bernard R. Glick/ Jack J. Pasternak, Molekulare Biotechnologie (1995), Spektrum Akademischer Verlag GmbH, Heidelberg Berlin Oxford, p. 342.). Thus, to achieve an optimal protein quantity, a two-step process is frequently used. First, the host cells are grown under optimal conditions up to a relatively high cell density. In the second step, transcription is then induced, depending on the type of the promoter employed. Especially suitable in the present context is a lactose - or IPTG (=isopropyl- β -D-thiogalactopyranoside) inducible tac promoter (deBoer et al., Proc. Natl. Acad. Sci. USA 80 (1983), 21-25). Termination signals for transcription are also described in the literature.

Regulatory elements for expressing the nucleic acid molecules according to the invention in plant cells are described in connection with the plant cells according to the invention.

Expression of the nucleic acid molecules according to the invention in prokaryotic cells, for example in *Escherichia coli* is important insofar as this allows the enzymatic activities of the enzymes encoded by these molecules to be characterized in greater detail. In particular, it is possible to characterize the product synthesized by the enzymes in question in the absence of other enzymes which participate in starch synthesis in the plant cell. This allows conclusions to be drawn of the function which the protein in question has during starch synthesis in the plant cell.

In addition, it is possible to introduce various mutations into the nucleic acid molecules according to the invention by customary techniques of molecular biology (see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), this leading to the synthesis of proteins with potentially altered biological properties. On the one hand, it is possible to generate deletion mutants in which nucleic acid molecules are generated by progressive deletions starting at the 5'- or at the 3'-end of the coding DNA sequence, which lead to the synthesis of suitably shortened proteins. For example, such deletions at the 5'-end of the nucleotide sequence allow amino acid sequences to be identified which are responsible for the translocation of the enzyme into the plastids (transit peptides). This permits the targeted production of enzymes which, due to the removal of the sequences in question, are no longer localized in the plastids, but in the cytosol, or which, due to the addition of other signal sequences, are localized in other compartments.

The exchange of the native transit peptide for another transit peptide which mediates localization in the plastids is also feasible. A plastid signal sequence which can be used is, for example, the spinach ferredoxin:NADP⁺ oxidoreductase (FNR). This sequence contains the 5' non-translated region and the flanking transit peptide sequence of the cDNA of the plastid protein spinach ferredoxin:NADP⁺ oxidoreductase (nucleotides -171 to +165; Jansen et al., Current Genetics 13, (1988), 517-522).

Another plastid signal sequence which can be used is, for example, the transit peptide of the maize waxy protein plus the first 34 amino acids of the mature waxy protein (Klösken et al., Mol. Gen. Genet. 217, (1989), 155-161). In addition, the transit peptide of the maize waxy protein (see above) may also be used without the first 34 amino acids of the mature waxy proteins.

On the other hand, the introduction of point mutations into the nucleic acid molecules according to the invention is also possible in positions where an altered amino acid sequence has an effect on, for example, enzyme activity or enzyme regulation. This allows for example mutants to be generated which have an altered k_{cat} and/or K_m value or which are no longer subject to the regulatory mechanisms via allosteric regulation or covalent modification which are normally present in the cell.

Moreover, it is possible to generate mutants which have an altered substrate or product specificity, such as, for example, mutants which use ADP-glucose-6-phosphate instead of ADP-glucose as substrate. Furthermore, it is possible to generate mutants which have an altered activity – temperature profile.

For carrying out recombinant manipulations in prokaryotic cells, the nucleic acid molecules according to the invention or parts of these molecules can be introduced into plasmids which permit mutagenesis or sequence modification by the recombination of DNA sequences. Base exchanges may be carried out on natural or synthetic sequences added with the aid of standard methods (cf. Sambrook et al., 1989, Molecular Cloning: A laboratory manual, 2nd Ed., Cold Spring Harbor Laboratory Press, NY, USA). For linking the DNA fragments to each other, adapters or linkers may be added to the fragments. Moreover, manipulations may be employed which provide suitable restriction cleavage sites or which remove excess DNA or restriction cleavage sites. Where insertions, deletions or substitutions are suitable, in-vitro mutagenesis, primer repair, restriction or ligation may be used. Analytical methods which are generally carried out are a sequence analysis, a restriction analysis and other methods of biochemistry and molecular biology.

Furthermore, the present invention relates to vectors which contain the above-described nucleic acid molecules according to the invention, the nucleic acid molecules being connected in sense orientation with regulatory elements which

ensure the transcription and synthesis of a translatable RNA in pro- or eukaryotic cells.

The meaning of the term "sense orientation" is known to the skilled person.

In a further embodiment, the invention relates to host cells, in particular prokaryotic or eukaryotic cells, which are transformed with an above-described nucleic acid molecule according to the invention or a vector according to the invention, and cells which are derived from such transformed cells and contain a nucleic acid molecule according to the invention or a vector according to the invention. These are preferably bacterial cells, especially preferably plant cells.

The present invention furthermore relates to a method for generating a host cell, wherein a host cell is genetically modified by introducing a nucleic acid molecule according to the invention and/or a vector according to the invention. The host cell may be of prokaryotic or else eukaryotic origin. The cells are preferably bacterial cells, especially preferably plant cells.

The term "genetically modified" means in connection with the present invention that the host cell, in particular a plant cell, is altered with regard to its genetic information by introducing a nucleic acid molecule according to the invention, and that the presence or the expression of the nucleic acid molecule according to the invention results in a phenotypic change. Phenotypic change in this context preferably means a measurable change in one or more cell functions. For example, genetically modified plant cells according to the invention show a reduced activity of the protein according to the invention or an increased activity of the protein according to the invention.

Subject matter of the invention are furthermore the proteins or bioactive fragments thereof which are encoded by the nucleic acid molecules according to the invention and methods for their production, wherein a host cell according to the invention is grown under conditions which permit the synthesis of the protein and the protein is subsequently isolated from the cultured cells and/or the culture medium. The

characteristic properties of the proteins according to the invention have already been described above in connection with the description of the corresponding nucleic acid molecules according to the invention.

The provision of the nucleic acid molecules according to the invention now allows to influence the starch metabolism of plants in an unprecedented manner with the aid of recombinant methods and to alter it in such a way that the synthesis of a modified starch results which is altered in comparison with starch synthesized in wild-type plants for example with regard to its physicochemical properties, in particular the amylose/amylopectin ratio, the degree of branching, the average chain length, the phosphate content, pastification behavior, and the size and/or shape of the starch granule (starch granule morphology). Due to an increase in the activity of the proteins according to the invention, for example by overexpressing suitable nucleic acid molecules, or by providing mutants which are no longer subject to the regulatory mechanisms naturally occurring in the cell and/or have different temperature dependences with regard to their activities, it is possible to increase the yield in suitably genetically engineered plants.

The economic importance of the possibility of influencing the starch synthesis in maize alone is obvious: maize is the most important starch plant worldwide. Approximately 80% of the starch produced worldwide each year is obtained from maize.

Thus, it is possible to express the nucleic acid molecules according to the invention in plant cells in order to increase the activity of the starch synthase in question. It is furthermore possible to modify the nucleic acid molecules according to the invention by methods known to the skilled person in order to obtain starch synthases according to the invention which are no longer subject to the regulatory mechanisms naturally occurring in the cell or show altered temperature dependences or substrate or product specificities.

When expressing the nucleic acid molecules according to the invention in plants, it is possible, in principle, for the protein synthesized to be localized in any compartment of the plant cell. For achieving localization in a particular compartment, the sequence which ensures localization in plastids must be deleted and, optionally, the remaining

coding region linked to DNA sequences which ensure localization in the compartment in question. Such sequences are known (see, for example, Braun et al., EMBO J. 11 (1992), 3219-3227; Wolter et al., Proc. Natl. Acad. Sci. USA 85 (1988), 846-850; Sonnewald et al., Plant J. 1 (1991), 95-106). Examples of plastid signal sequences have already been mentioned above in a different context.

The present invention thus also relates to transgenic plant cells which have been transformed with a nucleic acid molecule according to the invention or a vector according to the invention, and to transgenic plant cells derived from such transformed cells. Such cells contain a nucleic acid molecule according to the invention, the nucleic acid molecule preferably being linked to regulatory DNA elements which ensure transcription in plant cells, in particular to a promoter.

A multiplicity of techniques are available for introducing DNA into a plant host cell. These techniques encompass the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation means, the transformation of protoplasts by means of polyethylene glycol, the injection, the electroporation of DNA, the introduction of DNA by means of the biolistic approach, and other possibilities.

The use of the agrobacteria-mediated transformation of plant cells has been researched into intensively and described sufficiently in EP-A2-0120516; Hoekema, in: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasterdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant Sci. 4, 1-46 and An et al. EMBO J. 4, (1985), 277-287. For the transformation of potato, see, for example, Rocha-Sosa et al. (EMBO J. 8, (1989), 29-33.).

The transformation of monocotyledonous plants by means of *Agrobacterium*-based vectors has also been described (Chan et al., Plant Mol. Biol. 22, (1993), 491-506; Hiei et al., Plant J. 6, (1994) 271-282; Deng et al., Science in China 33, (1990), 28-34; Wilmink et al., Plant Cell Reports 11, (1992), 76-80; May et al., Bio/Technology 13, (1995), 486-492; Conner and Domisse, Int. J. Plant Sci. 153 (1992), 550-555; Ritchie et al., Transgenic Res. 2, (1993), 252-265). An alternative system for the transformation of monocotyledonous plants is the transformation by the biolistic

approach (Wan and Lemaux, *Plant Physiol.* 104, (1994), 37-48; Vasil et al., *Bio/Technology* 11 (1993), 1553-1558; Ritala et al., *Plant Mol. Biol.* 24, (1994), 317-325; Spencer et al., *Theor. Appl. Genet.* 79, (1990), 625-631), protoplast transformation, the electroporation of partially permeabilized cells, and the introduction of DNA by means of glass fibers. The transformation of maize, in particular, has been described repeatedly in the literature (cf., for example, WO 9506128A2, EP-A2-0513849, EP-A1-0465875, EP-A1-292435; Fromm et al., *Biotechnology* 8, (1990), 833-844; Gordon-Kamm et al., *Plant Cell* 2, (1990), 603-618; Koziel et al., *Biotechnology* 11 (1993), 194-200; Moroc et al., *Theor. Appl. Genet.* 80, (1990), 721-726).

The successful transformation of other cereal species has also been described, for example in the case of barley (Wan and Lemaux, see above; Ritala et al., see above; Krens et al., *Nature* 296, (1982), 72-74) and wheat (Nehra et al., *Plant J.* 5, (1994), 285-297).

Regulatory elements for the expression of the nucleic acid molecules according to the invention in plant cells are, in principle, any promoter, enhancer, terminator etc. which is active in plant cells. The promoter can be chosen in such a way that expression in the plants according to the invention is constitutive, or only in a particular tissue, at a particular point in time of the plant's development, or at a point in time determined by external factors. Relative to the plant, the promoter may be homologous or heterologous.

Examples of suitable promoters are the promoter of the cauliflower mosaic virus 35S RNA (see, for example, US5352605) and the Ubiquitin promoter (see, for example, US5614399) for constitutive expression, the patatin gene promoter B33 (Rocha-Sosa et al., *EMBO J.* 8 (1989), 23-29) for tuber-specific expression in potatoes, or a promoter which ensures expression only in photosynthetically active tissues, for example the ST-LS1 promoter (Stockhaus et al., *Proc. Natl. Acad. Sci. USA* 84 (1987), 7943-7947; Stockhaus et al., *EMBO J.* 8 (1989), 2445-2451), the Ca/b promoter (see, for example, US 5656496, US 5639952, Bansal et al., *Proc. Natl. Acad. Sci. USA* 89, (1992), 3654-3658) and the Rubisco SSU promoter (see, for example, US 5034322, US 4962028). However, it is also possible to use promoters which are only activated at a point in time which is determined by external factors

(see, for example, WO9307279A1). Promoters of heat shock proteins, which permit simple induction, may be of particular interest in this context. Furthermore, seed-specific promoters such as, for example, the *Vicia faba* USP promoter, ensure seed-specific expression in *Vicia faba* and other plants (Fiedler et al., *Plant Mol. Biol.* 22, (1993), 669-679; Bäumlein et al., *Mol. Gen. Genet.* 225, (1991), 459-467). Other promoters which can be employed are fruit-specific promoters, for example as described in WO9101373A1.

Promoters which are especially preferably used are those for endosperm-specific expression such as, for example, the glutelin promoter (Leisy et al., *Plant Mol. Biol.* 14, (1990), 41-50; Zheng et al., *Plant J.* 4, (1993), 357-366), the wheat HMG promoter, the USP promoter, the phaseolin promoter, or promoters of maize zein genes (Pedersen et al., *Cell* 29, (1982), 1015-1026; Quatroccio et al., *Plant Mol. Biol.* 15 (1990), 81-93). With the aid of endosperm-specific promoters, it is possible to increase the transcript quantity of the nucleic acid molecules according to the invention in the endosperm in comparison with the endosperm of corresponding wild-type plants.

The maize shrunken-1 promoter (sh-1) (Werr et al., *EMBO J.* 4, (1985), 1373-1380) is especially preferably used.

A termination sequence which serves for the correct termination of the transcription and for adding a poly-A tail to the transcript, which is understood to have a function in stabilizing the transcripts, may furthermore be present. Such elements have been described in the literature (cf., for example, Gielen et al., *EMBO J.* 8 (1989), 23-29) and are freely exchangeable.

In addition, it is possible to generate, with the aid of the nucleic acid molecules according to the invention, plant cells and plants in which the activity of a protein according to the invention is reduced. This leads to the synthesis of a starch with altered chemical and/or physical properties in comparison with starch from wild-type plant cells.

Another subject matter of the invention are therefore also transgenic plant cells in which the activity of a protein according to the invention is reduced in comparison with corresponding non-genetically-modified plant cells of wild-type plants.

The term "wild-type plant"/"wild-type plant cell" means for the purposes of the present invention that these are plant/plant cells which acted as starting material for generating the plants/plant cells according to the invention, i.e. whose genetic information corresponds to that of a plant/plant cell according to the invention apart from the genetic modification which has been introduced.

For example, plant cells with a reduced activity of a protein according to the invention can successfully be generated by expressing a corresponding antisense RNA, or by expressing a suitably constructed ribozyme which specifically cleaves transcripts which encode one of the proteins according to the invention. Moreover, a reduced activity can be achieved by introducing the DNA molecules which, via a cosuppression effect, result in a reduced expression of endogenous genes which encode a protein according to the invention. Moreover, plant cells with a reduced activity of a protein according to the invention can be generated by means of in-vivo mutagenesis. To this end, mutations or insertions can be introduced, via homologous recombination, into an endogenous gene which encodes the protein according to the invention. The mutation or insertion leads to a reduced expression of the endogenous gene which encodes a protein according to the invention, or to the synthesis of an inactive protein according to the invention.

For reducing the activity of a protein according to the invention in plant cells, an antisense RNA is preferred to be expressed.

To this end, it is possible to use, on the one hand, DNA molecules which encompass the entire sequence encoding a protein according to the invention inclusive of any flanking sequences which are present, and, on the other hand, DNA molecules which only encompass portions of the coding sequence, it being necessary for these portions to be long enough to cause an antisense effect in the cells. However, genomic DNA molecules which encode, for example, an intron, can furthermore also be used. In general, sequences up to a minimum length of 15 bp, preferably a length of 100-500 bp, may be used for an effective antisense inhibition, in particular sequences with a length of over 500 bp. As a rule, DNA molecules are used which

are shorter than 5000 bp, preferably sequences which are shorter than 2500 bp. DNA molecules which are homologous with regard to the plant species to be transformed are preferably used.

It is also possible to use DNA sequences which show a high degree of homology to the sequences of the DNA molecules according to the invention, but are not fully identical. The minimum homology should exceed approx. 65%. The use of sequences with homologies of between 95 and 100% is to be preferred.

Alternatively, a reduced activity of the protein according to the invention in the plant cells can also be achieved by a cosuppression effect. The method is known to the skilled worker and described, for example, by Jorgensen (*Trends Biotechnol.* 8 (1990), 340-344), Niebel et al., (*Curr. Top. Microbiol. Immunol.* 197 (1995), 91-103), Flavell et al. (*Curr. Top. Microbiol. Immunol.* 197 (1995), 43-46), Palaqui and Vaucheret (*Plant. Mol. Biol.* 29 (1995), 149-159), Vaucheret et al., (*Mol. Gen. Genet.* 248 (1995), 311-317), de Borne et al. (*Mol. Gen. Genet.* 243 (1994), 613-621) and in other sources.

Again, as in the case of the above-described antisense technology, both DNA molecules can be used in this context which encompass the entire coding region of the nucleic acid molecule according to the invention and DNA molecules which only encompass portions of the coding sequence. The use of introns is also possible.

The expression of ribozymes for reducing the activity of certain enzymes in cells is known to the skilled worker and described, for example, in EP-B1-0321201. The expression of ribozymes in plant cells was described, for example, by Feyter et al. (*Mol. Gen. Genet.* 250, (1996), 329-338).

Furthermore, a reduced activity in plant cells of the nucleic acid molecule according to the invention may also be achieved by "in-vivo mutagenesis", where a hybrid RNA-DNA-oligonucleotide ("chimeroplast") is introduced into cells by transforming cells (Kipp, P.B. et al., Poster Session at the 5th International Congress of Plant Molecular Biology, September 21-27, 1997, Singapore; R. A. Dixon and C.J. Arntzen, Meeting report on "Metabolic Engineering in Transgenic Plants", Keystone

Symposia, Copper Mountain, CO, USA; TIBTECH 15, (1997), 441-447; International Patent Application WO 9515972A1; Kren et al., Hepatology 25, (1997), 1462-1468; Cole-Strauss et al., Science 273, (1996), 1386-1389).

A portion of the DNA component of the RNA-DNA oligonucleotide is homologous to a nucleic acid sequence of an endogenous gene encoding a nucleic acid molecule according to the invention, but has, in comparison with the nucleic acid sequence of the endogenous gene, a mutation or contains a heterologous region surrounded by the homologous regions.

Base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous nucleic acid molecule followed by homologous recombination allows the mutation or heterologous region, present in the DNA component of the RNA-DNA oligonucleotide to be transferred into the endogenous gene of a plant cell. This leads to a reduced activity of a protein according to the invention.

The skilled person furthermore knows that the activity of a protein according to the invention can be achieved by expressing non-functional derivatives, in particular transdominant mutants, of such proteins and/or expressing antagonists/inhibitors of such proteins. Antagonists/inhibitors of such proteins include, for example, antibodies, antibody fragments or molecules with similar binding properties. For example, a cytoplasmic scFv antibody was used for modulating the activity of the phytochrome A protein in genetically modified tobacco plants (Owen, Bio/Technology 10, (1992), 790-794; Review: Franken, E., Teuschel, U. and Hain, R., Current Opinion in Biotechnology 8, (1997), 411-416; Whitelam, Trends Plant Sci. 1, (1996), 268-272).

The term "reduced activity" means for the purposes of the present invention a reduced expression of endogenous genes which encode a protein according to the invention and/or a reduced amount of proteins according to the invention in the cells, in particular a reduced enzymatic activity of the protein according to the invention in the cells.

Reduced expression can be established, for example, by measuring the amount of transcripts encoding the protein according to the invention, for example by Northern

blot analysis. "Reduced" preferably means in this context that the amount of transcripts compared with corresponding non-genetically modified cells is reduced by at least 50%, preferably by at least 70%, especially preferably by at least 85% and very especially preferably by at least 95%.

The reduced amount of proteins according to the invention can be determined, for example, by Western Blot analysis. "Reduced" preferably means in this context that the amount of proteins according to the invention compared with corresponding, non-genetically modified cells is reduced by at least 50%, preferably by at least 70%, especially preferably by at least 85% and very especially preferably by at least 95%.

The reduced enzymatic activity of the protein according to the invention can be determined, for example, as described by Denyer und Smith (Planta 186 (1992), 609-617). A reduced enzymatic activity compared with corresponding, non-genetically-modified cells preferably means in this context a reduction by at least 50%, preferably by at least 70%, especially preferably by at least 85% and very especially preferably by at least 95%.

What has been said above in a different context about the term "genetically modified" also applies here.

Subject matter of the present invention are therefore in particular also transgenic plant cells.

- a) which contain at least one DNA molecule which can lead to the synthesis of at least one antisense RNA which causes a reduced expression of endogenous genes which encode a protein according to the invention;
- b) which contain at least one DNA molecule which, via a cosuppression effect, leads to a reduced expression of endogenous genes which encode a protein according to the invention;
- c) which contain at least one DNA molecule which leads to the synthesis of at least one ribozyme which specifically cleaves transcripts of endogenous genes which encode a protein according to the invention; and/or
- d) which, due to an in-vivo mutagenesis, have a mutation or an insertion of a heterologous DNA sequence in at least one endogenous gene encoding a protein

according to the invention, the mutation or insertion causing reduced expression of the gene or the synthesis of an inactive protein according to the invention.

It is furthermore possible, with the aid of the nucleic acid molecules according to the invention, to generate plant cells and plants in which the activity of a protein according to the invention is increased. This leads to the synthesis of a starch of altered chemical and/or physical properties compared with starch from wild-type plant cells and wild-type plants.

Another subject matter of the invention are also transgenic plant cells in which the activity of a protein according to the invention is increased in comparison with corresponding, non-genetically-modified plant cells of wild-type plants.

For generating plant cells according to the invention which have an increased activity of the protein according to the invention compared with corresponding, non-genetically modified wild-type plant cells, nucleic acid molecules according to the invention are used in sense orientation, which comprise the coding region of a starch synthase according to the invention. In a further embodiment, it is also possible to use portions of the coding region, under the condition that they encode a catalytically active starch synthase protein. In an especially preferred embodiment, the nucleic acid molecules indicated under Seq ID No. 1 are used.

The term "increased activity" means for the purposes of the present invention increased expression of endogenous genes which encode a protein according to the invention and/or an increased quantity of protein according to the invention in the cells, in particular an increased enzymatic activity of the protein according to the invention in the cells.

The increased expression can be determined, for example, by measuring the amount of transcripts encoding the protein according to the invention, for example by Northern blot analysis. "Increased" in this context preferably means that the amount of transcripts compared with corresponding non-genetically modified cells is

increased by at least 50%, preferably by at least 100%, in particular by at least 500% and especially preferably by at least 1500%.

The increased amount of proteins according to the invention can be determined, for example, by Western blot analysis. "Increased" preferably means in this context that the amount of proteins according to the invention compared with corresponding non-genetically modified cells is increased by at least 50%, preferably by at least 100%, in particular by at least 500% and especially preferably by at least 1500%.

The increased enzymatic activity of the protein according to the invention can especially preferably be determined as described by Denyer and Smith (see above).

An increased enzymatic activity compared with corresponding, non-genetically modified cells preferably means in this context an increase by at least 50%, preferably by at least 100%, in particular by at least 500%, and especially preferably by at least 1500%.

In a preferred embodiment of the invention, the transgenic plant cells according to the invention with increased activity of a protein according to the invention compared with plant cells from wild-type plants are plant cells which originate from starch-storing tissues, preferably from tubers and the endosperm, in particular the endosperm of maize plants.

Surprisingly, it has been found that plant cells in which the activity of the protein according to the invention, in particular in plant cells of the endosperm, is increased compared with corresponding wild-type plants, synthesize a starch whose physicochemical properties are altered compared with starch synthesized in wild-type plants, in particular in the endosperm, so that it is better suited for specific purposes.

The plant cells according to the invention may belong to any plant species, i.e. to monocotyledonous or else dicotyledonous plants. They are preferably plant cells from agriculturally useful plants, i.e. plants which are grown by man for the purposes of nutrition or for technical, in particular industrial, purposes. The invention preferably relates to fiber-forming (for example flax, hemp, cotton), oil-storing (for example

rape, sunflowers, soya beans), sugar-storing (for example sugar beet, sugar cane, sugar millet, banana) and protein-storing plants (for example leguminous plants).

In a further preferred embodiment, the invention relates to plant cells from fodder plants (for example grasses used as animal feed, forage grasses, alfalfa, clover and the like), vegetable plants (for example tomatoes, lettuce, chicory).

In an especially preferred embodiment, the invention relates to plant cells from starch-storing plants (for example wheat, barley, oats, rye, potatoes, maize, rice, peas, cassava, mung bean), particularly preferred are plant cells from maize, rice, wheat and potatoes.

The plant cells according to the invention can be used for regenerating whole plants. The plants obtainable by regenerating the transgenic plant cells according to the invention are also a subject matter of the present invention.

Subject matter of the invention are furthermore plants which contain the above-described plant cells according to the invention. The plants according to the invention may be, in principle, plants of any plant species, i.e. both monocotyledonous or dicotyledonous plants. They are preferably plant cells from agriculturally useful plants, i.e. plants which are grown by man for the purposes of nutrition or for technical, in particular industrial, purposes. The invention preferably relates to plant cells from fibre-forming (for example flax, hemp, cotton), oil-storing (for example rape, sunflowers, soya beans), sugar-storing (for example sugar beet, sugar cane, sugar millet, banana) and protein-storing plants (for example leguminous plants).

In a further preferred embodiment, the invention relates to forage plants (for example pasture and forage grasses, alfalfa, clover and the like), vegetable plants (for example tomato, lettuce, chicory).

In an especially preferred embodiment, the invention relates to starch-storing plants (for example wheat, barley, oats, rye, potato, maize, rice, pea, cassava, mung bean), particularly preferred are maize, rice, wheat and potato plants.

In further preferred embodiment, the plants according to the invention show an increased activity of a protein according to the invention in plant cells of starch-storing tissues compared with corresponding non-genetically modified plant cells from wild-type plants, preferably in plant cells from tubers or the endosperm, especially preferably in plant cells of the endosperm of maize plants.

In a further embodiment of the invention, plants which contain the plant cells according to the invention with increased activity of the protein according to the invention, exhibit a higher yield and/or higher starch content compared with non-modified wild-type plants.

The term "higher yield" preferably means in this context an increased production of constituents, in particular starch, and/or biomass, in particular when the latter is measured by the fresh weight per plant.

The term "increased starch content" means in this context that the starch content in plant cells according to the invention is at least 10%, preferably at least 20%, in particular at least 30% and especially preferably at least 40% higher compared with plant cells of the non-modified wild-type plants.

Methods for determining the starch content are known to the skilled person.

Such an increase in yield and/or starch content preferably relates to harvestable plant organs such as seeds, fruits, storage roots, tubers, roots, flowers, buds, shoots, stems or timber.

In accordance with the invention, the increase in yield amounts to at least 3% based on the biomass and/or constituents, compared with corresponding, non-transformed plants of the same genotype when these plants are grown under the same conditions, preferably at least 5%, in particular at least 10% and especially preferably at least 20% or even 40% compared with wild-type plants.

The present invention also relates to methods for generating transgenic plants, wherein

- a) a plant cell is subjected to genetic modification by introducing a nucleic acid molecule according to the invention and/or a vector according to the invention; and
- b) a plant is regenerated from a cell; and, optionally,
- c) more plants are generated from the plant of b)

What has already been explained above in a different context also applies to the genetic modification introduced as described in step a). For example, genetically modified plant cells show a reduced activity of the protein according to the invention or an increased activity of the protein according to the invention.

Plants may be regenerated in accordance with step b) by methods known to the skilled person.

More plants may be generated in accordance with step c) of the methods according to the invention for example by vegetative propagation (for example using cuttings, tubers or by means of callus culture and regeneration of whole plants) or by generative propagation. Generative propagation is preferably done under controlled conditions, i.e. selected plants with specific properties are crossed with each other and propagated.

The present invention also relates to the plants obtainable by the methods according to the invention.

The present invention also relates to propagation material of plants according to the invention and of the transgenic plants generated in accordance with the methods according to the invention. The term propagation material encompasses those parts of the plant which are suitable for generating progeny by the vegetative or generative route. Examples which are suitable for vegetative propagation are cuttings, callus cultures, rhizomes or tubers. Other propagation material encompasses, for example, fruits, seeds, seedlings, protoplasts, cell cultures and the like. The propagation material is preferably tubers and seeds.

In a further embodiment, the present invention relates to harvestable parts of plants according to the invention such as fruits, storage roots, roots, flowers, buds, shoots or stems, preferably seeds or tubers, and to feedstuffs which contain these harvestable parts of plants, preferably seeds or tubers. Harvestable parts of plants according to the invention, preferably seeds or tubers and/or feedstuffs, may be characterized by an altered energy value, preferably an increased energy value.

The term "energy value" means to be in the present context in particular "digestible energy". The term "digestible energy" is defined as: digestible energy = feed gross energy minus fecal calorific value (Landwirtschaftliches Lehrbuch 2: Tierzucht, Ed.:D. Schmidt, 5th Edition, 1982, Eugen Ulmer GmbH & Co, p. 244). Gross energy means the total calorific value of a feedstuff which can be measured in Joule. Methods for determining the "energy value" or the "digestible energy" are known to the skilled person.

In accordance with the invention, the energy value is increased by at least 3%, preferably at least 10%, in particular at least 30% and especially preferably at least 60%.

Due to the expression, or additional expression, of a nucleic acid molecule according to the invention and/or due to the increased or reduced activity of a protein according to the invention, the transgenic plant cells and plants according to the invention synthesize a starch which is altered compared with starch synthesized in wild-type plants for example with regard to its physicochemical properties, in particular the amylose/amylopectin ratio, the degree of branching, the mean chain length, the phosphate content, the pastification behavior, the starch granule size and/or the starch granule shape. In particular, such a starch may be altered compared with wild-type starch with regard to the viscosity and/or the gel formation properties of sizes of this starch.

Subject matter of the present invention is also the starch obtainable from the transgenic plant cells according to the invention and/or the plants according to the invention and/or the propagation material according to the invention.

Due to the altered physicochemical properties, the starches according to the invention show altered functional properties. Important functional properties of starch, or its aqueous solutions, are the retrogradation behavior, the film-forming properties, the gel strength, the viscosity, the stability with regard to color, the enzymatic digestibility, the freeze-thaw stability, the stability to acids, the shear stability, the transparency, the water-binding capacity, the pastification properties and binding and adhesive properties. The starches according to the invention can be modified by processes known to the skilled worker and are suitable, in their unmodified or modified forms, for a variety of applications in the food or non-food sector.

In principle, the possible uses of the starch can be divided into two important sectors. One sector encompasses the hydrolysis products of the starch, mainly glucose and glucan units, which are obtained by enzymatic or chemical methods. They are used as starting materials for other chemical modifications and processes such as fermentation. Of importance here for reducing the outlay is the simplicity and inexpensive design of a hydrolysis process. Currently it is performed essentially enzymatically using amyloglucosidase. What would be feasible is a financial saving by using fewer enzymes. This could be caused by altering the structure of the starch, for example by increasing the granule surface area, better degradability due to a lower degree of branching, or a sterical structure which limits the accessibility for the enzymes employed. The other sector in which starch, due to its polymeric structure, is used is as a so-called native starch can be divided into two further fields of application:

1. Food Industry

Starch is a classic additive for various food, where essentially it serves the purpose of binding aqueous additives or causes increased viscosity or increased gel formation. Important characteristics are flowing and sorption behavior, swelling and pastification temperature, viscosity and thickening performance, solubility of the starch, transparency and paste structure, heat, shear and acid resistance, tendency to retrogradation, capability of film

formation, resistance to freezing/thawing, digestibility as well as the capability of complex formation with e.g. inorganic or organic ions.

2. Non-food Industry

In this vast area starch can be used as an adjuvant in various production processes or as an additive in technical products. The main field where starch is used as an adjuvant is the paper and cardboard industry. In this field, starch is mainly used for retention (holding back solids), for sizing filler and fine particles, as solidifying substance and for dehydration. In addition, the advantageous properties of starch with regard to stiffness, hardness, sound, grip, gloss, smoothness, tear strength as well as the surfaces are made use of.

2.1 Paper and cardboard industry

Within the paper production process, a differentiation can be made between four fields of application, namely surface, coating, mass and spraying.

The requirements on starch with regard to surface treatment are essentially a high degree of brightness, corresponding viscosity, high viscosity stability, good film formation as well as little formation of dust. When used in coating the solid content, a corresponding viscosity, a high capability to bind as well as a high pigment affinity play an important role. As an additive to the mass rapid, uniform, free-of-loss dispersion, high mechanical stability and complete retention in the paper pulp are of importance. When using the starch in spraying, corresponding content of solids, high viscosity as well as high capability to bind are also significant.

2.2 Adhesive industry

A major field of application is, for instance, in the adhesive industry, where the starch is used in four areas: the use as pure starch glue, the use in starch glues prepared with special chemicals, the use of starch as an additive to synthetic resins and polymer dispersions as well as the use of starches as extenders for synthetic adhesives. 90% of all starch-based adhesives are

used in the production of corrugated board, paper sacks and bags, composite materials for paper and aluminum, boxes and wetting glue for envelopes, stamps, etc.

2.3 Textile and textile care industry

Another possible use of starch as adjuvant and additive is in the production of textiles and textile care products. Within the textile industry, a differentiation can be made between the following four fields of application: the use of starch as a sizing agent, i.e. as an adjuvant for smoothing and strengthening the burring behavior for the protection against tensile forces active in weaving as well as for the increase of wear resistance during weaving, as an agent for textile improvement mainly after quality-deteriorating pretreatments, such as bleaching, dying, etc., as thickener in the production of dye pastes for the prevention of dye diffusion and as an additive for warping agents for sewing yarns.

2.4 Building industry

The fourth area of application of starch is its use as an additive in building materials. One example is the production of gypsum plaster boards, in which the starch mixed in the thin plaster pastifies with the water, diffuses at the surface of the gypsum board and thus binds the cardboard to the board. Other fields of application are admixing it to plaster and mineral fibers. In ready-mixed concrete, starch may be used for the deceleration of the sizing process.

2.5 Ground stabilization

Furthermore, starch is advantageous for the production of means for ground stabilization used for the temporary protection of ground particles against water in artificial earth shifting. According to state-of-the-art knowledge, combination products consisting of starch and polymer emulsions can be considered to have the same erosion- and encrustation-reducing effect as the products used so far; however, they are considerably less expensive.

2.6 Use of starch in plant protectives and fertilizers

Another field of application is the use of starch in plant protectives for the modification of the specific properties of these preparations. For instance, starches are used for improving the wetting of plant protectives and fertilizers, for the dosed release of the active ingredients, for the conversion of liquid, volatile and/or odorous active ingredients into microcrystalline, stable, deformable substances, for mixing incompatible compositions and for the prolongation of the duration of effect due to slower decomposition.

2.7 Drugs, medicine and cosmetics industry

Starch may also be used in the fields of drugs, medicine and in the cosmetics industry. In the pharmaceutical industry, the starch may be used as a binder for tablets or for the dilution of the binder in capsules. Furthermore, starch is suitable as disintegrant for tablets since, upon swallowing, it absorbs fluid and after a short time it swells so much that the active ingredient is released. For qualitative reasons, medical lubricating powders and medical powders for wounds are based on starch. In the field of cosmetics, starch is used, for example, as carrier of powder additives, such as scents and salicylic acid. A relatively vast field of application for starch is toothpaste.

2.8 Starch as an additive in coal and briquettes

Starch can also be used as an additive in coal and briquettes. By adding starch, coal can be quantitatively agglomerated and/or briquetted in high quality, thus preventing premature disintegrating of the briquettes. Barbecue coal contains between 4 and 6% added starch, calorated coal between 0.1 and 0.5%. Furthermore, starch becomes more and more important as a binding agent since adding it to coal and briquette can considerably reduce the emission of toxic substances.

2.9 Processing of ore and coal slurry

Furthermore, the starch may be used as a flocculating agent in the processing of ore and coal slurry.

2.10 Starch as an additive in casting

Another field of application is the use of starch as an additive to process materials in casting. For various casting processes cores produced from sands mixed with binding agents are needed. Nowadays, the most commonly used binding agent is bentonite mixed with modified starches, mostly swelling starches.

The purpose of adding starch is increased flow resistance as well as improved binding strength. Moreover, swelling starches may fulfill other prerequisites for the production process, such as dispersability in cold water, rehydratisability, good mixability in sand and high capability of binding water.

2.11 Use of starch in rubber industry

In the rubber industry starch may be used for improving the technical and optical quality. Reasons for this are improved surface gloss, grip and appearance. For this purpose, starch is dispersed on the sticky gummed surfaces of rubber substances before the cold vulcanization. It may also be used for improving printability of rubber.

2.12 Production of leather substitutes

Another field of application for the modified starch is the production of leather substitutes.

2.13 Starch in synthetic polymers

In the plastics market the following fields of application are emerging: the integration of products derived from starch into the processing process (starch is only a filler, there is no direct bond between synthetic polymer and starch) or, alternatively, the integration of products derived from starch into the production of polymers (starch and polymer form a stable bond).

The use of the starch as a pure filler cannot compete with other substances such as talcum. That changes when the specific starch properties become effective and the

property profile of the end products is thus clearly changed. One example is the use of starch products in the processing of thermoplastic materials, such as polyethylene. Thereby, starch and the synthetic polymer are combined in a ratio of 1 : 1 by means of coexpression to form a 'master batch', from which various products are produced by means of common techniques using granulated polyethylene. The integration of starch in polyethylene films may cause an increased substance permeability in hollow bodies, improved water vapor permeability, improved antistatic behavior, improved anti-block behavior as well as improved printability with aqueous dyes.

Another possibility is the use of the starch in polyurethane foams. Due to the adaptation of starch derivatives as well as due to the optimization of processing techniques, it is possible to specifically control the reaction between synthetic polymers and the hydroxy groups of the starch. The results are polyurethane films which get the following property profiles due to the use of starch: a reduced coefficient of thermal expansion, decreased shrinking behavior, improved pressure/tension behavior, increased water vapor permeability without a change in water acceptance, reduced flammability and cracking density, no drop off of inflammable parts, no halogen and reduced aging. Disadvantages that presently still exist are reduced pressure and impact strength.

Product development of film is not the only option any more. Also solid plastics products, such as pots, plates and bowls can be produced with starch content of more than 50%. Furthermore, the starch/polymer mixtures offer the advantage that they are biodegradable to a larger extent.

Furthermore, due to their extreme capability to bind water, starch graft polymers have gained utmost importance. These are products having a backbone of starch and a side lattice of a synthetic monomer grafted on according to the principle of radical chain mechanism. The starch graft polymers available nowadays are characterized by an improved binding and retaining capability of up to 1000 g water per g starch at a high viscosity. In the past few years these super absorbers have been more widely used - mainly in the hygiene field, e.g. in products such as diapers and sheets, as well as in the agricultural sector, e.g. in seed pellets.

Decisive factors for the use of the new starch modified by recombinant DNA techniques are, on the one hand, structure, water content, protein content, lipid content, fiber content, ashes/phosphate content, amylose/amylopectin ratio, distribution of the relative molar mass, degree of branching, granule size and shape as well as crystallization, and on the other hand, the properties resulting in the following features: flow and sorption behavior, pastification temperature, viscosity, thickening performance, solubility, paste structure and transparency, heat, shear and acid resistance, tendency to retrogradation, capability of gel formation, resistance to freezing/thawing, capability of complex formation, iodine binding, film formation, adhesive strength, enzyme stability, digestibility and reactivity.

The production of modified starch by genetically operating with a transgenic plant may modify the properties of the starch obtained from the plant in such a way as to render further modifications by means of chemical or physical methods superfluous. On the other hand, the starches modified by means of recombinant DNA techniques might be subjected to further chemical modification, which will result in further improvement of quality for certain of the above-described fields of application. These chemical modifications are principally known to the person skilled in the art. These are particularly modifications by means of

- heat treatment
- acid treatment
- oxidation and
- esterification

leading to the formation of phosphate, nitrate, sulfate, xanthate, acetate and citrate starches. Other organic acids may also be used for the esterification:

- formation of starch ethers

starch alkyl ether, O-allyl ether, hydroxylalkyl ether, O-carboxymethyl ether, N-containing starch ethers, P-containing starch ethers and S-containing starch ethers.

- formation of branched starches

- formation of starch graft polymers.

The present invention furthermore relates to a process for the production of a modified starch comprising the step of extracting the starch from an above-described plant (plant cell) according to the invention and/or from starch-storing parts of such a plant. Such a process preferably also comprises the step of harvesting the plants which have been grown, and/or starch-storing parts of these plants before extracting the starch, and especially preferably furthermore the step of growing plants according to the invention prior to harvesting. Methods for extracting the starch from plants or from starch-storing parts of plants are known to the skilled person. Moreover, methods for extracting the starch from various starch-storing plants are described, for example, in "Starch: Chemistry and Technology (Editor: Whistler, BeMiller and Paschall (1994), 2nd Edition, Academic Press Inc. London Ltd; ISBN 0-12-746270-8; see, for example, Chapter XII, page 412-468: maize and sorghum starches: production; by Watson; Chapter XIII, page 469-479: tapioca, arrowroot and sago starches: production; by Corbishley and Miller; Chapter XIV, page 479-490: potato starch: production and uses; by Mitch; Chapter XV, page 491 to 506: wheat starch: production, modification and uses; by Knight and Oson; and Chapter XVI, page 507 to 528: rice starch: production and uses; by Rohmer and Klem; maize starch: Eckhoff et al., Cereal Chem. 73 (1996) 54-57, the extraction of maize starch on an industrial scale is generally done by wet milling.). Apparatus usually used in processes for extracting starch from plant materials are separators, decanters, hydrocyclones, spray dryers and fluidized-bed dryers.

Subject matter of the present invention is furthermore starch obtainable by the above-described process according to the invention.

The invention furthermore relates to starch obtainable from the plant cells according to the invention and/or plants according to the invention and to starch obtainable from starch-storing tissues, in particular tubers and kernels, of plants according to the invention.

In a further embodiment, the present invention relates to the use of the starches according to the invention in the industrial sector, preferably for the production of foodstuffs, feedstuffs and paper.

Fig. 1: Plasmid map IR 65/87

LB: T-DNA left border

35S-T: 35S terminator (Frank et al., Cell 21, (1980), 285-294)

PAT: phosphinothricin resistance, EP-A2-0275957

35S-P: CaMV 35 S promoter (Frank et al., Cell 21, (1980), 285-294)

Ubiquitin P: ubiquitin promoter (Christensen et al., Plant Mol. Biol. 18, (1992), 675-689)

Ubi. intron: ubiquitin intron (Christensen et al., Plant Mol. Biol. 18, (1992), 675-689)

SS6: coding region of the nucleotide sequence indicated under SEQ ID No. 1

NOS: nos terminator (Depicker et al., J. Mol. Appl. Genet. 1, (1982), 561-573)

RB: T-DNA right border

Ampicillin: ampicillin resistance gene (Yanisch-Perron et al., Gene 33, (1985), 103-119)

Media and solutions used in the Examples:

20 x SSC	175.3 g NaCl
	88.2 g sodium citrate
	ad 1000 ml twice-distilled H ₂ O
	pH 7.0 with 10 N NaOH

YT	8 g Bacto yeast extract
	5 g Bacto tryptone
	5 g NaCl
	ad 1000 ml twice-distilled H ₂ O

Protoplast isolation medium (100 ml)

Cellulase Onozuka R S (Meiji Seika, Japan)	800 mg
Pectolyase Y 23	40 mg
KNO ₃	200 mg
KH ₂ PO ₄	136 mg
K ₂ HPO ₄	47 mg
CaCl ₂ 2H ₂ O	147 mg
MgSO ₄ 7H ₂ O	250 mg
Bovine serum albumin (BSA)	20 mg
Glucose	4000 mg
Fructose	4000 mg
Sucrose	1000 mg
pH	5.8
Osmolarity	660 mosm

Protoplast wash solution 1: as protoplast isolation solution, but without cellulase, pectolyase and BSA

Transformation buffer

a)	Glucose	0.5 M
	MES	0.1%
	MgCl ₂ 6H ₂ O	25 mM
	pH	5.8
	adjust to 600 mosm	
b)	PEG 6000 solution	
	Glucose	0.5 M
	MgCl ₂ 6H ₂ O	100 mM
	Hepes	20 mM
	pH	6.5

PEG 6000 is added to the above buffer under b) shortly before the solution is used (PEG 40% by weight). The solution is filtered through a 0.45 µm sterile filter.

W5 solution

CaCl ₂	125 mM
NaCl	150 mM
KCl	5 mM
Glucose	50 mM

Protoplast culture medium (data in mg/l)

KNO ₃	3000
(NH ₄) ₂ SO ₄	500
MgSO ₄ 7H ₂ O	350
KH ₂ PO ₄	400
CaCl ₂ 2H ₂ O	300

Fe-EDTA and trace elements as for Murashige-Skoog medium (Physiol. Plant, 15 (1962), 473).

m-Inositol	100
Thiamine HCl	1.0
Nicotinic acid amide	0.5
Pyridoxine HCl	0.5
Glycine	2.0
Glucuronic acid	750
Galacturonic acid	750
Galactose	500
Maltose	500
Glucose	36,000
Fructose	36,000
Sucrose	30,000
Asparagine	500

	42
Glutamine	100
Proline	300
Casein hydrolysate	500
2,4-Dichlorophenoxyacetic acid (2,4-D)	0.5
pH	5.8
Osmolarity	600 mosm

The following methods are used in the examples:

1. Transformation of maize

(a) Preparation of protoplasts of cell line DSM 6009

Protoplast isolation

2 - 4 days, preferably 3 days, after the last medium change in a protoplast suspension culture, the liquid medium is pipetted off and the cells which remain are washed with 50 ml protoplast washing solution 1 and again dried by pipetting off the washing solution. 10 ml of protoplast isolation medium are added to in each case 2 g of the cell biomass harvested. The resuspended cells and cell aggregates are incubated in the dark for 4 to 6 hours at $27 \pm 2^\circ\text{C}$, with gentle shaking (30 to 40 rpm).

Protoplast purification

As soon as at least 1 million protoplasts/ml have been released (observation under the microscope), the suspension is passed through a stainless-steel screen and a nylon screen of 200 and 45 μm mesh size, respectively. The combination of a 100 μm and a 60 μm screen is equally suitable for removing the cell aggregates. The protoplast-containing filtrate is assessed under the microscope. Usually, it contains 98-99% protoplasts. The remainder are undigested single cells. Protoplast preparations of this degree of purity are used for transformation experiments without additional gradient centrifugation.

The protoplasts are sedimented by centrifugation (100 rpm in the swing-bucket rotor ($100 \times g$, 3 min). The supernatant is discarded and the protoplasts are resuspended in washing solution 1. The centrifugation step is repeated and the protoplasts are then resuspended in the transformation buffer.

(b) Protoplast transformation

The protoplasts resuspended in transformation buffer are filled in 10 ml portions into 50 ml polyallomer tubes at a titer of $0.5 - 1 \times 10^6$ protoplasts/ml. The DNA used for the transformation is dissolved in Tris-EDTA (TE) buffer. 20 μ g of plasmid DNA are added per ml of protoplast suspension. The vector used is a plasmid which mediates resistance to phosphinothricin (cf., for example, EP-A2-0513 849). After addition of the DNA, the protoplast suspension is shaken carefully to distribute DNA homogeneously in the solution. Immediately thereafter, 5 ml of PEG solution are added dropwise.

The PEG solution is distributed homogeneously by carefully turning the tubes. Then, another 5 ml of PEG solution are added and the mixing step to achieve homogeneity is repeated. The protoplasts remain in the PEG solution for 20 minutes at $25 \pm 2^\circ\text{C}$. Then the protoplasts are sedimented by centrifuging for 3 minutes ($100 g$; 1000 rpm). The supernatant is discarded. The protoplasts are washed by carefully shaking them in 20 ml of W5 solution, whereupon they are recentrifuged. Then they are resuspended in 20 ml of protoplast culture medium, recentrifuged and resuspended again in culture medium. The titer is adjusted to $6 - 8 \times 10^5$ protoplasts/ml, and the protoplasts are cultured in 3 ml portions in Petri dishes ($\varnothing$ 60 mm, height 15 mm). The Petri dishes are sealed with Parafilm and then placed in the dark at $25 \pm 2^\circ\text{C}$.

(c) Protoplast culture

During the first 2 - 3 weeks after isolation and transformation, the protoplasts are cultured without addition of fresh medium. Once the cells regenerated from the protoplasts have developed into cell aggregates of over 20 - 50 cells, 1 ml of fresh protoplast culture medium which contains sucrose (90 g/l) as osmotic is added.

(d) Selection of transformed maize cells, and plant regeneration

3 - 10 days after the addition of fresh medium, the cell aggregates which have formed from protoplasts can be plated out on agar media supplemented with 100 mg/l L-phosphinothricin. N6 medium with the vitamins of the protoplast culture medium, 90 g/l sucrose and 1.0 mg/l 2,4-D is equally suitable as an analogous medium, for example with the macro- and microelements of the MS medium (Murashige and Skoog (1962), see above).

The calli originating from stably transformed protoplasts can continue growing unimpeded on the selective medium. After 3 - 5 weeks, preferably 4 weeks, the transgenic calli can be transferred to fresh selection medium which also contains 100 mg/l L-phosphinothricin, but which no longer contains any auxin. In the course of 3 - 5 weeks, approx. 50% of the transgenic maize calli which have the L-phosphinothricin acetyl transferase gene integrated into their genome differentiate the first plants on this medium in the presence of L-phosphinothricin.

(e) Growing transgenic regenerated plants

The embryogenic transformed maize tissue is grown on hormone-free N6 medium (Chu C.C. et al., Sci. Sin. 16 (1975), 659) in the presence of 5×10^{-4} M L-phosphinothricin. On this medium, maize embryos which sufficiently express the phosphinothricin acetal transferase gene (PAT gene) develop into plants. Untransformed embryos, or the ones whose PAT activity is only very weak, die. As soon as the leaves of the

in-vitro plants have reached a length of 4 - 6 cm, they can be transferred to soil. After agar residues are washed off the roots, the plants are planted into a 3:1:1:1 mixture of loam, sand, vermiculite and standard soil and adapted to the soil culture during the first 3 days after transplanting at a relative atmospheric humidity of 90 - 100%. They are grown on in a climate chamber with a 14-hour-photoperiod, approx. 25,000 Lux at plant level, at a day/night temperature of $23 \pm 1/17 \pm 1^{\circ}\text{C}$. The adapted plants are grown at an atmospheric humidity of $65 \pm 5\%$.

4. Radiolabeling of DNA fragments

DNA fragments were radiolabeled with the aid of a DNA Random Primer Labeling Kit by Boehringer (Germany) following the manufacturer's instructions.

The examples which follow illustrate the invention without imposing any form of limitation:

Example 1: Identification, isolation and characterization of a cDNA encoding a new isoform of a Zea mays starch synthase

For identifying a cDNA encoding a new isoform of Zea mays starch synthase, total RNA was first isolated from maize kernels (15 to 20 days after pollination) following the method of Logemann et al. (Anal. Biochem. 163, (1987), 21-26). Then, 1 mg of total RNA was used to prepare poly A⁺-RNA using the Oligotex-mRNA purification kit (Quiagen) following the manufacturer's instructions.

Starting from 5 µg of poly A⁺-RNA, a cDNA library was then constructed with the aid of the ZAP cDNA synthesis kit by Stratagene. The library contained approx. 9×10^5 independent recombinant phages with an average cDNA insert size of approx. 1.3 kb.

Then plaque-lifting was carried out on approx. 4×10^5 phages. To do this, Hybond N filters (Amersham) were used. After prehybridization for 4 hours at 42°C in buffer A

($5 \times$ SSC, 0.5% BSA, $5 \times$ Denhardt, 1% SDS, 40 mM phosphate buffer, pH 7.2; 100 mg/l herring sperm DNA, 25% formamide), the filters were hybridized with a radiolabeled (Random Primed DNA Labeling Kit, Boehringer Mannheim) EcoRI/XhoI fragment (total cDNA) of the *Solanum tuberosum* SSIII cDNA (GenBank Acc. No. X 94400). After 12 hours, the filters were washed three times for 20 minutes at 55°C in a buffer containing $3 \times$ SSC, 0.5% SDS. Then, an X-ray film was superimposed for approx. 14 hours.

Strongly hybridizing plaques were isolated, diluted, replaced and transferred to filters. These were again hybridized and washed as described above. After the isolated phages had been excised in-vivo following the manufacturer's instructions (Stratagene), various plasmids were isolated which were characterized by means of a restriction analysis. Then the DNA sequences of the plasmids which had the longest cDNA insertions were determined. One of these plasmids, which was termed pZm_SS6, contained the nucleotide sequence indicated in SEQ ID No. 1.

Example 2 Generation of a vector for transforming plants

Starting from a NotI/Bsp 120 I fragment of plasmid pZm_SS6 which contains the complete coding region of the nucleotide sequence indicated under SEQ ID No. 1, vector IR65/87 (see Figure 1), which is suitable, inter alia, for generating transgenic maize plants which have an increased activity (overexpression solution) or a reduced activity (cosuppression solution) of the starch synthase according to the invention, was generated by standard methods and deposited at the Deutsche Sammlung für Mikroorganismen on August 5, 1999, with the deposit number DSM 12970.

Vector IR65/87 was used for transforming maize plants in accordance with the above-described methods.

Then transgenic plants were selected which exhibited either increased activity or a reduced activity of the protein according to the invention compared with corresponding untransformed maize plants.

SEQUENCE LISTING

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..... in starch synthesis

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45 50 55

aat ccg agg agg gca tcg act tct aag agc aag ggc gtt gcc tct gga 663

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Ala Ser Val Ala Gly Gln Asn Glu Ser Val Gly Gly Phe His Gly Gln	
190 195 200	
cac gaa cca ata act ggt tct gag gaa cat ggc agt tca att gtt gat	1095
His Glu Pro Ile Thr Gly Ser Glu Glu His Gly Ser Ser Ile Val Asp	
205 210 215	
gca cca ata aaa ggc agg cta ttt gct gag gcc gtt gtg ggt cat aag	1143
Ala Pro Ile Lys Gly Arg Leu Phe Ala Glu Ala Val Val Gly His Lys	
220 225 230	
gat ttt act gaa tca gca gca gga aag gca agc agc gag aat gag gaa	1191
Asp Phe Thr Glu Ser Ala Ala Gly Lys Ala Ser Ser Glu Asn Glu Glu	
235 240 245 250	
ggg caa gct gtc tcg tta gaa gat gat gtc ggg ata agt aca gac gca	1239
Gly Gln Ala Val Ser Leu Glu Asp Asp Val Gly Ile Ser Thr Asp Ala	
255 260 265	
gat gaa gag ctt ccg gta tct gaa gat gat cca gaa gtg cta ctg agg	1287
Asp Glu Glu Leu Pro Val Ser Glu Asp Asp Pro Glu Val Leu Leu Arg	
270 275 280	
agg ctt caa gag ctt gct gat gag aat tat tcg act ggg aac aac tgt	1335
Arg Leu Gln Glu Leu Ala Asp Glu Asn Tyr Ser Thr Gly Asn Asn Cys	
285 290 295	
ttt gtt ttc cct gaa gta gtg aag gct gat tcg atg att gat ctt tac	1383

Phe	Val	Phe	Pro	Glu	Val	Val	Lys	Ala	Asp	Ser	Met	Ile	Asp	Leu	Tyr	
300						305					310					
tta	aac	cgc	agc	atg	tcg	gcc	tta	gct	agt	gag	tcc	gac	gtt	ttt	gta	1431
Leu	Asn	Arg	Ser	Met	Ser	Ala	Leu	Ala	Ser	Glu	Ser	Asp	Val	Phe	Val	
315					320					325					330	
aaa	gga	gca	ttc	aat	ggg	tgg	aga	tgg	aac	cgt	ttc	act	gaa	aca	atg	1479
Lys	Gly	Ala	Phe	Asn	Gly	Trp	Arg	Trp	Asn	Arg	Phe	Thr	Glu	Thr	Met	
				335					340						345	
cat	aga	agc	gaa	tta	aga	ggg	gat	tgg	tgg	tgc	tgc	aag	ctc	tac	att	1527
His	Arg	Ser	Glu	Leu	Arg	Gly	Asp	Trp	Trp	Cys	Cys	Lys	Leu	Tyr	Ile	
			350					355						360		
ccc	aag	cag	gca	tac	aga	cta	gac	ttt	gta	ttc	ttt	aac	ggg	gac	act	1575
Pro	Lys	Gln	Ala	Tyr	Arg	Leu	Asp	Phe	Val	Phe	Phe	Asn	Gly	Asp	Thr	
		365					370					375				
gtc	tat	gaa	aat	aac	aat	cac	aac	gat	ttt	ttc	ctg	gaa	ata	gaa	agt	1623
Val	Tyr	Glu	Asn	Asn	Asn	His	Asn	Asp	Phe	Phe	Leu	Glu	Ile	Glu	Ser	
	380					385					390					
gac	ata	gat	gaa	cac	tca	ttt	gag	gac	ttc	ttg	gtt	gaa	gaa	aag	cga	1671
Asp	Ile	Asp	Glu	His	Ser	Phe	Glu	Asp	Phe	Leu	Val	Glu	Glu	Lys	Arg	
395					400					405					410	
aag	gaa	ctc	gag	agg	ctt	gct	gca	gaa	gaa	gct	gaa	agg	aaa	aga	caa	1719
Lys	Glu	Leu	Glu	Arg	Leu	Ala	Ala	Glu	Glu	Ala	Glu	Arg	Lys	Arg	Gln	
				415					420						425	
gct	gag	gag	cgg	cac	agg	aga	gag	gaa	gaa	agg	gcc	gcg	atg	gaa	gct	1767
Ala	Glu	Glu	Arg	His	Arg	Arg	Glu	Glu	Glu	Arg	Ala	Ala	Met	Glu	Ala	
			430					435						440		
gac	agg	gca	caa	gca	agg	tct	gag	gtc	gag	atg	aag	aaa	gag	aaa	ttg	1815
Asp	Arg	Ala	Gln	Ala	Arg	Ser	Glu	Val	Glu	Met	Lys	Lys	Glu	Lys	Leu	
		445					450					455				
cgc	cag	atg	ttg	agt	tcg	gct	agc	aga	tat	gct	gag	aac	tta	tgg	tac	1863
Arg	Gln	Met	Leu	Ser	Ser	Ala	Ser	Arg	Tyr	Ala	Glu	Asn	Leu	Trp	Tyr	
	460					465					470					
ata	gaa	cct	aac	acc	tac	aga	gga	gga	gac	aga	gtt	aga	ttg	tac	tat	1911
Ile	Glu	Pro	Asn	Thr	Tyr	Arg	Gly	Gly	Asp	Arg	Val	Arg	Leu	Tyr	Tyr	
475					480					485					490	
aat	aga	agc	tcg	aga	tca	cta	atg	cat	aac	act	gag	att	tgg	atg	cat	1959
Asn	Arg	Ser	Ser	Arg	Ser	Leu	Met	His	Asn	Thr	Glu	Ile	Trp	Met	His	
				495					500						505	
gga	ggg	tat	aac	aac	tgg	att	gat	gga	ctc	tca	ata	gct	gaa	aga	ctt	2007
Gly	Gly	Tyr	Asn	Asn	Trp	Ile	Asp	Gly	Leu	Ser	Ile	Ala	Glu	Arg	Leu	
			510					515					520			
gtc	aaa	tct	cat	gaa	aag	gaa	ggc	gat	tgg	tgg	tat	gta	gaa	gtt	aca	2055
Val	Lys	Ser	His	Glu	Lys	Glu	Gly	Asp	Trp	Trp	Tyr	Val	Glu	Val	Thr	
		525					530					535				

tta cct gaa agg gca ttg gtg ttg gat tgg gtt ttt gct gat gga cca	2103
Leu Pro Glu Arg Ala Leu Val Leu Asp Trp Val Phe Ala Asp Gly Pro	
540 545 550	
cct ggg aat gca agg aat tat gat aac aat gga agg cag gat ttt cat	2151
Pro Gly Asn Ala Arg Asn Tyr Asp Asn Asn Gly Arg Gln Asp Phe His	
555 560 565 570	
gcc atc gtt cct aat aac ata tcg gat gat atc ttt tgg gtg gaa gaa	2199
Ala Ile Val Pro Asn Asn Ile Ser Asp Asp Ile Phe Trp Val Glu Glu	
575 580 585	
gaa cat agg atc ttt aca agg ctt caa caa gag aga aga gaa agg gag	2247
Glu His Arg Ile Phe Thr Arg Leu Gln Gln Glu Arg Arg Glu Arg Glu	
590 595 600	
agt gcc gaa aga ata aag gct gag aga tct gca aaa atg aag gct gag	2295
Ser Ala Glu Arg Ile Lys Ala Glu Arg Ser Ala Lys Met Lys Ala Glu	
605 610 615	
atg aag gaa aag act atg aga gcg ttt ctg ctc tca caa aaa cat att	2343
Met Lys Glu Lys Thr Met Arg Ala Phe Leu Leu Ser Gln Lys His Ile	
620 625 630	
gtg tat act gag cca ctc gaa gta cgt gca gga acc act gtg gac gtt	2391
Val Tyr Thr Glu Pro Leu Glu Val Arg Ala Gly Thr Thr Val Asp Val	
635 640 645 650	
ctt tat aat cct tct aac aca gtg ctg aat gga aag tca gag gtt tgg	2439
Leu Tyr Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Val Trp	
655 660 665	
ttc aga ggt tcc ttt aac cgt tgg act cat cca agt ggt ccc tta cca	2487
Phe Arg Gly Ser Phe Asn Arg Trp Thr His Pro Ser Gly Pro Leu Pro	
670 675 680	
cca cag aag atg gta aag gct gag aat agt tca cac tta cga aca aca	2535
Pro Gln Lys Met Val Lys Ala Glu Asn Ser Ser His Leu Arg Thr Thr	
685 690 695	
gtc agt gtt ccc ctg gat gca tat atg atg gac ttt gtt ttc tct gag	2583
Val Ser Val Pro Leu Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu	
700 705 710	
tcg gaa gaa ggt gga aga tat gac aat agg aac ggg atg gat tat cat	2631
Ser Glu Glu Gly Gly Arg Tyr Asp Asn Arg Asn Gly Met Asp Tyr His	
715 720 725 730	
att cct gtg tct gat tcg gtt gca agg gaa cct cca atg cat att gta	2679
Ile Pro Val Ser Asp Ser Val Ala Arg Glu Pro Pro Met His Ile Val	
735 740 745	
cac att gca gtg gaa atg gct cct atc gca aag gtt gga ggc ctt ggt	2727
His Ile Ala Val Glu Met Ala Pro Ile Ala Lys Val Gly Gly Leu Gly	
750 755 760	
gat gtt gtt aca agc ctt tca cga gct gtt cag gat tta ggc cat aaa	2775
Asp Val Val Thr Ser Leu Ser Arg Ala Val Gln Asp Leu Gly His Lys	
765 770 775	

gtt gag gtt att ctg ccg aag tat gat tgt tta atc cta agc agt gtg Val Glu Val Ile Leu Pro Lys Tyr Asp Cys Leu Ile Leu Ser Ser Val 780 785 790	2823
aag gat tta cac tac caa caa agt ttt gct tcg ggt ggc aca gag gta Lys Asp Leu His Tyr Gln Gln Ser Phe Ala Ser Gly Gly Thr Glu Val 795 800 805 810	2871
aaa gta tgg ttt gga aag gtt gaa gat ctg cca gtt tac ttc ttg gaa Lys Val Trp Phe Gly Lys Val Glu Asp Leu Pro Val Tyr Phe Leu Glu 815 820 825	2919
cca caa aat ggc atg ttt tgg gtt gga tgt gtg tat ggg aag aat gat Pro Gln Asn Gly Met Phe Trp Val Gly Cys Val Tyr Gly Lys Asn Asp 830 835 840	2967
gag agt aga ttt ggc ttc ttc tgt cat tct gct ctg gag ttt ctg ctc Glu Ser Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Leu Leu 845 850 855	3015
caa aaa gga tct tct cct gat atc ata cat tgt cat gac tgg tca agt Gln Lys Gly Ser Ser Pro Asp Ile Ile His Cys His Asp Trp Ser Ser 860 865 870	3063
gct ccc gtt gct tgg cta tac aag gaa cag tat gct ctt aat ggg ctg Ala Pro Val Ala Trp Leu Tyr Lys Glu Gln Tyr Ala Leu Asn Gly Leu 875 880 885 890	3111
gga aat ggt cgg att gta ttt acc atc cac aat ctt gag ttt gga gcg Gly Asn Gly Arg Ile Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala 895 900 905	3159
cat cac att ggc aag gca atg gca cat tgt gac aag gct aca act gtc His His Ile Gly Lys Ala Met Ala His Cys Asp Lys Ala Thr Thr Val 910 915 920	3207
tct gat aca tat tca aag gaa gtg gct gga cat gga gct att gca cct Ser Asp Thr Tyr Ser Lys Glu Val Ala Gly His Gly Ala Ile Ala Pro 925 930 935	3255
cac tac tat aaa ttc cat gga att cga aat gga att gat cct gac att His Tyr Tyr Lys Phe His Gly Ile Arg Asn Gly Ile Asp Pro Asp Ile 940 945 950	3303
tgg gat cca tat act gac aga ttt att ccg gtt cat tat aca tca gag Trp Asp Pro Tyr Thr Asp Arg Phe Ile Pro Val His Tyr Thr Ser Glu 955 960 965 970	3351
aat gtt ctt gag ggc aag ggt gct gca aaa aag gca ttg cag cag atg Asn Val Leu Glu Gly Lys Gly Ala Ala Lys Lys Ala Leu Gln Gln Met 975 980 985	3399
ctt gga tta cag caa act gat agc cct gtt gtt gga atc atc act cgt Leu Gly Leu Gln Gln Thr Asp Ser Pro Val Val Gly Ile Ile Thr Arg 990 995 1000	3447
cta aca gtg cag aag gga atc cac ctt atc aaa cat gca atg cat cga Leu Thr Val Gln Lys Gly Ile His Leu Ile Lys His Ala Met His Arg	3495

1005	1010	1015	
gct ctt gaa cgc aat ggg cag gtg gtt tta ctg ggt tct gca cca gat			3543
Ala Leu Glu Arg Asn Gly Gln Val Val Leu Leu Gly Ser Ala Pro Asp			
1020	1025	1030	
cat cgc ata caa ggt gac ttt aca aat tta gcc agt aag ctg cat ggt			3591
His Arg Ile Gln Gly Asp Phe Thr Asn Leu Ala Ser Lys Leu His Gly			
1035	1040	1045	1050
gaa tac cat ggc cgg gtg aag cta tgt tta acc tat gac gag cca ctg			3639
Glu Tyr His Gly Arg Val Lys Leu Cys Leu Thr Tyr Asp Glu Pro Leu			
1055	1060	1065	
tca cat ttg ctt att gct atg cgc tac gga tcc atc ccg att gtt cgg			3687
Ser His Leu Leu Ile Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg			
1070	1075	1080	
aaa act gga ggc ctg tac gac acc gtc ttt gac gtc gac gat gat aag			3735
Lys Thr Gly Gly Leu Tyr Asp Thr Val Phe Asp Val Asp Asp Asp Lys			
1085	1090	1095	
gat cgg gct caa gca caa ggc ctc gag cca aat gga ttc agt ttc gaa			3783
Asp Arg Ala Gln Ala Gln Gly Leu Glu Pro Asn Gly Phe Ser Phe Glu			
1100	1105	1110	
gga gct gat agc agt ggt gta gat tat gct ctc gac aga gct ata acc			3831
Gly Ala Asp Ser Ser Gly Val Asp Tyr Ala Leu Asp Arg Ala Ile Thr			
1115	1120	1125	1130
gcg ttt tat gat gcc cgc gac tgg ttc aac tcc ctt agc aag agg gta			3879
Ala Phe Tyr Asp Ala Arg Asp Trp Phe Asn Ser Leu Ser Lys Arg Val			
1135	1140	1145	
atg gag caa gat tgg tca tgg aat aga cct gca cta gac tac atg gag			3927
Met Glu Gln Asp Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Met Glu			
1150	1155	1160	
ttg tac cat tct gct cgc aaa aac tga tacattttac cacaaggga			3974
Leu Tyr His Ser Ala Arg Lys Asn			
1165	1170		
aacaggccca gtttgccag tttatgagca tctcagatgc aacacagagt gtatagttag			4034
agaatgccac ccgttgtagc ttactgccgt tacatgcatg tgtatacaca tctaaaaaaa			4094
aaaaaaaaaa aacggcacga gctcgtg			4121

<210> 2
 <211> 1031
 <212> PRT
 <213> Zea mays

<400> 2
 Glu Glu Glu Ala Asp Gln Asn Gln Ser Ser Ala Leu Pro Ser Ala Ser
 1 5 10 15

Met	Asp	Asp	Asp	Ser	Ile	Asp	Arg	Gln	Leu	Asp	Glu	Tyr	Arg	Gly	Lys
			20					25					30		
Ile	Ser	Ala	Leu	Val	Ser	Ser	Lys	Pro	Glu	Pro	Ser	Ser	Leu	Ala	Ser
		35					40					45			
Val	Ala	Gly	Gln	Asn	Glu	Ser	Val	Gly	Gly	Phe	His	Gly	Gln	His	Glu
	50					55					60				
Pro	Ile	Thr	Gly	Ser	Glu	Glu	His	Gly	Ser	Ser	Ile	Val	Asp	Ala	Pro
65					70					75					80
Ile	Lys	Gly	Arg	Leu	Phe	Ala	Glu	Ala	Val	Val	Gly	His	Lys	Asp	Phe
				85					90					95	
Thr	Glu	Ser	Ala	Ala	Gly	Lys	Ala	Ser	Ser	Glu	Asn	Glu	Glu	Gly	Gln
			100					105					110		
Ala	Val	Ser	Leu	Glu	Asp	Asp	Val	Gly	Ile	Ser	Thr	Asp	Ala	Asp	Glu
		115					120					125			
Glu	Leu	Pro	Val	Ser	Glu	Asp	Asp	Pro	Glu	Val	Leu	Leu	Arg	Arg	Leu
	130					135					140				
Gln	Glu	Leu	Ala	Asp	Glu	Asn	Tyr	Ser	Thr	Gly	Asn	Asn	Cys	Phe	Val
145					150					155					160
Phe	Pro	Glu	Val	Val	Lys	Ala	Asp	Ser	Met	Ile	Asp	Leu	Tyr	Leu	Asn
				165					170					175	
Arg	Ser	Met	Ser	Ala	Leu	Ala	Ser	Glu	Ser	Asp	Val	Phe	Val	Lys	Gly
			180					185					190		
Ala	Phe	Asn	Gly	Trp	Arg	Trp	Asn	Arg	Phe	Thr	Glu	Thr	Met	His	Arg
		195					200					205			
Ser	Glu	Leu	Arg	Gly	Asp	Trp	Trp	Cys	Cys	Lys	Leu	Tyr	Ile	Pro	Lys
	210					215					220				
Gln	Ala	Tyr	Arg	Leu	Asp	Phe	Val	Phe	Phe	Asn	Gly	Asp	Thr	Val	Tyr
225					230					235					240
Glu	Asn	Asn	Asn	His	Asn	Asp	Phe	Phe	Leu	Glu	Ile	Glu	Ser	Asp	Ile
				245					250					255	
Asp	Glu	His	Ser	Phe	Glu	Asp	Phe	Leu	Val	Glu	Glu	Lys	Arg	Lys	Glu
			260					265					270		
Leu	Glu	Arg	Leu	Ala	Ala	Glu	Glu	Ala	Glu	Arg	Lys	Arg	Gln	Ala	Glu
		275					280					285			
Glu	Arg	His	Arg	Arg	Glu	Glu	Glu	Arg	Ala	Ala	Met	Glu	Ala	Asp	Arg
	290					295					300				
Ala	Gln	Ala	Arg	Ser	Glu	Val	Glu	Met	Lys	Lys	Glu	Lys	Leu	Arg	Gln
305					310					315					320
Met	Leu	Ser	Ser	Ala	Ser	Arg	Tyr	Ala	Glu	Asn	Leu	Trp	Tyr	Ile	Glu
				325					330					335	

Pro Asn Thr Tyr Arg Gly Gly Asp Arg Val Arg Leu Tyr Tyr Asn Arg
 340 345 350
 Ser Ser Arg Ser Leu Met His Asn Thr Glu Ile Trp Met His Gly Gly
 355 360 365
 Tyr Asn Asn Trp Ile Asp Gly Leu Ser Ile Ala Glu Arg Leu Val Lys
 370 375 380
 Ser His Glu Lys Glu Gly Asp Trp Trp Tyr Val Glu Val Thr Leu Pro
 385 390 395 400
 Glu Arg Ala Leu Val Leu Asp Trp Val Phe Ala Asp Gly Pro Pro Gly
 405 410 415
 Asn Ala Arg Asn Tyr Asp Asn Asn Gly Arg Gln Asp Phe His Ala Ile
 420 425 430
 Val Pro Asn Asn Ile Ser Asp Asp Ile Phe Trp Val Glu Glu Glu His
 435 440 445
 Arg Ile Phe Thr Arg Leu Gln Gln Glu Arg Arg Glu Arg Glu Ser Ala
 450 455 460
 Glu Arg Ile Lys Ala Glu Arg Ser Ala Lys Met Lys Ala Glu Met Lys
 465 470 475 480
 Glu Lys Thr Met Arg Ala Phe Leu Leu Ser Gln Lys His Ile Val Tyr
 485 490 495
 Thr Glu Pro Leu Glu Val Arg Ala Gly Thr Thr Val Asp Val Leu Tyr
 500 505 510
 Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Val Trp Phe Arg
 515 520 525
 Gly Ser Phe Asn Arg Trp Thr His Pro Ser Gly Pro Leu Pro Pro Gln
 530 535 540
 Lys Met Val Lys Ala Glu Asn Ser Ser His Leu Arg Thr Thr Val Ser
 545 550 555 560
 Val Pro Leu Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Ser Glu
 565 570 575
 Glu Gly Gly Arg Tyr Asp Asn Arg Asn Gly Met Asp Tyr His Ile Pro
 580 585 590
 Val Ser Asp Ser Val Ala Arg Glu Pro Pro Met His Ile Val His Ile
 595 600 605
 Ala Val Glu Met Ala Pro Ile Ala Lys Val Gly Gly Leu Gly Asp Val
 610 615 620
 Val Thr Ser Leu Ser Arg Ala Val Gln Asp Leu Gly His Lys Val Glu
 625 630 635 640
 Val Ile Leu Pro Lys Tyr Asp Cys Leu Ile Leu Ser Ser Val Lys Asp

55

645	650	655
Leu His Tyr Gln Gln Ser Phe Ala Ser Gly Gly Thr Glu Val Lys Val 660 665 670		
Trp Phe Gly Lys Val Glu Asp Leu Pro Val Tyr Phe Leu Glu Pro Gln 675 680 685		
Asn Gly Met Phe Trp Val Gly Cys Val Tyr Gly Lys Asn Asp Glu Ser 690 695 700		
Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Leu Leu Gln Lys 705 710 715 720		
Gly Ser Ser Pro Asp Ile Ile His Cys His Asp Trp Ser Ser Ala Pro 725 730 735		
Val Ala Trp Leu Tyr Lys Glu Gln Tyr Ala Leu Asn Gly Leu Gly Asn 740 745 750		
Gly Arg Ile Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala His His 755 760 765		
Ile Gly Lys Ala Met Ala His Cys Asp Lys Ala Thr Thr Val Ser Asp 770 775 780		
Thr Tyr Ser Lys Glu Val Ala Gly His Gly Ala Ile Ala Pro His Tyr 785 790 795 800		
Tyr Lys Phe His Gly Ile Arg Asn Gly Ile Asp Pro Asp Ile Trp Asp 805 810 815		
Pro Tyr Thr Asp Arg Phe Ile Pro Val His Tyr Thr Ser Glu Asn Val 820 825 830		
Leu Glu Gly Lys Gly Ala Ala Lys Lys Ala Leu Gln Gln Met Leu Gly 835 840 845		
Leu Gln Gln Thr Asp Ser Pro Val Val Gly Ile Ile Thr Arg Leu Thr 850 855 860		
Val Gln Lys Gly Ile His Leu Ile Lys His Ala Met His Arg Ala Leu 865 870 875 880		
Glu Arg Asn Gly Gln Val Val Leu Leu Gly Ser Ala Pro Asp His Arg 885 890 895		
Ile Gln Gly Asp Phe Thr Asn Leu Ala Ser Lys Leu His Gly Glu Tyr 900 905 910		
His Gly Arg Val Lys Leu Cys Leu Thr Tyr Asp Glu Pro Leu Ser His 915 920 925		
Leu Leu Ile Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr 930 935 940		
Gly Gly Leu Tyr Asp Thr Val Phe Asp Val Asp Asp Asp Lys Asp Arg 945 950 955 960		

56

Ala Gln Ala Gln Gly Leu Glu Pro Asn Gly Phe Ser Phe Glu Gly Ala
 965 970 975

Asp Ser Ser Gly Val Asp Tyr Ala Leu Asp Arg Ala Ile Thr Ala Phe
 980 985 990

Tyr Asp Ala Arg Asp Trp Phe Asn Ser Leu Ser Lys Arg Val Met Glu
 995 1000 1005

Gln Asp Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Met Glu Leu Tyr
 1010 1015 1020

His Ser Ala Arg Lys Asn
 025 1030

<210> 3

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 3

Ser His Thr Ile Tyr Ala Ala Ser Asp Leu Phe Ile Ile Pro Ser Ile
 1 5 10 15

Phe Glu Pro Cys Gly Leu Thr Gln Met Ile Ala Met Arg Tyr Gly Ser
 20 25 30

<210> 4

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 4

Ser His Leu Ile Tyr Ala Gly Ala Asp Phe Ile Leu Val Pro Ser Ile
 1 5 10 15

Phe Glu Pro Cys Gly Leu Thr Gln Leu Thr Ala Met Arg Tyr Gly Ser
 20 25 30

<210> 5

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 5

Ser His Leu Ile Tyr Ala Gly Ala Asp Phe Ile Leu Val Pro Ser Ile

1	5	10	15												
Phe	Glu	Pro	Cys	Gly	Leu	Thr	Gln	Leu	Thr	Ala	Met	Arg	Tyr	Gly	Ser
			20					25					30		

<210> 6
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 6
Ala His Gln Met Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg
1 5 10 15

Phe	Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 7
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 7
Ala His Gln Met Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg
1 5 10 15

Phe	Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 8
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 8
Ala His Met Ile Thr Ala Gly Ala Asp Phe Met Leu Ile Pro Ser Arg
1 5 10 15

Phe	Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	His	Ala	Met	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 9
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 9

Ala His Met Ile Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly Thr
20 25 30

<210> 10

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 10

Ala His Leu Ile Met Ala Gly Ala Asp Val Leu Ala Val Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr
20 25 30

<210> 11

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 11

Ala His Lys Ile Ile Ala Gly Ala Asp Phe Ile Val Ile Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Val Gln Leu His Ala Met Pro Tyr Gly Thr
20 25 30

<210> 12

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 12

Ala His His Ile Met Ala Gly Ala Asp Leu Leu Ala Val Thr Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr
20 25 30

<210> 13

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 13

Ala	His	His	Ile	Met	Ala	Gly	Ala	Asp	Val	Leu	Ala	Val	Thr	Ser	Arg
1				5					10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 14

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 14

Ser	His	Arg	Ile	Thr	Ala	Gly	Cys	Asp	Ile	Leu	Leu	Met	Pro	Ser	Arg
1				5					10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Asn	Gln	Leu	Tyr	Ala	Met	Gln	Tyr	Gly	Thr
			20					25					30		

<210> 15

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 15

Ala	His	Arg	Ile	Thr	Ala	Gly	Ser	Asp	Ile	Leu	Leu	Met	Pro	Ser	Arg
1				5					10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Asn	Gln	Leu	Tyr	Ala	Met	Ser	Tyr	Gly	Thr
			20					25					30		

<210> 16

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 16

Ser	His	Arg	Ile	Thr	Ala	Gly	Cys	Asp	Ile	Leu	Leu	Met	Pro	Ser	Arg
1				5					10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Asn	Gln	Leu	Tyr	Ala	Met	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 17
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 17
 Ser His Arg Ile Thr Ala Gly Ala Asp Ile Leu Leu Met Pro Ser Arg
 1 5 10 15
 Phe Glu Pro Cys Ala Leu Asn Gln Leu Tyr Ala Met Lys Tyr Gly Thr
 20 25 30

<210> 18
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 18
 Ala His Arg Ile Thr Ala Gly Ala Asp Ile Ala Leu Met Pro Ser Arg
 1 5 10 15
 Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr
 20 25 30

<210> 19
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 19
 Ser His Arg Ile Thr Ala Gly Cys Asp Ile Leu Leu Met Pro Ser Arg
 1 5 10 15
 Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Gln Tyr Gly Thr
 20 25 30

<210> 20
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 20

61

Ser His Arg Ile Thr Ala Gly Cys Asp Ile Leu Leu Met Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Gln Tyr Gly Thr
20 25 30

<210> 21

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 21

Ala His Arg Ile Thr Ala Gly Ala Asp Val Leu Val Met Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr
20 25 30

<210> 22

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 22

Ala His Arg Ile Thr Ala Gly Ala Asp Ile Leu Leu Met Pro Ser Arg
1 5 10 15

Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr
20 25 30

<210> 23

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 23

Ala Arg Lys Leu Tyr Ala Ser Ser Asp Phe Ile Leu Met Pro Ser Tyr
1 5 10 15

Phe Glu Pro Cys Gly Leu Thr Gln Met Ile Gly Met Arg Tyr Gly Cys
20 25 30

<210> 24

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 24

Ala	His	Gln	Ile	Tyr	Ala	Gly	Ser	Asp	Met	Phe	Leu	Met	Pro	Ser	Lys
1					5				10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Thr	Gln	Leu	Tyr	Ala	Leu	Arg	Tyr	Gly	Cys
			20					25					30		

<210> 25

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 25

Ala	His	Gln	Ile	Tyr	Ala	Gly	Ala	Asp	Leu	Phe	Leu	Ile	Pro	Ser	Leu
1					5				10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Ser	Gln	Met	Ile	Ala	Leu	Arg	Tyr	Gly	Thr
			20					25					30		

<210> 26

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 26

Ala	His	Gln	Ile	Tyr	Ala	Gly	Ala	Asp	Leu	Phe	Leu	Ile	Pro	Ser	Leu
1					5				10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Gly	Gln	Leu	Ile	Ala	Leu	Gln	Tyr	Gly	Ala
			20					25					30		

<210> 27

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: motif VII

<400> 27

Ala	His	Gln	Ile	Tyr	Ala	Gly	Ala	Asp	Leu	Phe	Leu	Ile	Pro	Ser	Leu
1					5				10					15	

Phe	Glu	Pro	Cys	Gly	Leu	Gly	Gln	Leu	Ile	Ala	Leu	Gln	Tyr	Gly	Ala
			20					25					30		

<210> 28

<211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 28
 Ser His Leu Met Val Ala Gly Gly Asp Val Ile Leu Val Pro Ser Arg
 1 5 10 15
 Phe Glu Pro Cys Gly Leu Thr Gln Leu Tyr Gly Leu Gln Tyr Gly Thr
 20 25 30

<210> 29
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: motif VII

<400> 29
 Ala His Leu Ile Tyr Gly Ala Ala Asp Ile Ile Val Val Pro Ser Asn
 1 5 10 15
 Tyr Glu Pro Cys Gly Leu Thr Gln Met Ile Gly Leu Arg Tyr Gly Ala
 20 25 30

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated nucleic acid molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of:
 - 5 (a) nucleic acid molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;
 - (b) nucleic acid molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;
 - 10 (c) nucleic acid molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;
 - 15 (d) nucleic acid molecules whose nucleotide sequence deviates from the sequence of the nucleic acid molecules mentioned under (a), (b) or (c) due to the degeneracy of the genetic code; and
 - (e) nucleic acid molecules the sequence of which is more than 85% identical to the coding region of the nucleotide sequence shown in SEQ ID No. 1.
- 20 2. The nucleic acid molecule as claimed in claim 1 which is a DNA molecule.
3. The nucleic acid molecule as claimed in claim 1 which is an RNA molecule.
- 25 4. A vector comprising a nucleic acid molecule as claimed in any one of claims 1 to 3.
- 30 5. The vector as claimed in claim 4 comprising one or more regulatory elements which ensure the transcription of said nucleic acid molecules and/or the synthesis of a translatable RNA in a pro- and/or eukaryotic cell.

- 5 6. The vector as claimed in claim 4 , wherein said nucleic acid molecule is linked in sense orientation to regulatory elements which ensure the transcription and synthesis of a translatable RNA in pro- and/or eukaryotic cells, or wherein said nucleic acid molecule is linked in anti-sense orientation to regulatory elements which ensure the transcription and synthesis of a non-translatable RNA in pro- and/or eukaryotic cells.
- 10 7. A host cell host which is transformed with a nucleic acid molecule as claimed in claim 1 or a vector as claimed in any one of claims 4 to 6.
8. The host cell as claimed in claim 7 which is a plant cell.
- 15 9. An isolated protein or a bioactive fragment thereof encoded by a nucleic acid molecule as claimed in claim 1.
- 20 10. A method for producing a protein as claimed in claim 9 or a bioactive fragment thereof, in which a host cell as claimed in claim 7 is cultured under conditions which permit the synthesis of the protein, and the protein is isolated from the cultured cells and/or the culture medium.
- 25 11. The transgenic plant cell as claimed in claim 8, wherein said nucleic acid molecule which encodes a protein with the bioactivity of a starch synthase or a bioactive fragment thereof is under the control of regulatory elements which permit the transcription of a translatable mRNA in plant cells.
- 30 12. The transgenic plant cell as claimed in claim 8, wherein the activity of a protein or a bioactive fragment thereof as claimed in claim 9 is reduced in this plant cell compared with corresponding, non-genetically modified plant cells from wild-type plants.

13. The transgenic plant cell as claimed in claim 8, wherein the activity of a protein or a bioactive fragment thereof as claimed in claim 9 is increased in this plant cell compared with corresponding, non-genetically modified plant cells from wild-type plants.
- 5
14. A plant comprising plant cells as claimed in claim 8.
15. The plant as claimed in claim 14 which is a crop plant.
- 10
16. The plant as claimed in claim 14 or claim 15, which is a starch-storing plant.
17. The plant as claimed in any one of claims 14 to 16 which is a maize plant.
- 15
18. A method for generating a transgenic plant cell as claimed in claim 8, wherein a plant cell is subjected to genetic modification by introducing a nucleic acid molecule as claimed in of claim 1 and/or a vector as claimed in claim 4.
- 20
19. A method for generating a transgenic plant as claimed in claim 14, wherein
- (a) a plant cell is subjected to genetic modification by introducing a nucleic acid molecule as claimed in claim 1 and/or a vector as claimed in claim 4; and
- 25
- (b) a plant is regenerated from this cell; and, optionally,
- (c) more plants are generated from the plant of (b).
20. Propagation material of a plant as claimed in claim 14 comprising plant cells as claimed in claim 8.
- 30

21. Starch when obtained from plant cells as claimed in claim 8, from plants as claimed in claim 14, or from propagation material as claimed in claim 20.

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22. A method for producing a modified starch as claimed in claim 21 comprising the step of extracting the starch from a plant cell as claimed in claim 8, from a plant as claimed in claim 14 and/or from propagation material as claimed in claim 20.

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23. An isolated nucleic acid molecule, substantially as herein described with reference to any one of the examples or the written description.

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Dated this 10th day of February 2005

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