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Megnövekedett aminocukor tartalmú növények

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(54) **PLANTS HAVING AN INCREASED CONTENT OF AMINO SUGARS**

PFLANZEN MIT ERHÖHTEM AMINOZUCKERGEHALT

VEGETAUX POSSEDANT UN CONTENU ACCRU DE SUCRES AMINO

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Description

[0001] The present invention relates to plant cells and plants having an increased content of N-acetylated glucosamine derivatives. Furthermore, the present invention relates to plant cells and plants synthesizing glucosaminoglycans. The present invention also provides processes for producing said plants and compositions comprising said plant cells.

[0002] The amino sugar glucosamine, glucosamine derivatives and polymers comprising glucosamine derivatives are used, inter alia, as food supplements for the prophylaxis of joint disorders in animals and man. In the medical field, too, some glucosamine derivative-containing polymers are used for treating disorders.

[0003] WO 06 032538 describes transgenic plants which had been transformed with nucleic acid molecules coding for hyaluronan synthases. The synthesis of hyaluronan in the plants in question could be demonstrated unambiguously.

[0004] WO 98 35047 (US 6,444,878) describes a metabolic path for the synthesis of GlcNAc in plant cells where glucosamine is converted by a number of successive enzymatically catalyzed reaction steps with formation of the metabolites GlcNAc, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 1-phosphate into UDP-GlcNAc. A metabolic path which was described as an alternative for plants comprises the conversion of fructose 6-phosphate and glutamine into glucosamine 6-phosphate which is then converted by a number of successive enzymatically catalyzed reaction steps with formation of the metabolites glucosamine 1-phosphate and N-acetylglucosamine 1-phosphate into UDP-GlcNAc. The conversion of fructose 6-phosphate and glutamine into glucosamine 6-phosphate is catalyzed by a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase (GFAT) (Mayer et al., 1968, Plant Physiol. 43, 1097-1107). Relatively high concentrations of glucosamine 6-phosphate are toxic for plant cells (WO 98 35047).

[0005] WO 00 11192 describes the endosperm-specific overexpression of a nucleic acid molecule from corn coding for a protein having the enzymatic activity of a plant GFAT in transgenic corn plants with the aim of synthesizing a cationic starch having 2-amidoanhydroglucose molecules in plants. The metabolic path described which, according to the description of WO 00 11192, should result in the incorporation of 2-aminoanhydroglucose into the starch, comprises inter alia the incorporation of UDP-glucosamine by starch and/or glycogen synthases into the starch. It was possible to demonstrate increased amounts of UDP-glucosamine in the flour of endosperm of the transgenic corn plants in question overexpressing a nucleic acid molecule coding for a protein having the enzymatic activity of a plant GFAT translationally fused with a plastid signal peptide. When the protein having the enzymatic activity of a GFAT was expressed without signal peptide, it was possible to demonstrate an increased amount of glucosamine 1-phosphate in the corresponding flour from corn endosperm tissue. It was not possible to detect cationic starch or increased amounts of N-acetylated glucosamine derivatives, such as, for example, UDP-GlcNAc or N-acetylglucosamine 6-phosphate, in the transgenic plants.

[0006] The amino sugar beta-D-glucosamine (glucosamine) and/or derivatives of glucosamine are components of various polymers (glucosaminoglycans) which, inter alia, are essential components of the exoskeleton of arthropods, the extracellular matrix of mammals or the exopolysaccharides of some bacterial microorganisms. Thus, for example, N-acetyl-D-glucos-2-amine (N-acetylglucosamine, GlcNAc) is a glucosamine derivative acetylated at the nitrogen atom. GlcNAc is, for example, a molecular building block of hyaluronan (beta-1,4-[glucuronic acid beta-1,3-GlcNAc]_n), which is an essential component of the synovial fluid.

[0007] In the medical field, hyaluronan-containing products are currently used for the intra-articular treatment of arthrosis and as ophthalmics used for eye surgery. Derivatized cross-linked hyaluronan is used for treating joint disorders (Fong Chong et al., 2005, Appl Microbiol Biotechnol 66, 341-351). In addition, hyaluronan is a component of some rhinologics which, for example in the form of eye drops and nasal sprays, serve to moisten dry mucous membranes. Hyaluronan-containing solutions for injection are used as analgesics and antirheumatics. Patches comprising hyaluronan or derivatized hyaluronan are employed in wound healing. As dermatics, hyaluronan-containing gel implants are used for correcting skin deformations in plastic surgery. In cosmetic surgery, hyaluronan preparations are among the suitable skin filler materials. By injecting hyaluronan, for a limited period of time, it is possible to smooth wrinkles or to increase the volume of lips.

[0008] In cosmetic products, in particular in skin creams and lotions, hyaluronan is frequently used as a moisturizer by virtue of its high water-binding capacity. Furthermore, hyaluronan-containing preparations are sold as so-called nutraceuticals (food supplements) which can also be used in animals (for example dogs, horses) for the prophylaxis and alleviation of arthrosis.

[0009] The catalysis of the hyaluronan synthesis is effected by a single membrane-integrated or membrane-associated enzyme, i.e. hyaluronan synthase (DeAngelis, 1999, CMLS, Cellular and Molecular Life Sciences 56, 670-682). Hyaluronan synthase catalyzes the synthesis of hyaluronan from the substrates UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetylglucosamine (UDP-GlcNAc).

[0010] Hyaluronan used for commercial purposes is currently isolated from animal tissues (roostercombs) or prepared fermentatively using bacterial cultures.

[0011] Proteoglycans, a class of glycoproteins, are, inter alia, an essential component of cartilage and have, attached

to a core protein, glucosaminoglycans composed of repetitive disaccharide units. The repetitive disaccharide units for their part are covalently attached to the core protein via a characteristic carbohydrate binding sequence. Depending on the composition of the disaccharide units, a distinction is made, inter alia, of the glucosaminoglycans heparan/heparin sulfate, keratan sulfate and chondroitin/dermatan sulfate, whose disaccharide units each contain a molecule which is either glucosamine or a glucosamine derivative. In these substances, sulfate groups are introduced at various atoms or substituents of the disaccharide units, so that the respective substances mentioned are not uniform polymers but polymer groups summarized under the respective generic term. Here, the individual molecules of the polymer groups in question may differ both in the degree of sulfation and in the position of the monomers containing sulfate groups.

[0012] The synthesis of the disaccharide chain of the chondroitin/dermatan ([beta-1,4]-[glucuronic acid beta-1,4-N-acetylgalactosamine]_n) is catalyzed by a chondroitin synthase starting with UDP-GlcA and UDP-N-acetylgalactosamine, an epimer of UDP-GlcNAc (Kitagawa et al., 2001, J Biol Chem 276(42), 38721-38726). The glucuronic acid molecules of chondroitin can be converted by an epimerase into iduronic acid. If more than 10% of the glucuronic acid molecules are present as iduronic acid, the polymer is referred to as dermatan. The introduction of the sulfate groups in various positions of the disaccharide chain of the chondroitin or the dermatan is then catalyzed by further enzymes, resulting in chondroitin/dermatan sulfate. Here, the degree of sulfation may differ from molecule to molecule.

[0013] For some time, chondroitin sulfate has been considered as a potential active compound for treatment of osteoarthritis (Clegg et al., 2006, The New England Journal of Medicine 354(8), 795-808).

[0014] The synthesis of the disaccharide chain of heparin/heparan (heparosan) ([alpha-1,4]-[glucuronic acid beta-1,4-glucosamine]_n or [alpha-1,4]-[iduronic acid alpha-1,4-glucosamine]_n) is catalyzed by a heparin/heparosan synthase from UDP-GlcA and UDP-GlcNAc (DeAngelis und White, 2004, J. Bacteriology 186(24), 8529-8532). The glucuronic acid molecules of the heparin/heparosan can be converted by an epimerase into iduronic acid. The introduction of the sulfate groups in various positions of the disaccharide chain of the heparosan is then catalyzed by further enzymes, giving rise to heparin sulfate or heparan sulfate. Heparin sulfate has a considerably higher substitution by sulfate groups than heparan sulfate. Heparin sulfate has about 90% iduronic acid molecules, whereas in the case of heparan sulfate the fraction of glucuronic acid molecules predominates (Gallagher et al., 1992, Int. J. Biochem 24, 553-560). As in the case of chondroitin/dermatan sulfate, in the case of heparin/heparan sulfate, too, the degree of sulfation may differ from molecule to molecule.

[0015] Heparin sulfate is used, inter alia, as an anticoagulant, for example for the prophylaxis and treatment of thromboses.

[0016] Chondroitin/dermatan sulfate and heparin/heparan sulfate are currently produced by isolation from animal tissues. Chondroitin sulfate is mainly isolated from bovine or shark cartilage, and heparin/heparan sulfate is isolated from porcine intestine or bovine lungs. Since the disaccharide chains of chondroitin/dermatan sulfate or heparin/heparan sulfate have no uniform sulfation pattern, it is difficult to obtain a uniform specific product. Accordingly, the products are always mixtures of molecules with varying degrees of sulfation.

[0017] The glucosaminoglycan chitin ([beta-1,4-GlcNAc]_n) is one of the main components of the cell wall of fungi and the exoskeleton of insects, millipedes, arachnids and crustaceans and is a polymer which is insoluble in water. The enzyme chitin synthase catalyzes the synthesis of chitin by linking UDP-GlcNAc (Merzendorfer and Zimoch, 2003, J. Experimental Biology 206, 4393-4412).

[0018] As a raw material source for isolating chitin, use is to date mainly made of crustaceans (prawns, crabs) and fungi, such as, for example, *Aspergillus spec.*, *Penicillium spec.*, *Mucor spec.* WO 03 031435 describes, for example, a method for preparing GlcNAc by fermentation of yeasts. Depending on the method by which chitin is isolated from the raw material source in question, chitin contains in addition to GlcNAc also its deacetylated form glucosamine as a building block. If more than 50% of the building blocks are GlcNAc, the polymer is referred to as chitin, whereas polymers comprising more than 50% of glucosamine are referred to as chitosan. These days, glucosamine or derivatives thereof, such as, for example, GlcNAc, are produced by degradation of chitin. Chitin may either be deacetylated first, resulting in the formation of chitosan, or be degraded directly, resulting in the formation of GlcNAc.

[0019] Chitin can be deacetylated enzymatically with the aid of chitin deacetylases (Kafetzopoulos et al., 1993, Pro. Natl. Acad. Sci. 90, 2564-2568) or by chemical deacetylation.

[0020] The degradation of chitin or of chitosan can also take place both enzymatically (for example using chitinases, glucanases, beta-N-acetylglucosaminidases), and by chemical hydrolysis.

[0021] The degradation of chitosan or the deacetylation of GlcNAc results in the formation of glucosamine.

[0022] A substantial disadvantage of all methods for preparing amino sugars by degradation of chitin consists in the fact that, owing to incomplete hydrolysis and/or incomplete deacetylation, what is obtained is not a uniform product but a mixture of various mono- and oligomers.

[0023] An alternative process for preparing glucosamine with the aid of recombinant microorganisms, in particular *Escherichia coli*, which does not require the degradation of chitin, is described in US 2002/0160459.

[0024] For some time, glucosamine and glucosamine-containing substances, too, have been considered as potential active compounds for the treatment of osteoarthritis (Clegg et al., 2006, The New England Journal of Medicine 354(8),

795-808). Glucosamine or glucosamine-containing substances are also present in many food supplements. Foods enriched with GlcNAc are described, for example, in US 2006/0003965.

[0025] As already described, glucosaminoglycans, such as, for example chondroitin sulfate, heparin/heparan sulfate or chitin are currently isolated from animal tissues. In addition to the substances desired in each case, these tissues also contain other glucosaminoglycans. The separation of the individual glucosaminoglycans, if a complete separation is possible at all, is difficult and complicated. Furthermore, the potential presence, in animal tissues, of pathogenic microorganisms and/or of other substances, such as, for example, the BSE pathogen or the bird flu pathogen, which may cause diseases in man, represent a problem when using glucosaminoglycans isolated from animal tissue. The use of medicinal preparations contaminated with animal proteins may, in the patient, result in unwanted immunological reactions of the body (for hyaluronan preparations, see, for example, US 4,141,973), in particular if the patient is allergic to animal proteins.

[0026] A further problem during the isolation of glucosaminoglycans from animal tissues consists in the fact that the molecular weight of the glucosaminoglycans is frequently reduced during purification, since animal tissues also contain enzymes which degrade glucosaminoglycan.

[0027] Glucosamine or derivatives thereof isolated from crustaceans frequently contain substances (proteins) which may trigger an allergic reaction in man. Glucosamine or derivatives obtained from fungi may contain mycotoxins.

[0028] The amounts (yields) of glucosaminoglycans which can be obtained in satisfactory quality and purity from animal tissues are low (for example hyaluronan from roostercombs: 0.079% w/w, EP 0144019, US 4,782,046), which means that large amounts of animal tissues have to be processed.

[0029] The production of glucosaminoglycans with the aid of fermentation of bacteria is associated with high costs, since the bacteria have to be fermented in sealed sterile containers under complicated controlled cultivation conditions (for hyaluronan, see, for example, US 4,897,349). Furthermore, the amount of glucosaminoglycans which can be produced by fermentation of bacteria strains is limited by the existing production facilities. Here, it has also been taken into account that, owing to physical limitations, it is not possible to construct fermenters for relatively large culture volumes. In this context, mention may be made in particular of homogeneous mixing, required for efficient production, of fed-in substances (for example essential nutrient sources for bacteria, reagents for regulating the pH, oxygen) with the culture medium, which, if at all, can be ensured in large fermenters only with high technical expenditure.

[0030] Furthermore, substances prepared from animal raw materials are unacceptable for certain ways of life, such as, for example, veganism or for kosher food preparation.

[0031] Plants do not naturally produce glucosaminoglycans, such as, for example, hyaluronan, chitin, heparan/heparin sulfate, keratan sulfate or chondroitin/dermatan sulfate.

[0032] For the synthesis of glucosaminoglycans, it is necessary, inter alia, for sufficient amounts of acetylated glucosamine derivatives (in particular UDP-GlcNAc) and/or UDP-GlcA to be available as substrate for the respective enzymes involved in the synthesis. There is no information with regard to the amounts of N-acetylated glucosamines present in plant cells. WO 2005 035710 describes a process which allows the glucosamine content of plant material to be increased by drying. The highest glucosamine content in fresh, wet plant material was determined for chicory with 10 mg of glucosamine per 1 kg of fresh weight, which, at a molecular weight of 178 for glucosamine, corresponds to about 56 nmol of glucosamine per 1 gram fresh weight of plant material. WO 2005 035710 contains no information concerning the content of N-acetylated glucosamine derivatives in plants.

[0033] Furthermore, from the prior art described above, it is evident that the paths of glucosamine metabolism in plants have not yet been fully elucidated. In WO 00 11192, it was possible to generate plants by transformation with a nucleic acid molecule coding for a protein having the activity of a plant GFAT, which plants had an elevated content of glucosamine derivatives (UDP-glucosamine or glucosamine 1-phosphate); however, increased amounts of N-acetylated glucosamine derivatives were not found.

[0034] Accordingly, it is an object of the present invention to provide alternative sources of N-acetylated glucosamine derivatives and processes for preparing said alternative sources for N-acetylated glucosamine derivatives.

[0035] A first aspect of the present invention relates to plant cells or plants having a content of N-acetylated glucosamine derivatives of at least 2.50 μmol per gram of fresh weight, preferably of at least 5.00 μmol per gram of fresh weight, particularly preferably of at least 10.00 μmol per gram of fresh weight, very particularly preferably of at least 15.00 μmol per gram of fresh weight, especially preferably of at least 20.00 μmol per gram of fresh weight.

[0036] Preferably, plant cells according to the invention or plants according to the invention have a content of N-acetylated glucosamine derivatives of at most 250 μmol per gram of fresh weight, preferably of at most 200 μmol per gram of fresh weight, particularly preferably of at most 150 μmol per gram of fresh weight, very particularly preferably of at most 100 μmol per gram of fresh weight, especially preferably of at most 50 μmol per gram of fresh weight.

[0037] Compared to the prior art, plant cells according to the invention or plants according to the invention offer the advantage that they contain higher amounts of N-acetylated glucosamine derivatives. Compared to the production of N-acetylated glucosamine derivatives by fermentation of microorganisms or the isolation of N-acetylated glucosamines from animal raw material sources or fungi, plant cells according to the invention or plants according to the invention of

the present invention offer the advantage that plant cells according to the invention and plants according to the invention can be propagated infinitely in a vegetative or sexual manner, and that they continuously produce N-acetylated glucosamine derivatives. Furthermore, compared to known plants, plants according to the invention offer the advantage that they are better suitable for preparing glucosaminoglycans, such as, for example, chondroitin, hyaluronan, chitin, heparosan, since they contain a higher amount of substrates for the enzymes involved in the catalysis of the glucosaminoglycans mentioned (glucosaminoglycan synthases).

[0038] N-Acetylated glucosamine derivatives can be detected using methods known to the person skilled in the art (Morgan and Elson (1934, Biochem J. 28(3), 988-995). In the context of the present invention, for determining the content of N-acetylated glucosamine derivatives, use is preferably made of the method described under General Methods Item 4.

[0039] In the context of the present invention, the term "N-acetylated glucosamine derivatives" is to be understood as meaning all derivatives of glucosamine (2-amino-2-deoxyglucose), which also include epimers, such as, for example, galactosamine (2-amino-2-deoxygalactose) or mannosamine (2-amino-2-deoxymannose), which are measured using the method described under General Methods Item 4. The N-acetylated glucosamine derivatives are preferably N-acetylglucosamine phosphate (N-acetylglucosamine 1-phosphate and/or N-acetylglucosamine 6-phosphate), N-acetylglucosamine and/or UDP-N-acetylglucosamine.

[0040] Preferably plant cells according to the invention or plants according to the invention have an increased content of glucosamine phosphate (glucosamine 1-phosphate and/or glucosamines 6-phosphate) in addition to an increased content of N-acetylated glucosamine derivatives.

[0041] Plant cells according to the invention or plants according to the invention can be prepared, for example, by introducing foreign nucleic acid molecules coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase (GFAT) of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial GFAT.

[0042] In a preferred embodiment of the present invention, the plant cells according to the invention or the plants according to the invention are thus genetically modified plant cells and genetically modified plants, respectively.

[0043] Surprisingly, it has been found that plant cells or plants containing a nucleic acid molecule coding for a protein having the activity of a GFAT-2 or a protein having the activity of a bacterial GFAT contain considerably more N-acetylated glucosamine derivatives than plant cells or plants containing a nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform I (GFAT-1). As already mentioned, it was not possible to detect increased amounts of acetylated glucosamine derivatives in plants containing a nucleic acid molecule coding for a protein having the activity of a plant GFAT (WO 00 11192).

[0044] Accordingly, the present invention also provides genetically modified plant cells or genetically modified plants containing a foreign nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase (GFAT), wherein the foreign nucleic acid molecule codes for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT).

[0045] The genetic modification of a plant cell according to the invention or a plant according to the invention may be any genetic modification suitable for integrating a foreign nucleic acid molecule into a plant cell or plant.

[0046] Preferably, the foreign nucleic acid molecule is integrated into the genome; particularly preferably, the foreign nucleic acid molecule is stably integrated into the genome of plant cells according to the invention or plants according to the invention.

[0047] A large number of techniques for (stably) integrating nucleic acid molecules into a plant host cell for producing plant cells according to the invention or plants according to the invention is available. These techniques include the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as means of transformation, protoplast fusion, injection, electroporation of DNA, introduction of DNA by the biolistic approach and also further options (review in "Transgenic Plants", Leandro ed., Humana Press 2004, ISBN 1-59259-827-7).

[0048] The use of agrobacterium-mediated transformation of plant cells has been subject to in-depth studies and has been described exhaustively in EP 120516 and Hoekema, IN: The Binary Plant Vector System Offsetdrukkerij Kanter B.V. Alblasterdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant Sci. 4, 1-46 and in An et al. EMBO J. 4, (1985), 277-287. For the transformation of potatoes see, for example, Rocha-Sosa et al., EMBO J. 8, (1989), 29-33, for the transformation of tomato plants see, for example, US 5,565,347.

[0049] The transformation of monocotyledonous plants using vectors based on *Agrobacterium* transformation has been described, too (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). Alternative systems for transforming monocotyledonous plants are the transformation using 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), the protoplast transformation, the electroporation of partially permeabilized cells or the introduction of DNA using glass fibers. In particular the transformation of corn has been described several times in the literature (cf., for example, WO95/06128,

EP0513849, EP0465875, EP0292435; 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 transformation of other grasses, such as, for example, switchgrass (*Panicum virgatum*) has also been described (Richards et al., 2001, *Plant Cell Reporters* 20, 48-54).

[0050] The successful transformation of other cereal species has likewise already been described, for example for barley (Wan and Lemaux, loc. cit.; Ritala et al., loc. cit.; Krens et al., *Nature* 296, (1982), 72-74) and for wheat (Nehra et al., *Plant J.* 5, (1994), 285-297; Becker et al., 1994, *Plant Journal* 5, 299-307). All of the above methods are suitable in the context of the present invention.

[0051] Genetically modified plant cells and genetically modified plants having a foreign nucleic acid molecule can be distinguished from wild-type plant cells and wild-type plants, respectively, not having said foreign nucleic acid molecule, inter alia by the fact that they contain a foreign nucleic acid molecule which does not naturally occur in wild-type plant cells and wild-type plants, respectively. Such an integration of a foreign nucleic acid molecule into a plant cell or plant can be detected using methods known to the person skilled in the art, such as, for example, Southern blot analysis or by PCR.

[0052] In the context of the present invention, the term "stably integrated nucleic acid molecule" is to be understood as meaning the integration of a nucleic acid molecule into the genome of the plant. A stably integrated nucleic acid molecule is characterized in that, during the replication of the corresponding integration site, it is multiplied together with the nucleic acid sequences of the host which border on the integration site, so that the integration site in the replicated daughter DNA strand is surrounded by the same nucleic acid sequences as on the read mother strand which serves as a matrix for the replication.

[0053] The integration of a nucleic acid molecule into the genome of a plant cell or a plant can be demonstrated by genetic methods and/or methods of molecular biology. A stable integration of a nucleic acid molecule into the genome of a plant cell or into the genome of a plant is characterized in that in the progeny which has inherited said nucleic acid molecule, the stably integrated nucleic acid molecule is present in the same genomic environment as in the parent generation. The presence of a stable integration of a nucleic acid sequence in the genome of a plant cell or in the genome of a plant can be demonstrated using methods known to the person skilled in the art, inter alia with the aid of Southern blot analysis or the RFLP analysis (Restriction Fragment Length Polymorphism) (Nam et al., 1989, *The Plant Cell* 1, 699-705; Leister and Dean, 1993, *The Plant Journal* 4 (4), 745-750), with methods based on PCR, such as, for example, the analysis of differences in length in the amplified fragment (Amplified Fragment Length Polymorphism, AFLP) (Castiglioni et al., 1998, *Genetics* 149, 2039-2056; Meksem et al., 2001, *Molecular Genetics and Genomics* 265, 207-214; Meyer et al., 1998, *Molecular and General Genetics* 259, 150-160) or using amplified fragments cleaved using restriction endonucleases (Cleaved Amplified Polymorphic Sequences, CAPS) (Konieczny and Ausubel, 1993, *The Plant Journal* 4, 403-410; Jarvis et al., 1994, *Plant Molecular Biology* 24, 685-687; Bachem et al., 1996, *The Plant Journal* 9 (5), 745-753).

[0054] In the context of the present invention, the term "genome" is to be understood as meaning the entire genetic material present in a plant cell. It is known to the person skilled in the art that, in addition to the nucleus, other compartments (for example plastids, mitochondria) also contain genetic material.

[0055] A further preferred subject matter of the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention expressing a foreign nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT).

[0056] In the context of the present invention, the term "to express" or "expression" is to be understood as meaning the presence of transcripts (mRNA) coded for by a foreign nucleic acid molecule and/or the presence of proteins having the activity of a GFAT-2 or a bacterial GFAT.

[0057] An expression can be demonstrated, for example, by detection of specific transcripts (mRNA) of foreign nucleic acid molecules by Northern blot analysis or RT-PCR.

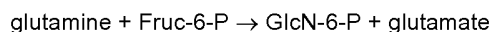
[0058] Whether plant cells or plants contain proteins having the activity of a GFAT-2 or proteins having the activity of a bacterial GFAT can be determined, for example, by immunological methods, such as Western blot analysis, ELISA (Enzyme Linked Immuno Sorbent Assay) or RIA (Radio Immune Assay). The person skilled in the art is familiar with methods for preparing antibodies which react specifically with a certain protein, i.e. which bind specifically to a certain protein (see, for example, Lottspeich and Zorbas (eds.), 1998, *Bioanalytik, Spektrum akad, Verlag, Heidelberg, Berlin*, ISBN 3-8274-0041-4). Some companies (for example Eurogentec, Belgium) offer the preparation of such antibodies as an order service.

[0059] In a further preferred embodiment of the present invention, plant cells according to the invention or plants according to the invention have an activity of a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or of coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT).

[0060] The activity of proteins having the activity of a GFAT-2 or proteins having the activity of a bacterial GFAT in extracts of plant cells according to the invention or plants according to the invention can be detected using methods

known to the person skilled in the art, such as, for example, described in Samac et al. (2004, Applied Biochemistry and Biotechnology 113-116, Humana Press, Editor Ashok Mulehandani, 1167-1182, ISSN 0273-2289). A preferred method for determining the amount of activity of a protein having the activity of a GFAT is given in General Methods, Item 8.

[0061] In the context of the present invention, the term "glutamine:fructose 6-phosphate amidotransferase (GFAT)" (E.C. 2.6.1.16), in the expert literature also referred to as glucosamine synthase, is to be understood as meaning a protein which synthesizes, from the starting materials glutamine and fructose 6-phosphate (Fruc-6-P), glucosamine 6-phosphate (GlcN-6-P). This catalysis proceeds according to the following reaction scheme:



[0062] In the context of the present invention, the term "glutamine:fructose 6-phosphate amidotransferase (GFAT)" is used as a generic term which includes all known isoforms.

[0063] A review article by Milewski (2002, Biochimica et Biophysica Acta 1597, 173-193) describes structural features of proteins having the activity of a GFAT. The amino acid sequence of all known proteins having the activity of a GFAT contains regions with conserved amino acid sequences. The amino acid sequence of proteins having the activity of a GFAT has an N-terminal glutamine binding domain and a C-terminal fructose 6-phosphate binding domain which are separated by a sequence of 40 to 90 non-conserved amino acids. Both domains are active even if they are present on separate amino acid molecules. Analyses of the crystal structure of a fragment comprising the N-terminal glutamine binding domain of the protein having the activity of a GFAT from *Escherichia coli* showed that the active center of this domain is located at the N-terminus and the amino acid Cys1 is involved in the hydrolysis of glutamine. The amino acids Arg73 and Asp123 interact with carboxyl and amino groups of the glutamine. This interaction is supported by the amino acids Thr76 and His77. The formation of hydrogen bonds with the amido group of the glutamine is attributed to the amino acids Gly99 and Trp74. The amino acids Asn98 and Gly99 stabilize the four-faced pocket of the active center. The amino acids 25 to 29 and 73-80 form flexible loops which, after binding of the substrate glutamine, contribute by a conformational change of the protein to the reaction catalyzed by a protein having the activity of a GFAT. Analysis of the crystal structure of the C-terminal fructose 6-phosphate binding domain of the protein having the activity of a GFAT from *Escherichia coli* showed that this domain is constructed of two topologically identical domains (amino acids 241 to 424 and 425 to 592) followed by a domain present at the C-terminal end as an irregular loop (amino acids 593 to 608), but which has only one active center. The amino acids Ser303, Ser347, Gln348, Ser349 and Thr352 are involved in substrate binding, whereas the amino acids Glu488, His504 and Lys603 are directly involved in the catalysis of the reaction of the protein having the activity of a GFAT.

[0064] In particular in animal organisms, it was possible to demonstrate two different isoforms of proteins having the activity of a GFAT (referred to in the literature as GFAT-1 and GFAT-2, respectively). Hu et al. (2004, J. Biol. Chem. 279(29), 29988-29993) describe differences of the respective isoforms of proteins having the activity of a GFAT. In addition to differences in the tissue-specific expression of the isoforms in question having the activity of a GFAT-1 and a GFAT-2, it was possible to show that both isoforms are regulated by phosphorylation by a cAMP-dependent protein kinase. The activity of a protein having the enzymatic activity of a GFAT-1 is inhibited by phosphorylation of a conserved serine residue (serine 205 in the GFAT-1 from the mouse, GenBank Acc No.: AF334736.1) of the amino acid sequence in question, whereas the activity of a protein having the activity of a GFAT-2 is increased by phosphorylation of a conserved serine residue (serine 202 in the GFAT-2 from the mouse, GenBank Acc No.: NM_013529) of the amino acid sequence in question. Both proteins having the activity of a GFAT-1 and proteins having the activity of a GFAT-2 are inhibited in a concentration-dependent manner by UDP-GlcNAc; however, for a protein having the activity of a GFAT-2, the inhibition by UDP-GlcNAc is lower (maximum reduction of activity by UDP-GlcNAc about 15%) compared to a protein having the activity of a GFAT-1 (maximum reduction of activity by UDP-GlcNAc by about 51% or 80%, respectively). There are indications that the inhibition of a protein having the activity of a GFAT-1 in animal organisms is based on the fact that at elevated UDP-GlcNAc concentrations there is an O-glucose-N-acetylglucosamine glycosylation of the proteins in question. Whether a regulation of the activity of proteins by O-glycosylation also takes part in plant cells is not yet fully understood (Huber and Hardin, 2004, Current Opinion in Plant Biotechnology 7, 318-322).

[0065] Proteins having the activity of a bacterial GFAT are distinguished by the fact that they are not inhibited by UDP-GlcNAc (Kornfeld, 1967, J. Biol. Chem. 242(13), 3135-3141).

[0066] Proteins having the activity of a GFAT-1, proteins having the activity of a GFAT-2, and even proteins having the activity of a bacterial GFAT are inhibited by the product glucosamine 6-phosphate formed in their reaction (Broschat et al., 2002, J. Biol. Chem. 277(17), 14764-14770; Deng et al., 2005, Metabolic Engineering 7, 201-214).

[0067] In the context of the present invention, the term "protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform I (GFAT-1)" is to be understood as meaning a protein which has the activity of a GFAT and whose activity is inhibited by phosphorylation by a cAMP-dependent protein kinase.

[0068] In the context of the present invention, the term "protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2)" is to be understood as meaning a protein which has the activity of a GFAT

and which is activated by phosphorylation by a cAMP-dependent protein kinase.

[0069] In the context of the present invention, the term "protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT)" is to be understood as meaning a protein which has the activity of a GFAT and whose activity is not inhibited by UDP-GlcNAc. Alternatively, "proteins having the activity of a bacterial GFAT" may also be referred to as "proteins having the activity of a non-eukaryotic GFAT".

[0070] In the context of the present invention, the term "foreign nucleic acid molecule" is to be understood as meaning such a molecule which either does not naturally occur in corresponding wild-type plant cells or which does not naturally occur in the specific spatial arrangement in wild-type plant cells or which is localized at a site in the genome of the wild-type plant cell where it does not naturally occur.

[0071] Preferably, the foreign nucleic acid molecule is a recombinant molecule which consists of various elements (nucleic acid molecules) whose combination or specific spatial arrangement does not naturally occur in plant cells.

[0072] In the context of the present invention, the term "recombinant nucleic acid molecule" is to be understood as meaning a nucleic acid molecule which has various nucleic acid molecules which are not naturally present in a combination as present in a recombinant nucleic acid molecule. Thus, recombinant nucleic acid molecules may, in addition to foreign nucleic acid molecules coding for a protein, have, for example, additional nucleic acid sequences which are not naturally present in combination with said protein-encoding nucleic acid molecules. Here, the additional nucleic acid sequences mentioned, which are present in a recombinant nucleic acid molecule in combination with a protein-encoding nucleic acid molecule, may be any sequences. They may, for example, represent genomic and/or plant nucleic acid sequences.

[0073] The additional nucleic acid sequences mentioned are preferably regulatory sequences (promoters, termination signals, enhancer, introns), particularly preferably regulatory sequences active in plant tissue, very particularly preferably tissue-specific regulatory sequences active in plant tissue.

[0074] Methods for generating recombinant nucleic acid molecules are known to the person skilled in the art and include genetic engineering methods, such as, for example, linking of nucleic acid molecules by ligation, genetic recombination or the novel synthesis of nucleic acid molecules (see, for example, Sambrook et al., Molecular Cloning, A Laboratory Manual, 3rd edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. ISBN: 0879695773; Ausubel et al., Short Protocols in Molecular Biology, John Wiley & Sons; 5th edition (2002), ISBN: 0471250929).

[0075] The present invention preferably provides genetically modified plant cells according to the invention or genetically modified plants according to the invention wherein the foreign nucleic acid molecules coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT are linked to regulatory elements initiating the transcription in plant cells (promoters). These may be homologous or heterologous promoters. The promoters may be constitutive, tissue-specific or development-specific promoters or promoters regulated by external factors (for example after the application of chemical substances, by action of abiotic factors, such as heat and/or cold, dryness, disease, etc.).

[0076] In general, any promoters active in plant cells are suitable for expressing a foreign nucleic acid molecule. Suitable promoters are, for example, the promoter of 35S RNA of the cauliflower mosaic virus or the ubiquitin promoter from corn or the *Cestrum* YLCV Promoter (Yellow Leaf Curling Virus; WO 01 73087; Stavalone et al., 2003, Plant Mol. Biol. 53, 703-713) for a constitutive expression, the patatingen promoter B33 (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) for a tuber-specific expression in potatoes or a fruit-specific promoter for tomato, such as, for example, the polygalacturonase promoter from tomato (Montgomery et al., 1993, Plant Cell 5, 1049-1062) or the E8 promoter from tomato (Metha et al., 2002, Nature Biotechnol. 20(6), 613-618) or the ACC oxidase promoter from peach (Moon and Callahan, 2004, J. Experimental Botany 55 (402), 1519-1528) 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) or for an endosperm-specific expression the HMWG promoter from wheat, the USP promoter, the phaseolin promoter, promoters of zein genes from corn (Pedersen et al., Cell 29 (1982), 1015-1026; Quatroccio et al., Plant Mol. Biol. 15 (1990), 81-93), a glutelin promoter (Leisy et al., Plant Mol. Biol. 14 (1990), 41-50; Zheng et al., Plant J. 4 (1993), 357-366; Yoshihara et al., FEBS Lett. 383 (1996), 213-218), a globulin promoter (Nakase et al., 1996, Gene 170(2), 223-226), a prolamin promoter (Qu and Takaiwa, 2004, Plant Biotechnology Journal 2(2), 113-125) or a shrunken-1 promoter (Werr et al., EMBO J. 4 (1985), 1373-1380). However, it is also possible to use promoters which are only active at a point in time determined by external factors (see, for example, WO 9307279). Of particular interest here may be promoters of heat-shock proteins which permit a simple induction. It is furthermore possible to use seed-specific promoters, such as, for example, the USP promoter from *Vicia faba* which ensures a 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).

[0077] The use of promoters present in the genome of algae-infecting viruses is also suitable for expressing nucleic acid sequences in plants (Mitra et al., 1994, Biochem. Biophys Res Commun 204(1), 187-194; Mitra and Higgins, 1994, Plant Mol Biol 26(1), 85-93; Van Etten et al., 2002, Arch Virol 147, 1479-1516).

[0078] In the context of the present invention, the term "tissue specific" is to be understood as meaning the substantial limitation of a manifestation (for example initiation of transcription) to a certain tissue.

[0079] In the context of the present invention, the terms "tuber, fruit or endosperm cell" are to be understood as meaning all cells present in a tuber, a fruit and in an endosperm of a seed, respectively.

[0080] In the context of the present invention, the term "homologous promoter" is to be understood as meaning a promoter which is naturally present in plant cells or plants used for the preparation of genetically modified plant cells according to the invention and genetically modified plants according to the invention, respectively, (homologous with respect to the plant cell or the plant) or as meaning a promoter which regulates the regulation of the expression of a gene in the organism from which the respective foreign nucleic acid molecule coding for a protein was isolated (homologous with respect to the nucleic acid molecule to be expressed).

[0081] In the context of the present invention, the term "heterologous promoter" is to be understood as meaning a promoter which is not naturally present in plant cells or plants used for the preparation of genetically modified plant cells according to the invention and in genetically modified plants according to the invention, respectively, (heterologous with respect to the plant cell or plant) or as meaning a promoter which is, in the organism from which the respective foreign nucleic acid molecule coding for a protein was isolated, not naturally present for regulating the expression of said foreign nucleic acid molecule (heterologous with respect to the nucleic acid molecule to be expressed).

[0082] Also present may be a termination sequence (polyadenylation signal) which serves to add a poly-A tail to the transcript. The poly-A tail is thought to act in stabilizing the transcripts. Such elements are described in the literature (cf. Gielen et al., EMBO J. 8 (1989), 23-29) and can be exchanged as desired.

[0083] It is also possible for intron sequences to be present between the promoter and the coding region of the foreign nucleic acid molecule. Such intron sequences may lead to stability of expression and an increased expression in plants (Callis et al., 1987, Genes Devel. 1, 1183-1200; Luehrsen, and Walbot, 1991, Mol. Gen. Genet. 225, 81-93; Rethmeier et al., 1997; Plant Journal 12(4), 895-899; Rose and Beliakoff, 2000, Plant Physiol. 122 (2), 535-542; Vasil et al., 1989, Plant Physiol. 91, 1575-1579; XU et al., 2003, Science in China Series C Vol.46 No.6, 561-569). Suitable intron sequences are, for example, the first intron of the sh1 gene from corn, the first intron of the poly-ubiquitin gene 1 from corn, the first intron of the EPSPS gene from rice or one of the first two introns of the PAT1 gene from *Arabidopsis*.

[0084] According to the invention, the foreign nucleic acid molecule coding for a protein having the enzymatic activity of a GFAT-2 may originate from any eukaryotic organism; preferably, said nucleic acid molecule originates from animals, particularly preferably from mammals and very particularly preferably from the mouse.

[0085] According to the invention, the foreign nucleic acid molecule coding for a protein having the enzymatic activity of a bacterial GFAT may originate from any non-eukaryotic organism or from a virus genome; preferably, said nucleic acid molecule originates from bacteria or viruses; particularly preferably, said nucleic acid molecule originates from *Escherichia coli*. Since amino acid sequences coding for viral proteins having the activity of a GFAT have a considerably higher identity with amino acid sequences coding for proteins having the activity of a bacterial GFAT and a considerably lower identity with proteins having the activity of a GFAT-1 or a GFAT-2, viral proteins having the activity of a GFAT are classed with the bacterial proteins having the activity of a GFAT (Landstein et al., 1998, Virology 250, 388-396).

[0086] With regard to viruses, the foreign nucleic acid molecule coding for a protein having the enzymatic activity of a GFAT preferably originates from an algae-infecting virus, with preference a virus which infects algae of the genus *Chlorella*, particularly preferably from a *Paramecium bursaria Chlorella* virus and very particularly preferably from a *Paramecium bursaria Chlorella* virus of an H1 strain.

[0087] Instead of a naturally occurring nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT, a nucleic acid molecule introduced into genetically modified plant cells according to the invention or genetically modified plants according to the invention may also have been generated by mutagenesis, where said mutagenized foreign nucleic acid molecule is characterized in that it codes for a protein having the activity of a GFAT-2 or a protein having the activity of a bacterial GFAT which has reduced inhibition by metabolites (for example of the glucosamine metabolism). In an exemplary manner, the preparation of such mutagenized nucleic acid molecules is described in Deng et al. (2005, Metabolic Engineering 7, 201-214; WO 04 003175) for a protein having the activity of a bacterial GFAT from *Escherichia coli*. Mutants of a protein having the activity of a GFAT-2 from the mouse are described, for example, in Hu et al. (2004, J. Biol. Chem. 279 (29), 29988-29993).

[0088] Nucleic acid molecules coding for a protein having the activity of a GFAT are known to the person skilled in the art and described in the literature. Thus, nucleic acid molecules coding for a protein having the activity of a bacterial GFAT are described, for example, for *Escherichia coli* (Dutka-Malen, 1988, Biochemie 70 (2), 287-290; EMBL acc No: L10328.1), *Bacillus subtilis* (EMBL acc No U21932), *Haemophilus influenzae* (EMBL acc Nos AB006424.1, BAA33071). Nucleic acid molecules coding for a protein having the activity of a bacterial GFAT are also described for viruses, such as, for example, the *Chlorella* virus k2 (EMBL acc No AB107976.1).

[0089] Nucleic acid molecules coding for a protein having the activity of a GFAT-2 are described inter alia from insects, for example for *Drosophila melanogaster* (NCBI acc No NM_143360.2), from vertebrates, for example for *Homo sapiens* (NCBI acc No BC000012.2, Oki et al., 1999, Genomics 57 (2), 227-34) or *Mus musculus* (EMBL acc No AB016780.1).

[0090] In a preferred embodiment, the present invention relates to genetically modified plant cells according to the invention and genetically modified plants according to the invention where the foreign nucleic acid molecule coding for

a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT is selected from the group consisting of

- a) nucleic acid molecules coding for a protein having the amino acid sequence given under SEQ ID NO 7 (GFAT-2) or a protein having the amino acid sequence given under SEQ ID NO 9 (bacterial GFAT);
- b) nucleic acid molecules coding for a protein whose sequence is at least 60%, preferably at least 70%, more preferably at least 80%, particularly preferably at least 90%, very particularly preferably at least 95% and most preferably at least 98% identical to the amino acid sequence shown under SEQ ID NO 7 (GFAT-2) or under SEQ ID NO 9 (bacterial GFAT);
- c) nucleic acid molecules comprising the nucleotide sequence shown under SEQ ID NO 6 (GFAT-2) or under SEQ ID NO 8 (bacterial GFAT) or under SEQ ID NO 10 (bacterial GFAT) or a sequence complementary thereto;
- d) nucleic acid molecules which are at least 60%, preferably at least 70%, more preferably at least 80%, particularly preferably at least 90%, very particularly preferably at least 95% and most preferably at least 98% identical to the nucleic acid sequences shown under a) or c);
- e) nucleic acid molecules which hybridize under stringent conditions with at least one strand of the nucleic acid sequences described under a) or c);
- f) nucleic acid molecules whose nucleotide sequence differs from the sequence of the nucleic acid molecules mentioned under a) or c) owing to the degeneracy of the genetic code and
- g) nucleic acid molecules which are fragments, allelic variants and/or derivatives of the nucleic acid molecules mentioned under a), b), c), d), e) or f).

[0091] In the context of the present invention, the term "hybridization" means a hybridization under conventional hybridization conditions, preferably under stringent conditions, as described, for example, in Sambrook et al. (Molecular Cloning, A Laboratory Manual, 3rd edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. ISBN: 0879695773) or Ausubel et al. (Short Protocols in Molecular Biology, John Wiley & Sons; 5th edition (2002), ISBN: 0471250929). With particular preference, "hybridization" means a hybridization under the following conditions:

hybridization buffer:

2xSSC; 10xDenhardt solution (Fikoll 400+PEG+BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na₂HPO₄; 250 µg/ml of herring sperm DNA; 50 µg/ml of tRNA; or 25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS

hybridization temperature:

T = 65 to 68°C
wash buffer: 0.1xSSC; 0.1% SDS
wash temperature: T = 65 to 68°C.

[0092] Nucleic acid molecules which hybridize with nucleic acid molecules coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT may originate from any organism; accordingly, they may originate from bacteria, fungi, animals, plants or viruses.

[0093] Nucleic acid molecules which hybridize with nucleic acid molecules coding for a protein having the activity of a GFAT-2 preferably originate from animals, particularly preferably from mammals and very particularly preferably from the mouse.

[0094] Nucleic acid molecules which hybridize with nucleic acid molecules coding for a protein having the activity of a bacterial GFAT preferably originate from bacteria or viruses, particularly preferably from *Escherichia coli*.

[0095] Nucleic acid molecules which hybridize with the molecules mentioned may be isolated, for example, from genomic or from cDNA libraries. Such nucleic acid molecules can be identified and isolated using the nucleic acid molecules mentioned or parts of these molecules or the reverse complements of these molecules, for example by hybridization according to standard methods (see, for example, Sambrook et al., Molecular Cloning, A Laboratory Manual, 3rd edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. ISBN: 0879695773; Ausubel et al., Short Protocols in Molecular Biology, John Wiley & Sons; 5th edition (2002), ISBN: 0471250929) or by amplification using PCR.

[0096] As hybridization sample for isolating a nucleic acid sequence coding for a protein having the activity of a GFAT-2, it is possible to use, for example, nucleic acid molecules having exactly or essentially the nucleic acid sequences described under SEQ ID NO 6 or fragments of these nucleic acid sequences. As hybridization sample for isolating a nucleic acid sequence coding for a protein having the activity of a bacteria, GFAT, it is possible to use, for example,

nucleic acid molecules having exactly or essentially the nucleic acid sequences described under SEQ ID NO 8 or fragments of these nucleic acid sequences.

[0097] The fragments used as hybridization samples may also be synthetic fragments or oligonucleotides prepared using the customary synthesis techniques, whose sequence is essentially identical to the nucleic acid molecule described in the context of the present invention. Once genes which hybridize with the nucleic acid sequences described in the context of the present invention are identified and isolated, the sequence should be determined and the properties of the proteins coded for by this sequence should be analyzed to determine whether they are proteins having the activity of a GFAT-2 or the activity of a bacterial GFAT. Methods of how to determine whether a protein has the activity of a protein having the activity of a GFAT-2 or having the activity of a bacterial GFAT are known to the person skilled in the art and described, inter alia, in the literature (bacterial GFAT: for example Deng et al., 2005, *Metabolic Engineering* 7, 201-214; Kornfeld, 1967, *J. Biol. Chem.* 242(13), 3135-3141; GFAT-2: for example Hu et al., 2004, *J. Biol. Chem.* 279 (29), 29988-29993). The molecules hybridizing with the nucleic acid molecules described in the context of the present invention comprise in particular fragments, derivatives and allelic variants of the nucleic acid molecules mentioned. In the context of the present invention, the term "derivative" means that the sequences of these molecules differ in one or more positions from the sequences of the nucleic acid molecules described above and are highly identical to these sequences. The differences to the nucleic acid molecules described above may, for example, be due to deletion (in particular deletion of N-and/or C-terminal regions), addition, substitution, insertion or recombination.

[0098] In the context of the present invention, the term "identity" means a sequence identity over the entire length of the coding region of a nucleic acid molecule or the entire length of an amino acid sequence coding for a protein of at least 60%, in particular an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90%, very particularly preferably of at least 95% and most preferably at least 98%. In the context of the present invention, the term "identity" is to be understood as meaning the number of identical amino acids/nucleotides (identity) with other proteins/nucleic acids, expressed in percent.

[0099] Preferably, the identity with respect to a protein having the activity of a GFAT-2 is determined by comparisons with the amino acid sequence given under SEQ ID NO 7 and the identity with respect to a nucleic acid molecule coding for a protein having the activity of a GFAT-2 as determined by comparisons of the nucleic acid sequence given under SEQ ID NO 6 with other proteins/nucleic acids with the aid of computer programs. Preferably, the identity with respect to a protein having the activity of a bacterial GFAT is determined by comparisons of the amino acid sequence given under SEQ ID NO 9 and the identity with respect to a nucleic acid molecule coding for a protein having the activity of a bacterial GFAT is determined by comparisons of the nucleic acid sequence given under SEQ ID NO 8 or SEQ ID NO 10 with other proteins/nucleic acids with the aid of computer programs. If sequences to be compared with one another are of different length, the identity is to be determined by determining the identity in percent of the number of amino acids/nucleotides which the shorter sequence shares with the longer sequence. Preferably, the identity is determined using the known and publicly available computer program ClustalW (Thompson et al., *Nucleic Acids Research* 22 (1994), 4673-4680). ClustalW is made publicly available by Julie Thompson (Thompson@EMBL-Heidelberg.DE) and Toby Gibson (Gibson@EMBL-Heidelberg.DE), European Molecular Biology Laboratory, Meyerhofstrasse 1, D 69117 Heidelberg, Germany. ClustalW can also be downloaded from various Internet pages, inter alia from IGBMC (Institut de Genetique et de Biologie Moléculaire et Cellulaire, B.P.163, 67404 Illkirch Cedex, France; <ftp://ftp-igbmc.u-strasbg.fr/pub/>) and from EBI (<ftp://ftp.ebi.ac.uk/pub/software/>) and all mirrored Internet pages of the EBI (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK).

[0100] Preferably, use is made of the ClustalW computer program of version 1.8 to determine the identity between proteins described in the context of the present invention and other proteins. Here, the parameters have to be set as follows: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3, GAOPEN=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET, ENDGAPS(OFF), NOPGAP, NOHGAP.

[0101] Preferably, use is made of the ClustalW computer program of version 1.8 to determine the identity for example between the nucleotide sequence of the nucleic acid molecules described in the context of the present invention and the nucleotide sequence of other nucleic acid molecules. Here, the parameters have to be set as follows: KTUPLE=2, TOPDIAGS=4, PAIRGAP=5, DNAMATRIX:IUB, GAOPEN=10, GAPEXT=5, MAXDIV=40, TRANSITIONS: unweighted.

[0102] Identity furthermore means that there is a functional and/or structural equivalence between the nucleic acid molecules in question or the proteins encoded by them. The nucleic acid molecules which are homologous to the molecules described above and represent derivatives of these molecules are generally variations of these molecules which represent modifications having the same biological function, i.e. coding for a protein having the activity of a GFAT-2 or the activity of a bacterial GFAT. They may be either naturally occurring variations, for example sequences from other species, or mutations, where these mutations may have occurred in a natural manner or were introduced by systematic mutagenesis. Furthermore, the variations may be synthetically produced sequences. The allelic variants may be either naturally occurring variants or synthetically produced variants or variants generated by recombinant DNA techniques. A special form of derivatives are, for example, nucleic acid molecules which differ from the nucleic acid

molecules described in the context of the present invention as a result of the degeneracy of the genetic code.

[0103] In a further preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where nucleic acid molecules coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT are characterized in that the codons of said nucleic acid molecules are different from the codons of the nucleic acid molecules which code for said protein having the activity of a GFAT-2 or said protein having the activity of a bacterial GFAT of the parent organism. Particularly preferably, the codons of the nucleic acid molecules coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT are changed such that they are adapted to the frequency of use of the codons of the plant cell or the plant into whose genome they are integrated or to be integrated.

[0104] As a result of the degeneracy of the genetic code, amino acids can be encoded by one or more codons. In different organisms, the codons coding for an amino acid are used at different frequencies. Adapting the codons of a coding nucleic acid sequence to the frequency of their use in the plant cell or in the plant into whose genome the sequence to be expressed is to be integrated may contribute to an increased amount of translated protein and/or to the stability of the mRNA in question in the particular plant cells or plants. The frequency of use of codons in the plant cells or plants in question can be determined by the person skilled in the art by examining as many coding nucleic acid sequences of the organism in question as possible for the frequency with which certain codons are used for coding a certain amino acid. The frequency of the use of codons of certain organisms is known to the person skilled in the art and can be determined in a simple and rapid manner using computer programs. Such computer programs are publicly accessible and provided for free *inter alia* on the Internet (for example <http://gcua.schoedl.de/>; <http://www.kazusa.or.jp/codon/>; <http://www.entelechon.com/eng/cutanalysis.html>). Adapting the codons of a coding nucleic acid sequence to the frequency of their use in the plant cell or in the plant into whose genome the sequence to be expressed is to be integrated can be carried out by *in vitro* mutagenesis or, preferably, by *de novo* synthesis of the gene sequence. Methods for the *de novo* synthesis of nucleic acid sequences are known to the person skilled in the art. A *de novo* synthesis can be carried out, for example, by initially synthesizing individual nucleic acid oligonucleotides, hybridizing these with oligonucleotides complementary thereto, so that they form a DNA double strand, and then ligating the individual double-stranded oligonucleotides such that the desired nucleic acid sequence is obtained. The *de novo* synthesis of nucleic acid sequences including the adaptation of the frequency with which the codons are used to a certain target organism can also be sourced out to companies offering this service (for example Entelechon GmbH, Regensburg, Germany).

[0105] All of the nucleic acid molecules mentioned are suitable for producing plant cells according to the invention or plants according to the invention.

[0106] The genetically modified plant cells according to the invention or the genetically modified plants according to the invention may, in principle, be plant cells and plants, respectively, of any plant species, i.e. both monocotyledonous and dicotyledonous plants. They are preferably crop plants, i.e. plants cultivated by man for the purpose of feeding man and animal or for producing biomass and/or for preparing substances for technical, industrial purposes. The genetically modified plant cells according to the invention or the genetically modified plants according to the invention are particularly preferably corn, rice, wheat, rye, oats, barley, manioc, potato, tomato, switchgrass (*Panicum virgatum*), sago, mung beans, peas, sorghum, carrots, eggplant, radish, oilseed rape, alfalfa, soybean, peanuts, cucumbers, pumpkins, melons, leek, garlic, cabbage, spinach, sweet potato, asparagus, zucchini, lettuce, artichokes, sweetcorn, parsnip, salsify, Jerusalem artichoke, banana, sugar beet, sugar cane, beetroot, broccoli, cabbage, onion, beet, dandelion, strawberry, apple, apricot, plum, peach, grapevines, cauliflower, celery, bell peppers, swede, rhubarb. They are preferably corn, rice, wheat, rye, oat or barley plants, very particularly preferably rice, tomato or potato plants.

[0107] In the context of the present invention, the term "potato plant" or "potato" is to be understood as meaning plant species of the genus *Solanum*, particularly tuber-producing species of the genus *Solanum* and in particular *Solanum tuberosum*.

[0108] In the context of the present invention, the term "tomato plant" or "tomato" is to be understood as meaning plant species of the genus *Lycopersicon*, in particular *Lycopersicon esculentum*.

[0109] In the context of the present invention, the term "rice plant" is to be understood as meaning plant species of the genus *Oryza*, in particular plant species of the genus *Oryza* agriculturally cultivated for commercial purposes, particularly preferably *Oryza sativa*.

[0110] As already discussed, plant cells according to the invention or plants according to the invention are suitable for producing glucosaminoglycans, such as, for example, chondroitin, hyaluronan, chitin, heparin (heparosan), since they contain a higher amount of substrates for the enzymes involved in the catalysis of the glucosaminoglycans mentioned.

[0111] Accordingly, the present invention furthermore relates to plant cells or plants synthesizing glucosaminoglycan, preferably at least 500 µg of glucosaminoglycan per gram of fresh weight, more preferably at least 1500 µg of glucosaminoglycan per gram of fresh weight, particularly preferably at least 3500 µg of glucosaminoglycan per gram of fresh weight, very particularly preferably at least 4000 µg of glucosaminoglycan per gram of fresh weight and especially preferably at least 5500 µg of glucosaminoglycan per gram of fresh weight. In this context, the glucosaminoglycan is preferably chondroitin, hyaluronan, chitin or heparin (heparosan), particularly preferably hyaluronan.

[0112] Plant cells according to the invention or plants according to the invention preferably have a glucosaminoglycan content of at most 25 000 μmol per gram of fresh weight, preferably at most 20 000 μmol per gram of fresh weight, particularly preferably at most 15 000 μmol per gram of fresh weight, very particularly preferably at most 10 000 μmol per gram of fresh weight, especially preferably at most 6500 μmol per gram of fresh weight.

[0113] Plant cells according to the invention or plants according to the invention which synthesize glucosaminoglycan can be produced, for example, by introducing foreign nucleic acid molecules coding for a protein having the activity of a GFAT and coding for a protein having the activity of a glucosaminoglycan synthase into a plant cell.

[0114] Accordingly, the present invention also relates to genetically modified plant cells or genetically modified plants containing a first foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or a bacterial GFAT and a second foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase.

[0115] In the context of the present invention, the term "protein having the activity of a glucosaminoglycan synthase" is to be understood as meaning a protein which uses UDP-GlcNAc or UDP-N-acetylgalactosamine, an epimer of UDP-GlcNAc, as substrate for synthesizing a glucosaminoglycan. The protein having the activity of a glucosaminoglycan synthase is preferably a hyaluronan synthase, chondroitin synthase, heparosan/heparin synthase, keratan synthase or chitin synthase.

[0116] Nucleic acid molecules and corresponding protein sequences coding for glucosaminoglycan synthases are known to the person skilled in the art and described as hyaluronan synthase for example from viruses (for example *Paramecium bursaria Chlorella Virus* 1, EMBL U42580.3, PB42580, US 20030235893), as chondroitin synthase for example from mammals (for example *Homo sapiens*, WO 03 012099, US 2005048604, US 2006052335), bacteria (for example *Escherichia coli*, US2003109693, EP 1283259, *Pasteurella multocida* US 2003104601), as chitin synthase for example from bacteria (for example *Azorhizobium caulinodans* EMBL CDS:AAB51164), from fungi (for example *Chaetomium globosum* EMBL CDS:EAQ92361, *Aspergillus nidulans* EMBL AB000125, *Arthroderma benhamiae* EMBL CDS:BAB32692, *Neurospora crassa* EMBL M73437.4), from insects (for example *Aedes aegypti* EMBL CDS:EAT46081, *Tribolium castaneum* EMBL CDS: AAQ55061), nematodes (for example *Dirofilaria immitis* EMBL AF288618, *Caenorhabditis elegans* EMBL AY874871), from viruses (for example *Chlorella virus* EMBL CDS: BAB83509, *Paramecium bursaria Chlorella virus* CVK2 EMBL CDS: BAE48153), as heparin/heparosan synthase for example from bacteria (for example *Pasteurella multocida* EMBL AF425591, AF439804, US 20030099967, *Escherichia coli* X77617.1).

[0117] The second foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase is preferably a recombinant nucleic acid molecule. Preferred embodiments of recombinant nucleic acid molecules have already been described and are to be used here in a corresponding manner.

[0118] In a further preferred embodiment, the second foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase is characterized in that the codons are modified compared to the codons of the nucleic acid molecule coding for said protein having the activity of a glucosaminoglycan synthase of the parent organism. Particularly preferably, the codons of the nucleic acid molecules coding for a protein having the activity of a glucosaminoglycan synthase are modified such that they are adapted to the frequency of use of the codons of the plant cell or the plant into whose genome they are integrated or to be integrated.

[0119] What was stated above for nucleic acid molecules coding for a protein having the activity of a GFAT-2 or a bacterial GFAT with respect to the modification of the codons of a nucleic acid molecule is to be applied here in a corresponding manner.

[0120] The present invention furthermore relates to plants containing plant cells according to the invention. Such plants may be generated by regeneration from plant cells according to the invention.

[0121] The present invention also relates to parts of plants according to the invention containing plant cells according to the invention.

[0122] In the context of the present invention, the term "plant parts" or "parts of plants" is to be understood as meaning, for example, processible plant parts used in the production of foodstuff or feedstuff, used as raw material source for industrial processes (for example for the isolation of glucosamine derivatives or glucosaminoglycans), as raw material source for the preparation of pharmaceuticals or as raw material source for the preparation of cosmetic products.

[0123] In the context of the present invention, the term "plant parts" or "parts of plants" is furthermore to be understood as meaning, for example, consumable plant parts which serve as food for man or which are used as animal feed.

[0124] Preferred "plant parts" or "parts of plants" are fruits, storage and other roots, flowers, buds, shoots, leaves or stalks, preferably seeds, fruits, grains or tubers.

[0125] The present invention also relates to propagation material of plants according to the invention. Preferably, propagation material according to the invention contains plant cells according to the invention, particularly preferably genetically modified plant cells according to the invention.

[0126] Here, the term "propagation material" comprises those components of the plant which are suitable for generating progeny via the vegetative or generative route. Suitable for vegetative propagation are, for example, cuttings, callus cultures, rhizomes or tubers. Other propagation material includes, for example, fruits, seeds, grains, seedlings, cell cultures, etc. The propagation material preferably takes the form of tubers, fruits, grains or seeds.

[0127] A further advantage of the present invention is the fact that parts of plants according to the invention have a higher content of N-acetylated glucosamine derivatives than known plants. Accordingly, plants according to the invention are particularly suitable for direct use as foodstuff/feedstuff or for preparing foodstuff/feedstuff having a prophylactic or therapeutic effect (for example for osteoarthritis prophylaxis). Since plants according to the invention have a higher content of N-acetylated glucosamine derivatives compared to known plants, the amounts of harvestable parts, propagation material, processible parts or consumable parts of plants according to the invention used for preparing foodstuff/feedstuff having an increased content of N-acetylated glucosamine derivatives can be reduced. If consumable parts of genetically modified plants according to the invention are consumed, for example, directly as so-called nutraceutical, a positive effect may be achieved even by the consumption of small amounts of substance. This may be of particular importance *inter alia* in the production of animal feed since animal feed with too high a content of plant components is unsuitable as feedstuff for various animal species. Furthermore, plant cells according to the invention or plants according to the invention have the advantage that they can also be used by vegans or for preparing kosher food. It is thus possible to administer food having an elevated content of N-acetylated glucosamines even to people following the ways of life mentioned.

[0128] It is known that N-acetylglucosamine has a stimulating effect on the growth of bifido bacteria (Liepke et al., 2002, Eur. J. Biochem. 269, 712-718). Furthermore, it has been shown that N-acetylglucosamine serves as a substrate for lactobacilli (for example *Lactobacillus casei* subspecies *paracasei*) from fish gut (Adolfo Bucio Galindo, 2004, Proefschrift, Wageningen Universiteit, ISBN 90-5808-943-6). Accordingly, N-acetylglucosamine has a positive effect on probiotic bacteria. Since plant cells according to the invention, plants according to the invention or parts of plants according to the invention have an elevated content of N-acetylglucosamine, they should consequently have a positive effect on the growth of probiotic bacteria and thus be suitable for use as a prebiotic foodstuff/feedstuff for man and animal.

[0129] The present invention furthermore relates to a process for producing a genetically modified plant which comprises the following steps:

- a) introduction of a foreign nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT) into a plant cell
- b) regeneration of a plant from plant cells obtained according to step a)
- c) if appropriate, generation of further plants with the aid of the plants according to step b).

[0130] The present invention furthermore relates to processes for producing a plant which synthesizes glucosaminoglycan, wherein

- a) a plant cell is genetically modified, where the genetic modification comprises the following steps i to ii in any order or carrying out any combinations of the following steps i to ii individually or simultaneously

- i) introducing a foreign nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT) into a plant cell
- ii) introducing a foreign nucleic acid molecule coding for a glucosaminoglycan synthase into a plant cell

- b) a plant is regenerated from plant cells comprising the genetic modification according to steps

- i) a) i
- ii) a) ii
- iii) a) i and a) ii,

- c) introducing into plant cells of plants according to step

- i) b) i a genetic modification according to step a) ii,
- ii) b) ii a genetic modification according to step a) i,

and regenerating a plant

- d) if appropriate, generating further plants with the aid of the plants obtained according to any of steps b) iii or c) i or c) ii.

[0131] With regard to the introduction of foreign nucleic acid molecules according to step a) of the process for producing a genetically modified plant or according to steps a) or c) of the process for producing a plant which synthesizes glucosaminoglycan into a plant cell, this introduction may, in principle, be any type of introduction of nucleic acid molecules

suitable for integrating a foreign nucleic acid molecule into a plant cell or plant. Such methods have already been described above and can be applied here in a corresponding manner.

[0132] With respect to the foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT according to step a) of the process for producing a genetically modified plant or with respect to the foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase according to step a) ii) of the process for producing a plant which synthesizes glucosaminoglycan, various possible embodiments of the respective nucleic acid molecules have already been described in the context with plant cells according to the invention and plants according to the invention. All these preferred embodiments which have already been described can also be used for carrying out the processes according to the invention mentioned.

[0133] The regeneration of the plants depending on the process according to step b) and/or c) of the processes according to the invention can be carried out using methods known to the person skilled in the art (described, for example, in "Plant Cell Culture Protocols", 1999, ed. by R.D. Hall, Humana Press, ISBN 0-89603-549-2).

[0134] The generation of further plants depending on the process according to the step c) or d) of the processes according to the invention can be carried out, for example, by vegetative propagation (for example via cuttings, tubers or via callus culture and regeneration of intact plants) or via generative propagation. In this context, generative propagation preferably takes place under controlled conditions, i.e. selected plants with specific characteristics are hybridized with one another and multiplied. The selection preferably takes place in such a manner that the plants, depending on the process according to step b) or d), have the modifications introduced in step a).

[0135] In a further preferred embodiment, processes according to the invention for producing a genetically modified plant are used for producing plants according to the invention.

[0136] The present invention also provides plants obtainable by processes according to the invention for preparing a genetically modified plant.

[0137] The present invention furthermore relates to a process for producing glucosaminoglycans which comprises the step of the extraction of glucosaminoglycans from genetically modified plant cells according to the invention, from genetically modified plants according to the invention, propagation material according to the invention, parts of plants according to the invention or plants obtainable by a process according to the invention for preparing a genetically modified plant which synthesizes glucosaminoglycan. The process according to the invention is preferably used for producing chondroitin, hyaluronan, chitin or heparin (heparosan), particularly preferably for producing hyaluronan.

[0138] Preferably, such a process also comprises the step of harvesting the cultivated genetically modified plant cells according to the invention, the genetically modified plants according to the invention, the propagation material according to the invention, the parts of plants according to the invention prior to the extraction of the glucosaminoglycan and particularly preferably furthermore the step of the cultivation of genetically modified plant cells according to the invention or genetically modified plants according to the invention prior to harvesting.

[0139] In contrast to bacterial or animal tissues, plant tissues do not contain any glucosaminoglycan-degrading enzymes. Accordingly, extraction of glucosaminoglycans from plant tissue is possible using relatively simple methods. If required, aqueous extracts of plant cells or tissues containing glucosaminoglycan can be purified further using methods known to the person skilled in the art, such as, for example, repeated precipitation with ethanol. A preferred method for purifying, for example, hyaluronan is described under General Methods Item 5.

[0140] The present invention also provides the use of genetically modified plant cells according to the invention, genetically modified plants according to the invention, propagation material according to the invention, parts of plants according to the invention or plants obtainable by a process according to the invention for producing a genetically modified plant which synthesizes glucosaminoglycan for producing glucosaminoglycans.

[0141] The present invention also provides the use of nucleic acid molecules coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT for preparing a genetically modified plant.

[0142] The present invention furthermore relates to a composition comprising genetically modified plant cells according to the invention.

[0143] Here, it is immaterial whether the plant cells are intact or no longer intact because they have been destroyed, for example, by processing. The compositions are preferably foodstuff, food supplements or feedstuff, pharmaceutical or cosmetic products.

[0144] The present invention preferably provides compositions according to the invention comprising recombinant nucleic acid molecules, the recombinant nucleic acid molecules being characterized in that they comprise nucleic acid molecules coding for a protein having the enzymatic activity of a GFAT-2 or a protein having the activity of a bacterial GFAT.

[0145] A stable integration of foreign nucleic acid molecules into the genome of a plant cell or plant results in the foreign nucleic acid molecules being flanked after integration into the genome of the plant cell or plant by genomic plant nucleic acid sequences. Accordingly, in a preferred embodiment, compositions according to the invention are characterized in that the recombinant nucleic acid molecules present in the composition according to the invention are flanked by genomic plant nucleic acid sequences.

[0146] Here, the genomic plant nucleic acid sequences may be any sequences naturally present in the genome of the

plant cell or plant used for preparing the composition.

[0147] That recombinant nucleic acid molecules which are present in the compositions according to the invention can be demonstrated using methods known to the person skilled in the art, such as, for example, methods based on hybridization or, preferably, methods based on PCR (Polymerase Chain Reaction).

[0148] Preferably, the compositions according to the invention comprise at least 0.05%, preferably at least 0.1%, particularly preferably at least 0.5%, very particularly preferably at least 1.0%, of N-acetylated glucosamine derivatives.

[0149] Preferably, the compositions according to the invention comprise at most 10%, preferably at most 5%, particularly preferably at most 3%, very particularly preferably at most 2%, of N-acetylated glucosamine derivatives.

[0150] Compositions according to the invention offer the advantage that they have an increased content of N-acetylated glucosamine derivatives or an increased content of glucosaminoglycans compared to compositions comprising not genetically modified plant cells. N-Acetylglucosamine has a stimulating effect on the growth of bifido bacteria (Liepke et al., 2002, Eur. J. Biochem. 269, 712-718). Furthermore, it has been shown that N-acetylglucosamine serves as substrate for lactobacilli (for example *Lactobacillus casei* subspecies *paracasei*) from fish gut (Adolfo Bucio Galindo, 2004, Proefschrift, Wageningen Universiteit, ISBN 90-5808-943-6). Accordingly, N-acetylglucosamine has a positive effect on probiotic bacteria. Since compositions according to the invention have increased N-acetylglucosamine contents, they should have a positive effect on the growth of probiotic bacteria.

[0151] The invention furthermore provides processes for preparing a composition according to the invention using plant cells according to the invention, plants according to the invention, propagation material according to the invention, parts of plants according to the invention or plants obtainable by a process according to the invention for producing a genetically modified plant. The processes for preparing a composition according to the invention are preferably processes for producing foodstuff, feedstuff or food supplements.

[0152] Processes for producing foodstuff, feedstuff, food supplements, pharmaceutical products or cosmetic products are known to the person skilled in the art and comprise *inter alia*, but are not exclusively limited to, the comminuting or the grinding of plants according to the invention or plant parts according to the invention.

[0153] The present invention also provides compositions obtainable by a process for preparing a composition according to the invention.

[0154] The present invention also relates to the use of genetically modified plant cells according to the invention or genetically modified plants according to the invention for preparing a composition according to the invention.

[0155] A preferred embodiment of compositions according to the invention are flours.

[0156] Parts of plants are frequently processed to flours. Examples of parts of plants which are used to prepare flours are, for example, tubers of potato plants and grains of cereal plants. To produce flours from cereal plants, the endosperm-containing grains of these plants are ground and sieved. In the case of other plants which do not contain any endosperm but, for example, tubers or storage roots, flour is often produced by comminuting, drying and subsequent grinding of the relevant parts of the plants. Plant cells according to the invention and plants according to the invention have an increased content of N-acetylated glucosamine derivatives or glucosaminoglycans compared to known plant cells or plants. Flours prepared from plant cells according to the invention, plants according to the invention, propagation material according to the invention or parts of plants according to the invention accordingly likewise contain an increased proportion of N-acetylated glucosamine derivatives or glucosaminoglycans.

[0157] Accordingly, the present invention furthermore relates to flours which obtainable from plant cells according to the invention, plants according to the invention or from parts of plants according to the invention. Preferred parts of plants according to the invention for producing flours are tubers and endosperm-containing grains. In the context of the present invention, particular preference is given to grains of plants of the (systematic) family Poaceae, especially preferably, the grains originate from corn, rice or wheat plants.

[0158] The present invention furthermore relates to flours according to the invention having a content of N-acetylated glucosamine derivatives of at least 10 μmol per gram, preferably at least 20 μmol per gram, more preferably at least 25 μmol per gram, particularly preferably at least 30 μmol per gram, very particularly preferably at least 35 μmol per gram and especially preferably at least 40 μmol per gram.

[0159] Flours according to the invention preferably have a content of N-acetylated glucosamine derivatives of at most 250 μmol per gram of fresh weight, preferably at most 200 μmol per gram of fresh weight, particularly preferably at most 150 μmol per gram of fresh weight, very particularly preferably at most 100 μmol per gram of fresh weight and especially preferably at most 50 μmol per gram of fresh weight.

[0160] In the context of the present invention, the term "flour" is to be understood as meaning a powder obtained by grinding plants or plant parts. If appropriate, the plants or plant parts are dried prior to grinding and, after grinding, further comminuted and/or sieved.

[0161] Compared to conventional flours, flours according to the invention have the advantage that they can be used for producing foodstuff, such as, for example, baked goods, having an increased content of N-acetylated glucosamine derivatives or glucosaminoglycans without it being necessary to add N-acetylated glucosamine derivatives or glucosaminoglycans obtained from animal or fungal raw material sources to the flour. The disadvantages of the use of N-

acetylated glucosamine derivatives or glucosaminoglycans isolated from the raw material sources mentioned, such as, for example, the risk that they may contain pathogens or allergenic substances, have already been mentioned further above.

[0162] The present invention furthermore provides a process for producing flours which comprises the step of grinding plant cells according to the invention, plants according to the invention or parts of plants according to the invention.

[0163] Flours can be produced by grinding parts of plants. It is known to the person skilled in the art how to produce flours. A process for producing flours preferably also comprises the step of harvesting the cultivated plants according to the invention or parts of plants according to the invention and/or the propagation material according to the invention and particularly preferably furthermore the step of the cultivation of plants according to the invention prior to harvesting.

[0164] In a further embodiment of the present invention, the process for producing flours comprises processing of plants according to the invention, of parts of plants according to the invention or of propagation material according to the invention. Here, processing may, for example, be heat treatment and/or drying. Heat treatment followed by drying of the heat-treated material is used, for example, when producing flours from storage roots, tubers such as, for example, from potato tubers, prior to grinding. Comminuting of plants according to the invention, of parts of plants according to the invention or of propagation material according to the invention may also constitute processing in the sense of the present invention. Removal of plant tissue, such as, for example, removal of the husk from the grains, prior to grinding also constitutes processing prior to grinding in the sense of the present invention.

[0165] In a further embodiment of the present invention, the process for producing flours comprises processing of the ground material after grinding.

[0166] Here, the ground material may, for example, be sieved after grinding, for example to produce different types of flour.

[0167] The present invention furthermore provides the use of plant cells according to the invention, plants according to the invention, of parts of plants according to the invention or of propagation material according to the invention for producing flours.

Description of the sequences

[0168]

- | | | |
|----|---------------|--|
| 30 | SEQ ID NO 1: | Nucleic acid sequence coding for a hyaluronan synthase of <i>Paramecium bursaria Chlorella Virus 1</i> . |
| | SEQ ID NO 2: | Amino acid sequence of a hyaluronan synthase of <i>Paramecium bursaria Chlorella Virus 1</i> . The amino acid sequence shown can be derived from SEQ ID NO 1. |
| 35 | SEQ ID NO 3: | Synthetic nucleic acid sequence coding for a hyaluronan synthase of <i>Paramecium bursaria Chlorella Virus 1</i> . The codons of the sequence shown were synthesized in a manner such that they are adapted to the use of codons in plant cells. The nucleic acid sequence shown codes for a protein having the amino acid sequence shown under SEQ ID NO 2. |
| | SEQ ID NO 4: | Nucleic acid sequence coding for a protein having the activity of a GFAT-1 from the mouse. |
| 40 | SEQ ID NO 5: | Amino acid sequence of a protein having the activity of a GFAT-1 from the mouse. The amino acid sequence shown can be derived from SEQ ID NO 4. |
| | SEQ ID NO 6: | Nucleic acid sequence coding for a protein having the activity of a GFAT-2 from the mouse. |
| 45 | SEQ ID NO 7: | Amino acid sequence of a protein having the activity of a GFAT-2 from the mouse. The amino acid sequence shown can be derived from SEQ ID NO 6. |
| | SEQ ID NO 8: | Nucleic acid sequence coding for a protein having the activity of a bacterial GFAT from <i>Escherichia coli</i> . |
| 50 | SEQ ID NO 9: | Amino acid sequence of a protein having the activity of a GFAT from <i>Escherichia coli</i> . The amino acid sequence shown can be derived from SEQ ID NO 8. |
| | SEQ ID NO 10: | Synthetic nucleic acid sequence coding for a protein having the activity of a GFAT from <i>Escherichia coli</i> . The codons of the sequence shown were synthesized in a manner such that they are adapted to the use of codons in plant cells. The nucleic acid sequence shown codes for a protein having the amino acid sequence shown under SEQ ID NO 9. |
| 55 | SEQ ID NO 11: | Nucleic acid sequence coding for a protein having the activity of a UDP-glucose dehydrogenase of <i>Paramecium bursaria Chlorella Virus 1</i> . |

(continued)

SEQ ID Amino acid sequence of a protein having the activity of a UDP-glucose dehydrogenase of *Paramecium*
 NO 12: *bursaria Chlorella* Virus 1. The amino acid sequence shown can be derived from SEQ ID NO 11.

5 SEQ ID Synthetic nucleic acid sequence coding for a protein having the activity of a UDP-glucose dehydrogenase
 NO 13: of *Paramecium bursaria Chlorella* Virus 1. The codons of the sequence shown were synthesized in a
 manner such that they are adapted to the use of codons in plant cells. The nucleic acid sequence shown
 codes for a protein having the amino acid sequence shown under SEQ ID NO 12.

SEQ ID Synthetic oligonucleotide which was used in example 6.

10 NO 14:

SEQ ID Synthetic oligonucleotide which was used in example 6.

NO 15:

SEQ ID Synthetic oligonucleotide which was used in example 15.

NO 16:

15 SEQ ID Synthetic oligonucleotide which was used in example 15.

NO 17:

20 **[0169]** The content of all cited publications including the accession numbers of nucleic acid molecules and amino acid
 sequences mentioned for sequence databases is incorporated by reference into the description of the application.

[0170] Methods which can be used in connection with the present invention are described below. These methods are
 specific embodiments; however, the present invention is not limited to these methods. It is known to the person skilled
 in the art that the invention can be carried out in the same manner by modifying the methods described and/or by
 replacing individual methods or parts of methods by alternative methods or alternative parts of methods.

25 General methods

1. Transformation of potato plants

30 **[0171]** Potato plants were transformed with the aid of *Agrobacterium*, as described in Rocha-Sosa et al. (EMBO J. 8,
 (1989), 23-29).

2. Transformation of tomato plants

35 **[0172]** Tomato plants were transformed with the aid of *Agrobacterium* according to the method described in US
 5,565,347.

3. Transformation of rice plants

40 **[0173]** Rice plants were transformed according to the method described by Hiei et al. (1994, Plant Journal 6(2),
 271-282).

4. Determination of the content of N-acetylated glucosamines

45 **[0174]** N-Acetylated glucosamine derivatives having a reducing end were determined similarly to the method of Elson
 and Morgan (1933, J Biochem. 27, 1824) and the improved colorimetric determination method of Reissig et al. (1955,
 Biol. Chem. 217, 959-966). The colorimetric determination method is based on a reaction of chromogen III (Mucken-
 schnabel et al., 1998, Cancer Letters 131, 13-20) with p-dimethylaminobenzaldehyde (DMAB, Ehrlich's reagent), yielding
 a red product whose concentration can be determined photometrically.

50 a) Work-up of the plant material

55 **[0175]** First, harvested plant material was comminuted. Depending on the amount of plant material used, comminution
 was carried out in a laboratory oscillating ball mill (MM200, from Retsch, Germany) for 30 seconds at 30 Hz or using a
 Warring blender at maximum speed for about 30 seconds. In general, 0.5 g of the comminuted plant material (for example
 leaf, tuber or rice grain) was mixed with 1 ml of a solution consisting of 7% perchloric acid, 5 mM EGTA and incubated
 on ice for 20 minutes. The mixture was then centrifuged (5 minutes at 16 000 x g, 4°C). The supernatant obtained after
 centrifugation was taken off and neutralized using a solution consisting of 5M KOH, 1 M TEA (adjusted pH 7.0) and then

centrifuged again (5 min at 16 000 x g, 4°C). After the end of the centrifugation, the supernatant was taken off, its volume was determined and the amount of N-acetylated glucosamine derivatives having a reducing end was determined using the method described under b).

b) Determination of the content of N-acetylated glucosamine derivatives having reducing ends

[0176] 20 µl of a solution consisting of 0.8M K₂B₄O₇, pH 9.6, are added to 100 µl of the plant extract obtained by the method described under a) and, after thorough mixing, heated at 95°C for 5 min. After cooling of the mixture to room temperature, 0.7 ml of Ehrlich's reagent (solution consisting of 10 g of DMAB in 12.5 ml of conc. HCl, 87.5 ml of glacial acetic acid, 1:10 diluted with glacial acetic acid) is added to the mixture, which is mixed again and incubated at 37°C for a further 30 minutes. The mixture is then centrifuged at 16 000 x g for 1 minute, and the optical density (OD) of the supernatant obtained after centrifugation is subsequently determined in a photometer at 585 nm.

c) Calculation of the concentration of N-acetylated glucosamine derivatives

[0177] First, a calibration curve was established using defined amounts of N-acetylglucosamine 6-phosphate. To this end, the OD of solutions comprising 0 mM, 0.1 mM, 0.5 mM, 1 mM, 5 mM and 10 mM of N-acetylglucosamine 6-phosphate was determined according to the method described under b).

[0178] The calibration curve was established in Microsoft Excel by fitting a second order polynomic trend/regression line of the formula $y = ax^2 + bx + c$ or $y = x^2 + px + q$ to the points measured for the individual concentrations. To calculate the values, the equation obtained was resolved for x, resulting in: $x = -p/2 - \text{square root}(p^2 / 4 - q)$, where $p = b / a$, $q = (c - y) / a$ and y is the measured OD of the unknown sample. Taking into account the fresh weight employed, the volume used and taking into account any dilution factor used, the contents were calculated in µmol (of the solution measured) or in µmol per g of fresh weight.

5. Isolation of glucosaminoglycans from plant tissue using the example of hyaluronan

[0179] To detect the presence of hyaluronan and to determine the hyaluronan content in plant tissue, plant material was worked up as follows: 200 µl of water (demineralized, conductivity = 18 MΩ) were added to about 0.3 g of leaf or tuber material, and the mixture was comminuted in a laboratory oscillating ball mill (MM200, from Retsch, Germany) (30 sec at 30 Hz). A further 800 µl of water (demineralized, conductivity = 18MΩ) were then added, and the mixture was mixed well (using, for example, a Vortex mixer). Cell debris and insoluble components were separated from the supernatant by centrifuging at 16 000 x g for 5 minutes. An aliquot of the supernatant obtained was used to determine the amount of hyaluronan.

[0180] In the case of tomato fruits, in each case a whole ripe tomato fruit was worked up. To this end, the weight of the tomato fruit was determined, the tomato was comminuted in a Warring blender with a little water, the comminuted sample was freed from cell debris by centrifugation at 3600 x g for 30 minutes and the volume of the extract was determined. An aliquot of the supernatant obtained was used to determine the amount of hyaluronan.

6. Purification of glucosaminoglycans using the example of hyaluronan

[0181] After addition of 100 ml of water (demineralized, conductivity = 18 MΩ), about 100 grams of plant material were comminuted in a Warring blender at maximum speed for about 30 seconds. If relatively large parts of plants, such as, for example, tubers or tomato fruits, were used for isolation, they were cut beforehand into pieces of a size of about 1 cm³. The cell debris was then removed using a tea sieve. The cell debris which had been separated off was once more suspended in 300 ml of water (demineralized, conductivity = 18 MΩ) and again removed using a tea sieve. The two suspensions obtained (100 ml + 300 ml) were combined and centrifuged at 13 000 x g for 15 minutes. NaCl was added to the centrifugation supernatant obtained until a final concentration of 1% had been reached. After the NaCl had gone into solution, precipitation was carried out by addition of twice the volume of ethanol followed by thorough mixing and incubation at -20°C overnight. The mixture was then centrifuged at 13 000 x g for 15 minutes. The sedimented precipitate obtained after this centrifugation was dissolved in 100 ml of buffer (50 mM TrisHCl, pH 8, 1 mM CaCl₂) and proteinase K was then added to a final concentration of 100 µg/ml and the solution was incubated at 42°C for 2 hours. This was followed by 10 minutes of incubation at 95°C. Once more, NaCl was added to this solution until a final concentration of 1% had been reached. After the NaCl had gone into solution, another precipitation was carried out by addition of twice the volume of ethanol, thorough mixing and incubation at -20°C for about 96 hours. This was followed by 15 minutes of centrifugation at 13 000 x g. The sedimented precipitate obtained after this centrifugation was dissolved in 30 ml of water (demineralized, conductivity = 18 MΩ), and once more, NaCl was added to a final concentration of 1%. By adding twice the volume of ethanol, thorough mixing and incubation at -20°C overnight, another precipitation was carried out. The

precipitate obtained after subsequent centrifugation at 13 000 x g for 15 minutes was dissolved in 20 ml of water (demineralized, conductivity = 18 MΩ).

[0182] Further purification was carried out by centrifugal filtration. To this end, in each case 5 ml of the dissolved precipitate were applied to a membrane filter (CentriconAmicon, pore width 10 000 NMWL, Prod. No. UCF8 010 96), and the sample was centrifuged at 2200 x g until only about 3 ml of the solution above the filter remained. Two more times, in each case 3 ml of water (demineralized, conductivity = 18 MΩ) were then added to the solution above the membrane and in each case re-centrifuged under identical conditions until, at the end, only about 3 ml of the solution above the filter remained. The solutions still present above the membrane after centrifugal filtration were taken off, and the membrane was rinsed repeatedly (three to five times) with about 1.5 ml of water (demineralized, conductivity = 18 MΩ). All solutions which were still present above the membrane and the solutions obtained from rinsing were combined, NaCl was added to a final concentration of 1%, after the NaCl had gone into solution, twice the volume of ethanol was added, the sample was mixed and a precipitate was obtained by storage at -20°C overnight. The precipitate obtained after subsequent centrifugation at 13 000 x g for 15 minutes was dissolved in 4 ml of water (demineralized, conductivity = 18 MΩ) and then freeze-dried (24 hours under a pressure of 0.37 mbar, freeze drying apparatus Christ Alpha 1-4, from Christ, Osterode, Germany).

7. Detection of hyaluronan and determination of the hyaluronan content

[0183] Hyaluronan was detected using a commercial test (Hyaluronic Acid (HA) test kit from Corgenix, Inc., Colorado, USA, Prod. No. 029-001) according to the instructions of the manufacturer which are herewith incorporated into the description by way of reference. The test principle is based on the availability of a protein which binds specifically to hyaluronan (HABP) and is carried out similarly to an ELISA, where a color reaction indicates the hyaluronan content in the sample examined. Accordingly, for the quantitative determination of hyaluronan, the samples to be measured should be employed in a concentration such that it is within the stated limits (for example: dilution of the sample in question or use of less water for extracting hyaluronan from plant tissue, depending on whether a limit was exceeded or not reached).

8. Determination of the activity of a GFAT

[0184] The activity of a protein having the activity of a GFAT is determined as described in Rachel et al. (1996, J. Bacteriol. 178 (8), 2320-2327).

[0185] To distinguish whether a protein has the activity of a GFAT-1 or GFAT-2, the method described in Hu et al. (2004, J. Biol. Chem. 279 (29), 29988-29993) is used.

9. Detection of N-acetylated glucosamine derivatives by mass spectroscopy

[0186] To detect N-acetylated glucosamine derivatives by mass spectroscopy, plant tissue was worked up as under General Methods Item 4 a). To obtain an extract as free of salt as possible, the respective samples were, prior to the examination by mass spectroscopy, initially frozen at -20°C and thawed during centrifugation (16 000 x g at room temperature). For the measurement, the supernatant was diluted 1:20 with a methanol:water mixture in a ratio of 1:1 (volume/volume).

[0187] To increase the detection sensitivity for weak signals (peaks), MS spectra with three different detector sensitivities were recorded. However, in this case the response of the detector is no longer linear, which is noted when the signal intensities (peak areas) of different metabolites are compared and which should be taken into account. To ensure that the measurements can be compared with one another, it was ensured that the individual samples gave identical signal intensities (in cps, counts per second) at the same detector setting.

[0188] The areas of the resulting signals (peak areas) assigned to the different metabolites are stated relatively to the peak area of hexoses (m/z=179) in %. The ratio of the signal intensities (peak areas) in different samples can be used to infer the concentration ratios of the corresponding N-acetylated glucosamine derivatives in relation to the concentration of hexoses in the sample in question.

[0189] MS-MS measurements of the individual samples and of individual corresponding reference substances (glucosamine, N-acetyl glucosamine, glucosamine 6-phosphate glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 1-phosphate, UDP-N-acetylglucosamine) were carried out in parallel. In this way, it is possible to assess whether the signal (peak) used for determining the area is a signal generated exclusively by a specific metabolite or by specific isomeric metabolites having the same mass, or whether the signal in question can be assigned only partially to the corresponding metabolite or the corresponding specific isomeric metabolites having the same mass.

[0190] MS and MS-MS spectra were recorded in the negative mode using a Q-STAR Pulsar i hybrid mass spectrometer from Applied Biosystems fitted with a nano-electrospray source. The ions detected were mainly deprotonated ions with a single charge.

[0191] The measurements were carried out under the following conditions:

Mass range	50-700 Da.
Detector sensitivity:	2000, 2050 and 2100.

[0192] For each of the three detector settings, it was ensured that the samples had similar signal intensities (in cps, counts per second).

Examples

1. Acquisition of nucleic acid sequences coding for a protein having the activity of a GFAT-1 from the mouse

[0193] The nucleic acid sequence coding for a protein having the activity of a GFAT-1 (glutamine:fructose 6-phosphate amidotransferase or glucosamine 6-phosphate synthase, EC 2.6.1.16) was purchased from BioCat GmbH, Heidelberg, Germany (Art. No. MMM1013-65346, cDNA clone MGC:58262, IMAGE:6742987). This is a clone which is produced by the I.M.A.G.E. consortium (<http://image.llnl.gov>) and distributed by BioCat GmbH. The cDNA coding for a protein having the activity of a GFAT-1 was cloned into the vector pCMV Sport 6 from Invitrogen. The plasmid was named IC 365-256. The nucleic acid sequence coding for the protein having the activity of a GFAT-1 from *Mus musculus* is shown under SEQ ID NO 4.

[0194] To facilitate subsequent cloning steps, the coding sequence of the GFAT-1 was excised using *Xho* I and *Eco* RV from the plasmid IC 365-256 and cloned into the plasmid pME9, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 367-256.

[0195] The plasmid pME9 is a pBlueSkript vector from Stratagene (Prod. No. 212207) where, in contrast to the pBlueSkript vector mentioned, pME9 contains a modified Multiple Cloning Site (MCS) which, in addition to the MCS present in the pBlueSkript vector, has an additional *Pac* I restriction site at both ends of the MCS.

2. Acquisition of a nucleic acid sequence coding for a protein having the activity of a GFAT-2 from a mouse

[0196] The nucleic acid sequence coding for a protein having the activity of a GFAT-2 (glutamine:fructose 6-phosphate amidotransferase or glucosamine 6-phosphate synthase, EC 2.6.1.16) was purchased from Invitrogen (Clone ID 4167189, cDNA clone MGC:18324, IMAGE:4167189). This is a clone produced by the I.M.A.G.E. consortium (<http://image.llnl.gov>) and distributed by Invitrogen. The cDNA coding for a protein having the activity of a GFAT-2 was cloned into the vector pCMV Sport 6 from Invitrogen. The plasmid was named IC 369-256. The nucleic acid sequence coding for the protein having the activity of a GFAT-2 from *Mus musculus* is shown under SEQ ID NO 6.

3. Synthesis of the nucleic acid sequences coding for a protein having the activity of a bacterial GFAT from *Escherichia coli*

[0197] The nucleic acid sequence coding for a protein having the activity of a bacterial GFAT (glutamine:fructose 6-phosphate amidotransferase or glucosamine 6-phosphate synthase, *glms*, EC 2.6.1.16) from *Escherichia coli* was synthesized by Entelechon GmbH and cloned into the vector pCR4Topo from Invitrogen (Prod. No. K4510-20). The plasmid obtained was named IC 373-256. The synthetic nucleic acid sequence coding for the protein having the activity of a bacterial GFAT from *Escherichia coli* is shown under SEQ ID NO 10. The corresponding nucleic acid sequence originally isolated from *Escherichia coli* is shown under SEQ ID NO 8.

4. Synthesis of nucleic acid molecules coding for a hyaluronan synthase of *Paramecium bursaria Chlorella* Virus 1

[0198] The nucleic acid sequence coding for a hyaluronan synthase of *Paramecium bursaria Chlorella* Virus 1 was synthesized by Medigenomix GmbH (Munich, Germany) and cloned into the vector pCR2.1 from Invitrogen (Prod. No. K2000-01). The plasmid obtained was named IC 323-215. The synthetic nucleic acid sequence coding for the HAS protein from *Paramecium bursaria Chlorella* Virus 1 is shown under SEQ ID NO 3. The corresponding nucleic acid sequence originally isolated from *Paramecium bursaria Chlorella* Virus 1 is shown under SEQ ID NO 1.

5. Synthesis of nucleic acid molecules coding for a protein having the activity of a UDP-glucose dehydrogenase of *Paramecium bursaria Chlorella* Virus 1

[0199] The nucleic acid sequence coding for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella* Virus 1 was synthesized by Entelechon GmbH and cloned into the vector pCR4Topo

from Invitrogen (Prod. No. K4510-20). The plasmid obtained was named IC 339-222. The synthetic nucleic acid sequence coding for the protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1* is shown under SEQ ID NO 13. The corresponding nucleic acid sequence originally isolated from *Paramecium bursaria Chlorella Virus 1* is shown under SEQ 10 NO 11

6. Preparation of the plant expression vector IR 47-71

[0200] The plasmid pBinAR is a derivative of the binary vector plasmid pBin19 (Bevan, 1984, Nucl Acids Res 12: 8711-8721) which was constructed as follows:

A 529 bp fragment comprising the nucleotides 6909-7437 of the 35S promoter of the cauliflower mosaic virus was isolated as *EcoR I/Kpn I* fragment from the plasmid pDH51 (Pietrzak et al, 1986 Nucleic Acids Res. 14, 5858) and ligated between the *EcoR I* and *Kpn I* restriction sites of the polylinker of pUC18. This gave the plasmid pUC18-35S. With the aid of the restriction endonucleases *Hind III* and *Pvu II*, a 192 bp fragment comprising the polyadenylation signal (3'-terminus) of the *octopine synthase* gene (Gen 3) of the T-DNA of the Ti plasmid pTiACH5 (Gielen et al, 1984, EMBO Journal 3, 835-846) (nucleotides 11749-11939) was isolated from the plasmid pAGV40 (Herrera-Estrella et al, 1983 Nature, 303, 209-213). After addition of *Sph I* linkers to the *Pvu II* restriction site, the fragment was ligated between the *Sph I* and *Hind III* restriction sites of pUC18-35S. This gave the plasmid pA7. From this plasmid, the entire polylinker comprising the 35S promoter and the OCS terminator was excised with *EcoR I* and *Hind III* and ligated into the appropriately cut vector pBin19. This gave the plant expression vector pBinAR (Hofgen and Willmitzer, 1990, Plant Science 66, 221-230).

[0201] The promoter of the Patatin gene B33 from *Solanum tuberosum* (Rocha-Sosa et al., 1989, EMBO J. 8, 23-29) was ligated as *Dra I* fragment (nucleotides -1512 - +14) into the vector pUC19, which had been cut with *Sst I* and whose ends had been blunted with the aid of T4-DNA polymerase. This gave the plasmid pUC19-B33. Using *EcoR I* and *Sma I*, the B33 promoter was excised from this plasmid and ligated into the appropriately cut vector pBinAR. This gave the plant expression vector pBinB33.

[0202] To facilitate further cloning steps, the MCS (Multiple Cloning Site) was widened. To this end, two complementary oligonucleotides were synthesized, heated at 95°C for 5 minutes and slowly cooled to room temperature, and the double-stranded fragment obtained was cloned into the *Sal I* and *Kpn I* restriction sites of pBinB33. The oligonucleotides used for this purpose had the following sequence:

5'-TCG ACA GGC CTG GAT CCT TAA TTA AAC TAG TCT CGA GGA GCT CGG
TAC-3'

5'-CGA GCT CCT CGA GAC TAG TTT AAT TAA GGA TCC AGG CCT G-3'

[0203] The plasmid obtained was named IR 47-71.

7. Preparation of the plant expression vector pBinARHyg

[0204] Using the restriction endonucleases *EcoR I* and *Hind III*, the fragment comprising the 35S promoter, the OCS terminator and the entire multiple cloning site was excised from the plasmid pA7 and cloned into the vector pBIBHyg (Becker, 1990, Nucleic Acids Res. 18, 203) which had been cut with the same restriction endonucleases. The plasmid obtained was named pBinARHyg.

8. Preparation of the cloning vector IC 317-204

[0205] Nucleic acid fragments comprising the OCS terminator were isolated from the plasmid IR 47-71 using the restriction endonucleases *Xho I* and *Hind III* and cloned into the vector pBlueScript KS (from Stratagene, Prod. No. 212207), which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 306-204.

[0206] Nucleic acid fragments comprising the B33 promoter were isolated from the plasmid IR 47-71 using the restriction endonucleases *Bam HI* and *Eco RI* and cloned into the vector pBlueScript KS (from Stratagene, Prod. No. 212207), which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 314-204. From IC 306-204, the OCS terminator was isolated using the restriction endonuclease *Bam HI* and cloned into the plasmid IC 314-204, which had been cut with the same restriction endonuclease. The plasmid obtained was named IC 317-204.

9. Preparation of the plant expression vector IC 341-222 comprising a coding nucleic acid sequence for a hyaluronan synthase of *Paramecium bursaria Chlorella Virus 1*

[0207] By restriction digestion with *BamH* I and *Xho* I, nucleic acid molecules comprising the coding sequence of hyaluronan synthase were isolated from the plasmid IC 323-215 and cloned into the *BamH* I and *Xho* I restriction sites of the plasmid IR 47-71. The plant expression vector obtained was named IC 341-222.

10. Preparation of the plant expression vectors 349-222 comprising coding nucleic acid sequences for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1*

[0208] Using restriction digestion with *BamH* I and *Kpn* I, nucleic acid molecules comprising the coding sequence for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1* were isolated from the plasmid IC 339-222 and cloned into the plasmid pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 342-222.

[0209] By restriction digestion with *Xba* I and *Kpn* I, nucleic acid molecules comprising the coding sequence for a protein having the activity a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1* were isolated from the plasmid IC 342-222 and cloned into the expression vector-pBinAR Hyg, which had been cut with *Xba* I and *Kpn* I. The plasmid obtained was named IC 349-222.

11. Preparation of the plant expression vectors IC 376-271 comprising coding nucleic acid sequences for a protein having the activity of a GFAT-1 from the mouse and for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1*.

[0210] A nucleic acid fragment comprising the B33 promoter and the OCS terminator, which fragment had been isolated from IC 317-204 by restriction digestion using *Eco* RI, was cloned into the *Eco* RI restriction site of the plasmid IC 349-222. Here, head-to-head orientation of the promoters (25S and B33) was ensured. The vector obtained was named IC 354-222.

[0211] To obtain a plant expression vector comprising a nucleic acid sequence coding for a protein having the activity of a GFAT-1 from the mouse, the coding sequence of the protein having the activity of a GFAT-1 from the mouse was isolated by restriction digestion with *Xho* I and *Eco* RV from IC 365-256 and cloned into the plasmid IC 354-222, which had been cut with *Xho* I and *Ecl*136 II. The plant expression vector obtained was named IC 376-256.

12. Preparation of the plant expression vector IC 372-256 comprising coding nucleic acid sequences for a protein having the activity of a GFAT-2 from the mouse and for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1*

[0212] A nucleic acid fragment comprising the coding sequence of the protein having the activity of a GFAT-2 from the mouse was isolated from IC 369-256 by restriction digestion with *Xho* I and *Eco* RV and cloned into the plasmid IC 354-222, which had been cut with *Xho* I and *Ecl*136 II. The plant expression vector obtained was named IC 372-256.

13. Preparation of the plant expression vector 375-271 comprising coding nucleic acid sequences for a protein having the activity of a GFAT from *Escherichia coli* and for a protein having the activity of a UDP-glucose dehydrogenase from *Paramecium bursaria Chlorella Virus 1*

[0213] A nucleic acid fragment comprising the coding sequence of the protein having the activity of a GFAT from *Escherichia coli* was isolated from IC 373-256 by restriction digestion with *Xho* I and *Eco* RV and cloned into the plasmid IC 354-222, which had been cut with *Xho* I and *Ecl*136 II. The plant expression vector obtained was named IC 375-271.

14. Preparation of the plant expression vector IC 398-311 comprising a coding nucleic acid sequence for a protein having the activity of a GFAT from *Escherichia coli*

[0214] By restriction digestion with *Ecl* 136 I and *Xho* I, the coding sequence of the protein having the activity of a bacterial GFAT from *E. coli* was isolated from the plasmid IC 373-256 and ligated into the *Sma* I and *Sal* I restriction sites of the vector pBinAR Hyg. The plant expression vector obtained was named IC 398-311.

15. Preparation of the plant expression vector IC 386-299

[0215] By PCR using genomic DNA isolated from leaves of rice (*Oryza sativa*, cultivar M202) using DNA polymerase

(Expand High Fidelity PCR Systems, Roche Prod. No.: 1732641), the DNA of the prolamin promoter from rice (EMBL Accession NO D63901, Sha et al., 1996, Biosci. Biotech. Biochem. 60, 335 - 337, Wu et al., 1998, Plant Cell Physiol. 39(8), 885 - 889) was isolated. The amplicon obtained from this PCR reaction was cloned into the vector pCR 2.1 using the TA cloning kit (Invitrogen Prod. No.: KNM2040-01). The plasmid obtained was named MI 4-154. Conditions used for the amplification of the DNA coding for the prolamin promoter: The conditions and buffers stated by the manufacturer and 50 ng of total DNA were used.

0.83 μ M dNTP mix

0.25 μ M Primer prol-F1

5'-AAAACTAGTTCTACATCGGCTTAGGTGTAGCAACACG

0.25 μ M primer prol-R1

5'-AAAAGATATCTGTTGTTGGATTCTACTACTATGCTTCAA

Reaction conditions:

[0216]

Step 1	15 sec
Step 2	60°C 15 sec
Step 3	72°C 45 sec

[0217] First, the reaction according to steps 1 to 3 was carried out using 35 repetitions (cycles). After the reaction had ended, the reaction mixture was cooled to 4°C. Subsequent cloning into the vector pCR 2.1 using the TA cloning kit (Invitrogen Prod. No.: KNM2040-01) was carried out following the conditions stated by the manufacturer. The plasmid comprising the prolamin promoter from rice was named MI 4-154.

[0218] A nucleic acid fragment comprising the coding sequence of the protein having the activity of a GFAT-2 from the mouse was isolated by restriction digestion using the restriction endonucleases Not I and *Kpn* I from the plasmid IC 369-256 and cloned into the vector pMCS5 (purchased from MoBiTec), which had been digested with *Not* I and *Kpn* I. The plasmid obtained was named IC 385-299. In the next step, the nucleic acid fragment comprising the coding sequence of the protein having the activity of a GFAT-2 from the mouse was isolated by restriction digestion with the restriction endonucleases *Xho* I and *Hpa* I from IC 385-299 and cloned into the plasmid MI 9-154, which had been cut with *Xho* I and *Ecl*136 II. The plant expression vector obtained was named IC 386-299. Starting vector for the preparation of the vector MI 9-154 is the plasmid ML 18-56 (WO 05/030941). An MCS synthesized by two oligonucleotides and having the appropriate sticky ends and comprising the restriction sites *Pst* I, *Sac* I, *Bln* I, *Xho* I, *Hpa* I, *Spe* I and *Hind* III was introduced into the plasmid ML 18-56, which had been digested with *Hind* III and *Pst* I. The vector obtained was named MI 8-154.

[0219] By digestion with *Eco* RV and *Spe* I, the prolamin promoter was isolated from MI 4-154 and ligated into the vector MI 8-154, which had been digested with *Hpa* I and *Spe* I. The vector obtained was named MI 9-154.

16. Potato plants comprising a nucleic acid molecule coding for a protein having the activity of a bacterial GFAT

a) Transformation of potato plants

[0220] Potato plants (cultivar Désirée) were transformed by the method given in General Methods Item 1 using the plant expression vector IC 398-311, which comprises a coding nucleic acid sequence for a protein having the activity of a bacterial GFAT from *Escherichia coli* under the control of the promoter of the patatin gene B33 from *Solanum tuberosum* (Rocha-Sosa et al., 1989, EMBO J. 8, 23-29). The transgenic lines obtained, which are transformed with the plasmid IC 398-311, were named 432 ES.

b) Analysis of lines 432 ES

[0221] Plants of the line 432 ES were cultivated in a greenhouse in soil in 6 cm pots. In each case about 0.3 g to 0.8 g of leaf material, harvested from individual plants, was worked up according to the method described under General Methods Item 4, and the content of N-acetylated glucosamine derivatives was determined. For individual plants having an increased content of N-acetylated glucosamine derivatives, the following results were obtained:

Table 1: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in leaves of independent transgenic plants of the line 432 ES. Column 1 refers in each case to the plant, independently obtained from the transformation, from which the material was harvested ("wt" refers to plants which have not been transformed).

5	Plant	$\mu\text{mol/g FW}$	Plant	$\mu\text{mol/g FW}$
	432ES 1	2.97	432ES 25	4.45
	432ES 2	0.51	432ES 26	1.81
10	432ES 4	2.19	432ES 27	1.75
	432ES 5	3.99	432ES 28	0.45
	432ES 6	6.20	432ES 32	4.56
	432ES 7	2.98	432ES 33	3.64
15	432ES 8	0.48	432ES 35	3.64
	432ES 9	11.48	432ES 37	6.67
	432ES 10	0.30	432ES 38	0.95
20	432ES 11	6.89	432ES 40	8.69
	432ES 12	5.45	432ES 42	1.47
	432ES 13	0.23	432ES 43	5.41
	432ES 14	0.80	432ES 44	6.33
25	432ES 15	1.75	432ES 45	3.39
	432ES 16	4.87	wt 1	0.05
	432ES 18	3.38	wt 2	0.26
30	432ES 19	6.38	wt 3	0.17
	432ES 21	1.42		
	432ES 22	9.73		
35	432ES 23	5.88		

[0222] These results show that plants having a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT have a considerably higher content of N-acetylated glucosamine derivatives than correspondingly non-transformed wild-type plants.

17. Rice plants comprising a nucleic acid molecule coding for a protein having the activity of a GFAT-2

a) Transformation of rice plants

[0223] Rice plants (variety M202) were transformed according to the method given under General Methods Item 3 with the plant expression vector IC 386-299, which comprises a coding nucleic acid sequence for a protein having the activity of a GFAT-2 from the mouse under the control of the promoter of the 13 kDa prolamin polypeptide. The transgenic lines obtained, which are transformed with the plasmid IC 386-299, were named GAOS0788.

b) Analysis of the lines GAOS0788

[0224] Independent plants, originating from the transformation with the plasmid IC 386-299, of the line GAOS0788 were cultivated in soil in a greenhouse. From each plant, about 20-25 ripe seeds (grains) were harvested, the husks were removed with a dehusker (Laboratory Paddy sheller, Grainman, Miami, Florida, USA) and about 7 brown rice seeds (pools) of each line were comminuted in a laboratory oscillating ball mill (MM200, from Retsch, Germany, 30 sec at 30 Hz), resulting in a flour. Using the method described under General Methods Item 4, the content of N-acetylated glucosamine derivatives was then determined. For individual plants having an increased content of N-acetylated glucosamine derivatives, the following results were obtained:

Table 2: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in pools of ripe seeds of independent transgenic plants of the line GAOS0788. Column 1 refers to the plant, independently obtained from the transformation, from which material was harvested (here, "control" refers to plants transformed with a plasmid having no nucleic acid molecule coding for a protein having the activity of a GFAT. Non-detectable amounts are marked "n.d.".

Plant	$\mu\text{mol/g FW}$	Plant	$\mu\text{mol/g FW}$
GAOS0788-00101	14.50	GAOS0788-02301	7.82
GAOS0788-00202	17.36	GAOS0788-02401	20.89
GAOS0788-00301	14.46	GAOS0788-02501	6.67
GAOS0788-00501	23.07	GAOS0788-02601	7.34
GAOS0788-00602	7.75	GAOS0788-02701	4.31
GAOS0788-00701	4.44	GAOS0788-02802	8.02
GAOS0788-00802	17.43	GAOS0788-02901	4.74
GAOS0788-00901	10.13	GAOS0788-03001	4.36
GAOS0788-01001	6.38	GAOS0788-03101	1183
GAOS0788-01202	8.32	GAOS0788-03202	2.76
GAOS0788-01401	8.64	GAOS0788-03302	12.82
GAOS0788-01502	2.97	Control	n.d.
GAOS0788-01602	8.15	Control	n.d.
GAOS0788-01701	16.50		
GAOS0788-02002	5.65		
GAOS0788-02202	5.15		

c) Analysis of individual seeds of the plants GAOS0788-02401 and GAOS0788-00501

[0225] The seeds harvested in Example b) originated from plants obtained directly after transformation, which plants were thus heterozygotic with respect to the respective integration sites of the T-DNAs in question. Accordingly, as a result of Mendel's laws of inheritance, the seed pools analyzed contained seeds comprising various amounts of the T-DNAs in question, it also being possible for individual seeds not having any T-DNAs integrated by transformation to be present in the respective pools. Thus, single, individual brown seeds from the plants of the line GAOS0788-02401 and plants of the line GAOS0788-00501 were each examined by the method described under General Methods Item 4 for their content of N-acetylated glucosamine derivatives. The following results were obtained:

Table 3: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) of individual seeds of the plants from lines GAOS0788-02401 and GAOS0788-00501. In each case, column 1 refers to the plant, independently obtained from the transformation, from which individual seeds were harvested and analyzed (here, "control" refers to seeds of plants transformed with a construct comprising no nucleic acid molecule coding for a protein having the activity of a GFAT). Non-detectable amounts are marked "n.d.".

Sample	$\mu\text{mol/g FW}$
GAOS0788-02401 seed 1	n.d
GAOS0788-02401 seed 2	22.41
GAOS0788-02401 seed 3	38.47
GAOS0788-02401 seed 4	16.57
GAOS0788-02401 seed 5	17.67
GAOS0788-02401 seed 6	3.79

(continued)

Sample	$\mu\text{mol/g FW}$
GAOS0788-02401 seed 7	10.14
GAOS0788-02401 seed 8	18.70
GAOS0788-00501 seed 1	n.d
GAOS0788-00501 seed 2	17.20
GAOS0788-00501 seed 3	19.89
GAOS0788-00501 seed 4	15.47
GAOS0788-00501 seed 5	9.31
GAOS0788-00501 seed 6	20.88
GAOS0788-00501 seed 7	25.31
GAOS0788-00501 seed 8	31.92
GAOS0788-00501 seed 9	28.82
GAOS0788-00501 seed 10	43.35
Control seed 1	n.d
Control seed 2	n.d
Control seed 3	n.d
Control seed 4	n.d

[0226] The results obtained show that flours from seeds (grains) of rice plants having a nucleic acid molecule coding for a protein having the activity of a GFAT-2 have a considerably higher content of N-acetylated glucosamine derivatives compared to flours produced from plants having no nucleic acid molecule coding for a protein having the activity of a GFAT-2.

18. Synthesis of N-acetylated glucosamine derivatives in tomato plants transformed with nucleic acid molecules coding for various isoforms of a protein having the activity of a GFAT

a) Production of tomato plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1

[0227] Tomato plants (cultivar MoneyMaker) were transformed by the method given under General Methods Item 2 with the plant expression vector IC 376-271, which comprises a coding nucleic acid sequence for a protein having the activity of a UDP-glucose dehydrogenase and a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1. The transgenic lines obtained, which are transformed with the plasmid 376-271, were named 420 ES. Proteins having the activity of a UDP-glucose dehydrogenase catalyze the synthesis of UDP-GlcA from UDP-glucose. In addition to GlcNAc, some glucosaminoglycan synthases, such as, for example, hyaluronan synthase, require UDP-GlcA as substrate.

b) Analysis of the lines 420 ES

[0228] Plants of the line 420 ES were cultivated in hydroculture in pots in a greenhouse. In each case about 5 g of plant material, harvested from individual plants, were worked up using the method described under General Methods Item 4, and the content of N-acetylated glucosamine derivatives was determined. Here, per plant, a plurality of independent measurements were carried out for each worked-up sample. The following results were obtained for individual plants:

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
420ES 1 a	0.15	0.15
420ES 1 b	0.10	
420ES 1 c	0.15	
420ES 2 a	0.11	0.10
420ES 2 b	0.09	
420ES 2 c	0.10	
420ES 3 a	0.29	0.28

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
420ES 16 a	0.08	0.07
420ES 16 b	0.06	
420ES 16 c	0.07	
420ES 17 a	0.08	0.07
420ES 17 b	0.07	
420ES 17 c	0.08	
420ES 18 a	0.07	0.08

Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]	Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]
420ES 3 b	0.30	0.18	420ES 18 b	0.09	0.03
420ES 3 c	0.25		420ES 18 c	0.09	
420ES 4 a	0.20		420ES 19 a	0.03	
420ES 4 b	0.19	0.09	420ES 19 b	0.00	0.06
420ES 4 c	0.16		420ES 19 c	0.05	
420ES 5 a	0.10		420ES 20 a	0.04	
420ES 5 b	0.08	0.27	420ES 20 b	0.07	0.08
420ES 5 c	0.09		420ES 20 c	0.05	
420ES 6 a	0.24		420ES 22 a	0.08	
420ES 6 c	0.29	1.31	420ES 22 b	0.07	0.13
420ES 7 b	1.12		420ES 22 c	0.08	
420ES 7 c	1.50		420ES 23 a	0.14	0.05
420ES 8 a	0.05	0.06	420ES 23 b	0.11	
420ES 8 b	0.05		420ES 23 c	0.13	
420ES 8 c	0.06	0.02	420ES 24 a	0.05	0.06
420ES 9 a	0.02		420ES 24 b	0.04	
420ES 9 b	0.01		420ES 24 c	0.05	
420ES 9 c	0.02	0.04	420ES 25 a	0.05	0.09
420ES 10 a	0.05		420ES 25 b	0.07	
420ES 10 b	0.03		420ES 25 c	0.06	
420ES 10 c	0.05	0.06	420ES 26 a	0.13	0.08
420ES 11 a	0.06		420ES 26 b	0.06	
420ES 11 b	0.10		420ES 26 c	0.08	
420ES 11 c	0.03	0.08	420ES 27 a	0.09	0.01
420ES 12 a	0.09		420ES 27 b	0.10	
420ES 12 b	0.06		420ES 27 c	0.05	
420ES 13 a	0.02	0.02	420ES 28 a	0.01	0.08
420ES 13 b	0.01		420ES 28 b	0.02	
420ES 13 c	0.03		420ES 28 c	0.01	
420ES 14 a	0.02	0.03	420ES 29 a	0.09	0.03
420ES 14 b	0.04		420ES 29 b	0.07	
420ES 14 c	0.04		420ES 29 c	0.07	
420ES 15 a	0.05	0.06	420ES 30 a	0.04	0.10
420ES 15 b	0.06		420ES 30 b	0.03	
420ES 15 c	0.06		420ES 30 c	0.01	
			wt 7a	0.09	0.01
			wt 7b	0.11	
			wt 7c	0.09	
			wt 12 a	0.02	0.01
			wt 12 b	n.d	
			wt 12 c	0.03	

Table 4: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in leaves of independent transgenic plants of the line 420 ES.

[0229] Column 1 refers to the plant, independently originating from the transformation, from which the material was

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harvested (here, "wt" refers to non-transformed plants). The extension of the names of the plants by a, b or c denotes independent measurements carried out for the worked-up sample in question. Non-detectable amounts are marked "n.d.".

[0230] These results show that plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1 and coding for a protein having the activity of a UDP-glucose dehydrogenase have a content of N-acetylated glucosamine derivatives which is slightly higher than that of corresponding non-transformed wild-type plants.

c) Production of tomato plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2

[0231] Tomato plants (cultivar Moneymaker) were transformed by the method given under General Methods Item 2 with the plant expression vector IC 372-256 comprising a coding nucleic acid sequence for a protein having the activity of a UDP-glucose dehydrogenase and a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2. The transgenic lines obtained, which are transformed with the plasmid IC 372-256, were named 421 ES.

d) Analysis of lines 421 ES

[0232] Plants of the line 421 ES were cultivated in hydroculture in pots in a greenhouse. In each case about 5 g of plant material, harvested from individual plants, were worked up using the method described under General Methods Item 4, and the content of N-acetylated glucosamine derivatives was determined. Here, per plant, a plurality of independent measurements were carried out for each worked-up sample. The following results were obtained for individual plants:

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
421ES 1 a	0.60	0.67
421ES 1 b	0.61	
421ES 1 c	0.80	
421ES 3 a	1.07	1.10
421ES 3 b	1.07	
421ES 3 c	1.17	
421ES 4 a	2.22	2.00
421ES 4 b	1.88	
421ES 4 c	1.89	

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
421ES 27 a	0.11	0.13
421ES 27 b	0.16	
421ES 27 c	0.12	
421ES 28 a	0.02	0.01
421ES 28 b	n.d.	
421ES 28 c	0.01	
421ES 29 a	0.35	0.40
421ES 29 b	0.46	
421ES 29 c	0.39	

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
421ES 5 a	0.79	0.87
421ES 5 b	1.07	
421ES 5 c	0.74	
421ES 6 a	0.62	0.74
421ES 6 b	0.76	
421ES 6 c	0.85	
421ES 7 a	1.20	1.01
421ES 7 b	1.01	
421ES 7 c	0.84	
421ES 9 a	0.35	0.40
421ES 9 b	0.36	
421ES 9 c	0.48	
421ES 10 a	0.08	0.16
421ES 10 b	0.18	
421ES 10 c	0.22	
421ES 11 a	2.96	2.78
421ES 11 b	2.61	
421ES 11 c	2.78	
421ES 12 a	1.13	0.96
421ES 12 b	0.82	
421ES 12 c	0.93	
421ES 19 a	0.04	0.04
421ES 19 b	0.03	
421ES 21 a	0.21	0.25
421ES 21 b	0.36	
421ES 21 c	0.19	
421ES 23 a	0.01	0.01
421ES 23 b	0.01	
421ES 23 c	0.02	
421ES 26 a	0.18	0.16
421ES 26 b	0.19	
421ES 26 c	0.10	

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
421ES 31 a	0.14	0.14
421ES 31 b	0.16	
421ES 31 c	0.11	
421ES 32 a	0.04	0.03
421ES 32 b	0.01	
421ES 32 c	0.05	
421ES 33 a	0.12	0.11
421ES 33 b	0.08	
421ES 33 c	0.13	
421ES 34 a	0.32	0.34
421ES 34 b	0.37	
421ES 34 c	0.34	
421ES 35 a	0.20	0.21
421ES 35 b	0.24	
421ES 35 c	0.17	
421ES 36 a	0.07	0.06
421ES 36 b	0.07	
421ES 36 c	0.03	
421ES 37 a	0.12	0.12
421ES 37 b	0.11	
421ES 37 c	0.14	
421ES 38 a	0.32	0.34
421ES 38 b	0.34	
421ES 38 c	0.37	
wt 8 a	n.d.	n.d.
wt 8 c	n.d.	
wt 13 a	n.d.	n.d.
wt 13 b	n.d.	
wt 13 c	n.d.	

Table 5: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in leaves of independent transgenic plants of the line 421 ES. Column 1 refers to the plant, independently originating from the transformation, from which the material was harvested (here, "wt" refers to non-transformed plants). The extension of the names of the plants by a, b or c denotes independent measurements carried out for the worked-up sample in question. Non-detectable amounts are marked "n.d."

[0233] These results show that plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 and coding for a protein having the activity of a UDP-glucose dehydrogenase have a content of N-acetylated glucosamine derivatives which is considerably higher than that of correspondingly non-transformed wild-type plants.

e) Production of tomato plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT

[0234] Tomato plants (cultivar MoneyMaker) were transformed by the method given under General Methods Item 2 with the plant expression vector IC 375-271 comprising a coding nucleic acid sequence for a protein having the activity of a UDP-glucose dehydrogenase and a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT. The transgenic lines obtained, which are transformed with the plasmid IC 375-271, were named 422 ES.

f) Analysis of lines 422 ES

[0235] Plants of the line 422 ES were cultivated in hydroculture in pots in a greenhouse. In each case about 5 g of plant material, harvested from individual plants, were worked up using the method described under General Methods Item 4, and the content of N-acetylated glucosamine derivatives was determined. Here, per plant, a plurality of independent measurements were carried out for each worked-up sample. The following results were obtained for individual plants:

Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]	Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]
422ES 2 a	13.96	14.50	422ES 13 a	13.11	10.89
422ES 2 b	13.39		422ES 13 b	10.54	
422ES 2 c	16.13		422ES 13 c	9.02	
422ES 3 a	0.28	0.29	422ES 14 a	7.68	7.75
422ES 3 b	0.29		422ES 14 b	8.05	
422ES 3 c	0.30		422ES 14 c	7.52	
422ES 4 a	0.20	0.18	422ES 16 a	14.02	14.45
422ES 4 b	0.13		422ES 16 b	13.35	
422ES 4 c	0.21		422ES 16 c	15.98	
422ES 5 a	10.57	9.97	422ES 17 a	10.79	9.72
422ES 5 b	9.74		422ES 17 b	9.99	
422ES 5 c	9.60		422ES 17 c	8.37	
422ES 6 a	16.58	16.20	422ES 18 a	3.09	4.20
422ES 6 b	16.11		422ES 18 b	4.55	
422ES 6 c	15.91		422ES 18 c	4.96	
422ES 7 a	3.13	2.99	422ES 19 a	6.43	5.99
422ES 7 b	2.64		422ES 19 b	4.94	
422ES 7 c	3.19		422ES 19 c	6.59	

Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]	Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]
422ES 8 a	16.50	14.70	422ES 20 a	15.85	15.50
422ES 8 b	14.32		422ES 20 b	15.87	
422ES 8 c	13.27		422ES 20 c	14.79	
422ES 9 a	9.76	9.72	422ES 21 a	0.32	0.35
422ES 9 b	9.33		422ES 21 c	0.38	
422ES 9 c	10.07		wt 9 a	0.36	0.23
422ES 11 a	5.80	5.40	wt 9 b	0.19	
422ES 11 b	5.34		wt 9 c	0.13	
422ES 11 c	5.05		wt 14 a	n.d.	n.d.
422ES 12 a	11.57	12.23	wt 14 b	n.d.	
422ES 12 b	11.65		wt 14 c	n.d.	
422ES 12 c	13.46				

Table 6: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in leaves of independent transgenic plants of the line 422 ES. Column 1 refers to the plant, independently originating from the transformation, from which the material was harvested (here, "wt" refers to non-transformed plants). The extension of the names of the plants by a, b or c denotes independent measurements carried out for the worked-up sample in question. Non-detectable amounts are marked "n.d.".

g) Analysis of fruits of lines 420 ES, 421 ES and 422 ES

[0236] Ripe fruits were harvested from selected plants of lines 420 ES, 421 ES and 422 ES. Various whole tomato fruits of individual plants were harvested and worked up using the method described under General Methods Item 4, and the content of N-acetylated glucosamine derivatives was determined. Here, independent measurements were carried out for different fruits of a plant. The following results were obtained for individual plants:

Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]	Plant	$\mu\text{mol/g FW}$	Mean [$\mu\text{mol/g FW}$]
420ES 2 I	0.01	0.01	422ES 2 I	3.17	4.26
420ES 2 II	0.01		422ES 2 II	3.74	
420ES 3 I	0.07	0.06	422ES 2 III	5.79	
420ES 3 II	0.06		422ES 2 IV	4.90	
420ES 4 I	0.04	0.04	422ES 2 V	3.73	
420ES 4 II	n.d		422ES 5 I	2.12	3.41
420ES 4 III	0.04		422ES 5 II	1.76	
420ES 6 I	0.09	0.05	422ES 5 III	1.99	
420ES 6 II	0.03		422ES 5 IV	3.26	
420ES 6 III	0.04		422ES 5 V	5.27	

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]	Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
420ES 7 I	0.01	0.03	422ES 5 VI	4.49	
420ES 7 II	0.04		422ES 5 VII	4.95	
420ES 7 III	0.05		422ES 6 I	7.41	7.41
420ES 7 IV	0.03		422ES 9 I	3.67	3.34
420ES 8 I	0.03	0.04	422ES 9 II	3.02	
420ES 8 II	0.04		422ES 11 I	2.55	1.98
420ES 8 III	0.04		422ES 11 II	1.92	
420ES 12 I	0.00	0.04	422ES 11 III	1.47	
420ES 12 II	0.07		422ES 12 I	3.76	7.65
420ES 17 I	0.05	0.05	422ES 12 II	9.80	
420ES 17 II	0.06		422ES 12 III	9.39	
420ES wt 7 I	n.d.	0.03	422ES 13 I	5.79	5.31
420ES wt 7 II	0.04		422ES 13 II	5.04	
420ES wt 7 III	0.03		422ES 13 III	5.11	
421ES 4 I	0.94	0.86	422ES 14 I	4.08	3.50
421ES 4 II	0.85		422ES 14 II	2.93	
421ES 4 III	0.67		422ES 16 I	2.62	3.60
421ES 4 IV	0.79		422ES 16 II	2.72	
421ES 4 V	1.02		422ES 16 III	5.45	
421ES 5 I	0.35	0.53	422ES 17 I	7.25	7.57
421ES 5 II	0.72		422ES 17 II	7.89	
421ES 5 III	0.67		422ES 18 I	n.d.	2.30
421ES 5 IV	0.45		422ES 18 II	2.56	
421ES 5 V	0.48		422ES 18 III	2.04	
421ES 21 I	2.02	1.17	422ES wt 9 I	0.02	0.02
421ES 21 II	0.92		422ES wt 9 II	0.01	
421ES 21 III	0.96		422ES wt 9 III	0.00	
421ES 21 IV	0.79		422ES wt 9 IV	0.05	
421ES 25 I	0.61	0.76	422ES wt 9 V	n.d.	
421ES 25 II	0.75		422ES wt14 I	0.05	0.05
421ES 25 III	0.91		422ES wt14 II	n.d.	
421ES 27 I	0.86	0.89	422ES wt14 III	n.d.	
421ES 27 II	0.91				
421ES 27 III	0.90				
421ES 29 I	0.48	0.76			
421ES 29 II	0.52				
421ES 29 III	0.52				
421ES 29 IV	1.53				
421ES 33 I	0.74	0.67			
421ES 33 II	0.83				
421ES 33 III	0.45				

Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]	Plant	$\mu\text{mol/g}$ FW	Mean [$\mu\text{mol/g}$ FW]
421ES 35 I	0.48	0.77			
421ES 35 II	0.79				
421ES 35 III	0.87				
421ES 35 IV	0.95				
421ES 38 I	0.97	1.21			
421ES 38 II	1.35				
421ES 38 III	1.29				
421ES wt 13 I	0.03	0.04			
421ES wt 13 II	0.05				
421ES wt 13 III	n.d				

Table 7: Amount of N-acetylated glucosamine derivatives (in μmol per gram of fresh weight) measured in fruits of independent transgenic plants of lines 420 ES, 421 ES and 422 ES. Column 1 refers to the plant, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). The extension of the names of the plants by Latin numerals denotes different fruits of the plant in question. Non-detectable amounts are marked "n.d."

[0237] These results show that plants having a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT and coding for a protein having the activity of a UDP-glucose dehydrogenase have a considerably higher content of N-acetylated glucosamine derivatives than correspondingly non-transformed wild-type plants. Compared to plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1 and coding for a protein having the activity of a UDP-glucose dehydrogenase, plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 and coding for a protein having the activity of a UDP-glucose dehydrogenase have an even higher content of N-acetylated glucosamine derivatives. This is true both for leaf material and for fruits of the plants in question.

h) Analysis of N-acetylated glucosamine derivatives of line 422 ES by mass spectroscopy

[0238] Extracts of individual different fruits of the plant with the name 422 ES 13 were examined by mass spectroscopy according to the method described under General Methods Item 9 for the presence of N-acetylated glucosamine derivatives. The following results were obtained:

Detector	Sample	Mass (m/z) with associated metabolites				
		178 GlcN	220 GlcNAc	258 GlcN-P	300 GlcNAc-P	302.5 UDP-GlcNAc
cps: 9.5-10 e4 (d.v.: 2000)	422 ES 13 I	0.08	5.08	0.56	1.44	0.14
	422 ES 13 II	0.09	6.41	0.61	1.48	0.14
	422 ES 13 III	0.09	5.95	1.05	1.51	0.18
	wt	0.06	0.05	0.10	0.06	0.00
cps: 1.6-1.8 e5 (d.v.: 2050)	422 ES 13 I	0.37	10.69	1.91	4.31	0.42
	422 ES 13 II	0.37	13.92	2.10	4.49	0.45
	422 ES 13 III	0.30	12.96	3.43	3.98	0.55
	wt	0.30	0.25	0.49	0.38	0.03
cps: 2.0-2.2 e5 (d.v.: 2100)	422 ES 13 I	0.71	18.77	3.95	8.70	0.73
	422 ES 13 II	0.68	21.81	3.88	8.32	0.67
	422 ES 13 III	0.48	19.82	6.25	7.14	0.84
	wt	0.55	0.53	1.05	0.94	0.05

Table 8: Detection of the metabolites glucosamine (GlcN), N-acetylglucosamine (GlcNAc), glucosamine phosphate (GlcN-P), N-acetylglucosamine phosphate (GlcNAc-P) and UDP-N-acetylglucosamine (UDP-GlcNAc) in fruits of the plant 422 ES 13 by mass spectroscopy. What is shown is the proportion of the signal intensity (peak area) obtained for the stated metabolite in the mass spectrum, based on the signal intensity for hexoses ($m/z=179$) obtained in the same measurement, in percent. The different measurements were carried out at the stated detector settings with respect to sensitivity ("d.v.") and signal intensity ("cps") (column 1). Column 2 denotes the plant, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). The extension of the names of the plants by Latin numerals denote different fruits of the plant in question.

[0239] In parallel, via MS-MS measurements of samples 422 ES 13 I and fruits of a wild-type plant (wt) using reference substances (glucosamine, N-acetylglucosamine, glucosamine 6-phosphate, glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 1-phosphate, UDP-N-acetylglucosamine) it was analyzed whether the detected signal intensities (peak areas) in question of the MS spectra were really due to the presence of the corresponding metabolite or the corresponding isomeric metabolites of the same mass, or whether the signal intensities in question in the MS spectrum were possibly caused by interference by signals of other substances. The following observations were made:

Glucosamine (GlcN, $m/z=178$): The highest amounts of GlcN detected in the MS spectra of samples 422 ES 13 I and wt were in the range of the lower detection limit. In the MS spectrum, no significant differences between the sample 422 ES 13 I and the wt samples were noticed. Accordingly, it was not possible to determine with any degree

of certainty whether the samples contained GlcN.

N-Acetylglucosamine (GlcNAc, $m/z=220$): The most significant differences in the MS spectra of samples 422 ES 13 and the wt sample were found for this metabolite. In the MS spectra of samples 422 ES 13 I, 422 ES 13 II and 422 ES 13 III, considerable amounts of GlcNAc were detected. The corresponding MS-MS spectrum for the sample 422 ES 13 I corresponds to the spectrum of the reference substance (N-acetylglucosamine) and has, if any, only very small amounts of substances which may interfere with the relevant signal in the MS spectrum. In contrast, in the MS spectrum of the wt sample the signal intensity for $m/z=220$ was very low. The MS-MS spectrum of the wt sample showed that GlcNAc is only present in traces, if at all. The MS-MS spectrum very clearly showed that the signal intensity determined for $m/z=220$ of the wt sample in the MS spectrum was the result mainly of other substances interfering with the signal.

Glucosamine phosphates (GlcN-P, $m/z=258$): The signal intensity of the MS spectra for the wt sample is considerably lower than for samples 422 ES 13 I, 422 ES 13 II and 422 ES 13 III. All samples measured by MS-MS show that the signal for $m/z=258$ is not only due to the presence of GlcN-P but also to interference of the signal by other substances. The MS-MS spectrum of the wt sample showed that only traces of GlcN-P are present, if any. In contrast, the corresponding signal for sample 422 ES 13 I in the MS-MS spectrum showed the presence of significant amounts of GlcN-P in the relevant signal of the MS spectrum.

N-Acetylglucosamine phosphate (GlcNAc-P, $m/z=300$): For the wt sample, the signal intensities for $m/z=300$ in the MS spectrum are substantially lower than for the samples 422 ES 13 I, 422 ES 13 II and 422 ES 13 III. The values determined by MS-MS for the wt sample show that, if any, only traces of GlcNAc-P are present. In contrast, for sample 422 ES 13 I it was possible to demonstrate by MS-MS measurement that the predominant part of the signal intensity determined for $m/z=300$ in the MS spectrum of this sample is due to GlcNAc-P. UDP-N-Acetylglucosamine (UDP-GlcNAc, $m/z=302.5$): In the wild-type, the signal intensities of the MS spectrum are considerably lower than in samples 422 ES 13 I, 422 ES II and 422 ES III. The corresponding MS-MS spectra show that in all samples a certain part of the signal intensity of the MS spectra is not only due to the presence of UDP-GlcNAc, but also due to signal interference by other substances. However, the MS-MS measurements showed that compared to the signal-interfering substances, the proportion of UDP-GlcNAc in the MS spectra of sample 422 ES 13 I is substantially higher than for the wt sample.

19. Production of plants which synthesize glucosaminoglycans

[0240] To determine whether plants having an increased content of N-acetylated glucosamine derivatives are suitable for producing plants having an increased glucosaminoglycan content, at first plants expressing a glucosaminoglycan synthase (hyaluronan synthase) were generated.

a) Plants comprising a nucleic acid molecule coding for a protein having the activity of a hyaluronan synthase

[0241] Potato plants (cultivar Désirée) and tomato plants (cultivar Moneymaker) were transformed using the method given under General Methods Item 1 (potato plants) and under General Methods Item 2 (tomato plants) respectively, with the plants expression vector IC 341-222 which comprises a coding nucleic acid sequence for a protein having the activity of a hyaluronan synthase from *Paramecium bursaria* *Chlorella* Virus 1 under the control of the promoter of the patatin gene B33 from *Solanum tuberosum* (Rocha-Sosa et al., 1989, EMBO J. 8, 23-29). The transgenic lines obtained, which are transformed with the plasmid IC 341-222, were named 365 ES (potato plants) and 367 ES (tomato plants), respectively.

b) Analysis of the lines 365 ES

[0242] Individual plants of the line 365 ES were cultivated in soil in 6 cm pots in a greenhouse. In each case about 0.3 g of material of potato tubers of the individual plants was worked up using the method described under General Methods Item 5. The amount of the hyaluronan present in the respective plant extracts was determined using the method described under General Methods Item 7. Here, the supernatant obtained after centrifugation was diluted 1:10 to determine the hyaluronan content. The following results were obtained for selected plants:

Table 9: Amount of hyaluronan (in μg per gram of fresh weight) produced by independent selected transgenic plants of line 365 ES. Column 1 refers to the plant from which tuber material was harvested (here, "wt" refers to non-transformed plants). Column 2 states the value for the amount of hyaluronan determined in leaves of the plants in question. Non-detectable amounts are marked "n.d."

Plant	Hyaluronan [$\mu\text{g/g}$ FW]
365 ES 13	47
365 ES 74	68
wt	n.d.

c) Analysis of plants of line 367 ES

[0243] From different selected tomato plants of line 367 ES which had been cultivated in soil in a greenhouse, in each case 1 leaf was harvested and frozen in liquid nitrogen. Further work-up and determination of the hyaluronan content was carried out as described in Example 19b) for tubers of potato plants. The following results were obtained:

Table 10: Amount of hyaluronan (in μg per gram of fresh weight) produced in leaves of independent selected transgenic plants of lines 367 ES. Column 1 refers to the plant from which leaf material was harvested (here, "wt" refers to non-transformed plants). Column 2 states the value of the amount of hyaluronan determined in leaves of the plants in question.

Plant	Hyaluronan [$\mu\text{g/g}$ FW]
367 ES 25	57.19
367 ES 42	88.99
wt	0.06

20. Plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a UDP-glucose dehydrogenase and a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase

[0244] Some glucosaminoglycan synthases (such as, for example, hyaluronan synthase) require, as substrate, N-acetylated glucosamine derivatives and UDP-GlcA. Accordingly, we first generated plants having an increased activity of a protein having the activity of a UDP-glucose dehydrogenase and an increased activity of a protein having the activity of a hyaluronan synthase.

a) Production of potato plants

[0245] Potato plants of line 365 ES 74 (see Example 19 b)) were transformed using the method given under General Methods Item 1 with the plant expression vector IC 349-222 comprising a coding nucleic acid sequence for a protein having the activity of a UDP-glucose dehydrogenase under the control of the 35S promoter. The transgenic lines obtained, which are transformed with the plasmid IC 349-222, were named 423 ES.

b) Analysis of plants of line 423 ES

[0246] Plants of line 423 ES were cultivated in soil in 6 cm pots in a greenhouse. In each case about 0.3 g to 0.8 g of leaf material, harvested from individual plants, was worked up using the method described under General Methods Item 5, and the content of Hyaluronan was determined using the method described under General Methods Item 7. For individual plants having an increased content of N-acetylglucosamine derivatives, the following results were obtained:

Plant	Hyaluronan [µg/g FW]
423ES 1	328.75
423ES 3	210.38
423ES 5	340.99
423ES 6	250.88
423ES 7	214.53
423ES 8	309.22
423ES 9	253.31
423ES 10	229.61
423ES 11	234.40
423ES 12	480.22
423ES 13	253.63
423ES 14	221.77
423ES 15	202.46
423ES 17	281.46
423ES 18	310.41
423ES 19	268.91
423ES 20	394.04
423ES 21	462.64
423ES 24	438.33
423ES 25	419.50
423ES 26	342.89
423ES 27	383.32
423ES 28	236.83

Plant	Hyaluronan [µg/g FW]
423ES 49	285.19
423ES 51	213.97
423ES 53	328.76
423ES 54	358.23
423ES 55	154.06
423ES 59	276.32
423ES 60	498.70
423ES 61	300.97
423ES 62	292.08
423ES 65	230.38
423ES 67	267.54
423ES 68	370.08
wt 1	0.38
wt 2	0.12
wt 3	0.07
wt 4	n.d.
wt 5	0.47
wt 6	n.d.
wt 7	0.05
wt 8	0.05
wt 9	0.10
wt 10	n.d.
365ES 74-1	348.43

Plant	Hyaluronan [µg/g FW]
423ES 29	332.63
423ES 32	254.88
423ES 33	283.31
423ES 35	276.60
423ES 36	308.85
423ES 38	307.72
423ES 41	259.89
423ES 43	244.62
423ES 47	229.25
423ES 48	238.22

Plant	Hyaluronan [µg/g FW]
365ES 74-2	214.59
365ES 74-3	391.88
365ES 74-4	442.60
365ES 74-5	293.01
365ES 74-6	323.47
365ES 74-7	464.21
365ES 74-8	341.32
365ES 74-9	338.93
365ES 74-10	438.55

Table 11: Amount of hyaluronan (in µg per gram of fresh weight) measured in leaves of independent transgenic plants of line 423 ES. Column 1 refers in each case to the plants, independently originating from the transformation, from which material was harvested (here, "wt 1" to "wt 10" refer to independent non-transformed plants). For comparison, values for 10 different progeny of plants of line 365 ES used as starting line for the transformation (365 ES-1 to 365 ES-10) are shown. Non-detectable amounts are marked "n.d.".

[0247] It can be seen from the results that plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a UDP-glucose-dehydrogenase and a nucleic acid molecule coding for a protein having the activity of a hyaluronan synthase do not synthesize any statically significant increased amounts of hyaluronan compared to plants having only a nucleic acid molecule coding for a protein having the activity of a hyaluronan synthase.

21. Plants synthesizing increased amounts of glucosaminoglycan

a) Production of tomato plants synthesizing increased amounts of glucosaminoglycan

[0248] Tomato plants of lines 367 ES 25 (see example 19 c)), having a nucleic acid molecule coding for a hyaluronan synthase were transformed again using the method given under General Methods Item 2 with the plant expression vectors IC 372-256 or IC 375-271 comprising nucleic acid molecules coding for different isoforms of proteins having the activity of a GFAT.

[0249] The transgenic tomato plants obtained after transformation of line 367 ES 25, with the plasmid IC 372-256 (GFAT-2), were named 399 ES.

[0250] The transgenic tomato plants obtained after transformation of line 367 ES 25 with the plasmid IC 375-271 (bacterial GFAT), were named 405 ES.

b) Analysis of lines 399 ES and 405 ES

[0251] Ripe fruits were harvested from different tomato plants of lines 399 ES and 405 ES cultivated in soil in a greenhouse, and the hyaluronan content was determined as described under General Methods Item 7. The following results were obtained:

Table 12: Amount of hyaluronan ("HA" in μg per gram of fresh weight) measured in fruits of independent transgenic plants of lines 399 ES and 405 ES. Column 1 refers to the plants, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). For comparison, values of different progeny of plants of line 367 ES used as starting line for the transformation are shown. The extensions of the names of the plants by Latin numerals denote different fruits of the plant in question. Non-detectable amounts are marked "n.d.".

Sample	HA [$\mu\text{g/g}$ FW]	Mean [$\mu\text{g/g}$ FW]	Sample	HA [$\mu\text{g/g}$ FW]	Mean [$\mu\text{g/g}$ FW]
399ES 1 I	63.38	87.02	405ES 5 I	207.20	254.94
399ES 1 II	96.45		405ES 5 II	302.67	
399ES 1 III	101.23		405ES 10 I	1232.38	1074.94
399ES 11 I	388.83	292.79	405ES 10 II	917.50	
399ES 11 I	244.01		wt I	0.86	0.46
399ES 11 III	254.91		wt II	0.06	
399ES 11 IV	285.72		367ES 25-8 I	136.67	155.70
399ES 11 V	297.99		367ES 25-8 II	174.72	
399ES 11 VI	285.29		367ES 25-9 I	37.76	
wt I	0.02	0.01			
wt II	0.02				
wt III	0.01				
wt IV	n.d.				

(continued)

	Sample	HA [$\mu\text{g/g}$ FW]	Mean [$\mu\text{g/g}$ FW]		Sample	HA [$\mu\text{g/g}$ FW]	Mean [$\mu\text{g/g}$ FW]
5	367ES 25-1 I	9.77	12.04				
	367ES 25-1 II	8.21					
	367ES 25-1 III	18.04					
10	367ES 25-1 IV	13.86					
	367ES 25-1 V	10.33					
	367ES 25-2 I	9.31	11.96				
	367ES 25-2 II	10.55					
15	367ES 25-2 III	11.53					
	367ES 25-2 IV	16.54					
	367ES 25-2 V	11.86					
20	367ES 25-3 I	6.99	8.51				
	367ES 25-3 II	7.94					
	367ES 25-3 III	9.23					
	367ES 25-3 IV	7.09					
25	367ES 25-3 V	11.28					

[0252] These results show that plants comprising foreign nucleic acid molecules coding for a glucosaminoglycan synthase and coding for a protein having the activity of a UDP-glucose dehydrogenase and coding for a protein having the activity of a GFAT-2 or a bacterial GFAT synthesize considerably higher amounts of glucosaminoglycans than plants having only a foreign nucleic acid molecule coding for a glucosaminoglycan synthase.

c) Production of potato plants synthesizing increased amounts of glucosaminoglycan

[0253] Potato plants of lines 365 ES 74 (see example 19 b)) comprising a nucleic acid molecule coding for a hyaluronan synthase were transformed again using the method stated under General Methods Item 1 with the plant expression vectors IC 376-271, IC 372-256 or IC 375-271 comprising nucleic acid molecules coding for different isoforms of proteins having the activity of a GFAT.

[0254] The transgenic potato plants obtained after transformation of line 365 ES 74 with the plasmid IC 376-271 (GFAT-1), were named 409 ES.

[0255] The transgenic potato plants obtained after transformation of line 365 ES 74 with the plasmid IC 372-256 (GFAT-2), were named 396 ES.

[0256] The transgenic potato plants obtained after transformation of line 365 ES 74 with the plasmid IC 375-271 (bacterial GFAT), were named 404 ES.

d) Analysis of lines 396 ES, 404 ES and 409 ES

[0257] Leaf and/or tuber material was harvested from different potato plants of lines 396 ES (GFAT-2), 404 ES (bacterial GFAT) and 409 ES (GFAT-1) cultivated in soil in a greenhouse, and the hyaluronan content was determined as described under General Methods Item 7. The following results were obtained for plants of line 409 ES:

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
409 ES 2	54.01	
409 ES 3	68.75	212.24
409 ES 4	59.80	111.54
409 ES 5	26.90	
409 ES 6	38.01	182.39
409 ES 7	25.80	95.68
409 ES 8	51.92	99.35
409 ES 9	48.43	168.61
409 ES 10	52.52	

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
409 ES 26	38.81	
409 ES 27	24.71	126.74
409 ES 28	66.95	
409 ES 29	79.58	164.66
wt-1	n.d.	
wt-2	n.d.	
wt-3	n.d.	
wt-4	n.d.	
365 ES 74-1	25.19	

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
409 ES 13	55.87	
409 ES 14	45.91	143.96
409 ES 15	52.76	
409 ES 16	60.28	
409 ES 22	69.47	114.97
409 ES 23	108.67	

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
365 ES 74-2	31.15	
365 ES 74-3	72.96	
365 ES 74-4	35.98	
365 ES 74-5	40.18	123.66
365 ES 74-6	37.70	

Table 13: Amount of hyaluronan ("HA" in μg per gram of fresh weight) measured in leaves and tubers of independent transgenic plants of line 409 ES. Column 1 refers to the plants, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). Values for different progeny of plants of line 365 ES 74, which was used as starting line for the transformation, are shown for comparison. Non-detectable amounts are marked "n.d.".

[0258] The following results were obtained for plants of line 396 ES:

Table 14: Amount of hyaluronan ("HA" in μg per gram of fresh weight) measured in leaves and tubers of independent transgenic plants of line 396 ES. Column 1 refers to the plants, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). Values for different progeny of plants of line 365 ES 74, which was used as starting line for the transformation, are shown for comparison.

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]	Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
396 ES 2		470.93	396 ES 51	1160.57	
396 ES 9		735.40	396 ES 57	428.33	
396 ES 11	938.33		396 ES 57	807.97	
396 ES 15		393.64	365 ES 74-1	265.10	
396 ES 16	416.43		366 ES 74-2		91.84
396 ES 17	426.79		365 ES 74-3	193.50	
396 ES 23	271.85		367 ES 74-4		175.48
396 ES 24	443.57		365 ES 74-5	73.90	

(continued)

Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]	Plant	HA in leaves [$\mu\text{g/g}$ FW]	HA in tubers [$\mu\text{g/g}$ FW]
396 ES 25	801.58		368 ES 74-6		168.68
396 ES 28		484.76	365 ES 74-7	67.58	
396 ES 30		224.06	369 ES 74-8		121.89
396 ES 32	941.89		365 ES 74-9	62.23	
396 ES 33		1295.98	370 ES 74-10		275.24
396 ES 34			365 ES 74-11	134.56	
396 ES 36	204.49		wt-1	0.07	2.27
396 ES 36	860.54		wt-2	0.11	
396 ES 42		1445.51	wt-3	0.12	1.07
396 ES 44		1312.56	wt-4	0.04	0.78
396 ES 48	461.05		wt-5	0.10	
396 ES 49	538.75		wt-5	0.24	
396 ES 50	619.23				

[0259] The following results were obtained for plants of 404 ES:

Table 15: Amount of hyaluronan (in μg per gram of fresh weight) measured in leaves of independent transgenic plants of line 404 ES. Column 1 refers to the plants, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). Values for different progeny of plants of line 365 ES 74, which was used as starting line for the transformation, are shown for comparison.

Plant	Hyaluronan in leaves [$\mu\text{g/g}$ FW]	Plant	Hyaluronan in leaves [$\mu\text{g/g}$ FW]
404 ES 1	801.14	404 ES 37	533.89
404 ES 6	365.15	404 ES 38	651.12
404 ES 7	218.42	404 ES 39	353.74
404 ES 8	521.92	404 ES 40	371.88
404 ES 9	366.46	404 ES 42	849.43
404 ES 10	226.83	404 ES 43	479.34
404 ES 11	231.39	404 ES 44	921.11
404 ES 13	1547.12	404 ES 46	846.81
404 ES 14	616.79	404 ES 48	302.54
404 ES 15	832.32	wt-1	0.20
404 ES 20	581.11	wt-2	0.30
404 ES 21	489.73	wt-3	0.19
404 ES 23	817.91	wt-4	0.39
404 ES 24	434.06	wt-5	0.20
404 ES 26	205.00	365 ES 74-1	72.44
404 ES 28	359.96	365 ES 74-2	135.60
404 ES 29	1146.68	365 ES 74-3	19.56

(continued)

Plant	Hyaluronan in leaves [μg/g FW]		Plant	Hyaluronan in leaves [μg/g FW]
404 ES 34	310.76		365 ES 74-4	114.83
404 ES 35	1388.51		365 ES 74-5	73.77
404 ES 36	1095.11			

[0260] These results show that plants comprising foreign nucleic acid molecules coding for a glucosaminoglycan synthase and coding for a protein having the activity of a UDP-glucose dehydrogenase and coding for a protein having the activity of a GFAT-2 or a bacterial GFAT synthesize considerably higher amounts of glucosaminoglycan than plants comprising foreign nucleic acid molecules coding for a glucosaminoglycan synthase and coding for a protein having the activity of a UDP-glucose dehydrogenase and coding for a protein having the activity of a GFAT-1.

e) Production of plants comprising foreign nucleic acid molecules coding for a hyaluronan synthase and a protein having the activity of a bacterial GFAT

[0261] Potato plants of line 365 ES 74 (see example 19 b)), comprising a nucleic acid molecule coding for a hyaluronan synthase were transformed again using the method given under General Methods Item 1 with the plant expression vector IC 398-311 comprising nucleic acid molecules coding for a protein having the activity of a bacterial GFAT. The lines originating from this transformation were named 433 ES.

f) Analysis of line 433 ES

[0262] Leaf and/or tuber material was harvested from different potato plants of line 433 ES cultivated in soil in a greenhouse, and the hyaluronan content was determined as described under General Methods Item 7. The following results were obtained for plants of line 433 ES:

Table 16: Amount of hyaluronan ("HA" in μg per gram of fresh weight) measured in leaves and tubers of independent transgenic plants of line 433 ES. Column 1 refers to the plants, independently originating from the transformation, from which material was harvested (here, "wt" refers to non-transformed plants). Values for different progeny of plants of line 365 ES 74, which was used as starting line for the transformation, are shown for comparison. The values for line 365 ES 74 correspond to those in Table 14, since all plants were cultivated simultaneously in a greenhouse.

Plant	HA in leaves [μg/g FW]	HA in tubers [μg/g FW]		Plant	HA in leaves [μg/g FW]	HA in tubers [μg/g FW]
433ES 1	111.84	126.70		433ES 28	1850.99	294.98
433ES 3	303.34	203.16		433ES 30	2512.40	
433ES 4	3142.41			433ES 31	3337.54	
433ES 5	312.98	825.96		433ES 32	1583.60	
433ES 7	1492.94			433ES 34	3552.44	
433ES 8	914.03			433ES 35	5419.43	
433ES 9	1858.68			433ES 36	902.01	
433ES 10	357.90			433ES 37	829.35	
433ES 11	5962.82			433ES 38	1536.55	
433ES 12		662.99		wt-1	0.40	n.d.
433ES 13	626.52	624.33		wt-2	0.34	n.d.
433ES 14	665.23			wt-3	n.d.	
433ES 15	601.36			365 ES 74-1	265.1	
433ES 16	3416.94			366 ES 74-2		91.84

(continued)

Plant	HA in leaves [μg/g FW]	HA in tubers [μg/g FW]	Plant	HA in leaves [μg/g FW]	HA in tubers [μg/g FW]
433ES 18	781.02		365 ES 74-3	193.5	
433ES 19	3294.09		367 ES 74-4		175.48
433ES 20	1348.85	975.18	365 ES 74-5	73.9	
433ES 21	937.92		368 ES 74-6		168.68
433ES 22	1086.45		365 ES 74-7	67.58	
433ES 23	1327.28		369 ES 74-8		121.89
433ES 24	340.80	76.00	365 ES 74-9	62.23	
433ES 25	1529.95		370 ES 74-10		275.24
433ES 26	375.53		365 ES 74-11	134.56	
433ES 27		425.65			

[0263] These results show that plants comprising foreign nucleic acid molecules coding for a glucosaminoglycan synthase and coding for a protein having the activity of a bacterial GFAT synthesize considerably higher amounts of glucosaminoglycan than plants having only foreign nucleic acid molecules coding for a glucosaminoglycan synthase.

22. Summary of the results

[0264] The results in Example 16 show that plants comprising a nucleic acid molecule coding for a protein having the activity of a bacterial GFAT have considerably increased contents of N-acetylated glucosamine derivatives compared to non-transformed wild-type plants.

[0265] The results in Example 17 show that plants comprising a nucleic acid molecule coding for a protein having the activity of a GFAT-2 have considerably higher contents of N-acetylated glucosamine derivatives than non-transformed wild-type plants.

[0266] All transformed plants described in Example 18 have, in addition to nucleic acid molecules coding for different isoforms of a protein having the activity of a GFAT, in each case the same nucleic acid molecule coding for a protein having the activity of a UDP-glucose dehydrogenase. Accordingly, the essential difference of the transformed plants described in Example 18 are the different foreign nucleic acid molecules coding for the different isoforms of a protein having the activity of a GFAT. Example 18 b) shows that the content of N-acetylated glucosamine derivatives in plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1 is increased only slightly compared to non-transformed plants.

[0267] Furthermore, it can be seen from Example 18 d) that plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 have a considerably higher content of N-acetylated glucosamine derivatives than non-transformed wild-type plants. The content of N-acetylated glucosamine derivatives in plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 is also considerably higher than in plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1.

[0268] Furthermore, it can be seen from Examples 18 f) and g) that plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT have even higher contents of N-acetylated glucosamine derivatives than plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2.

[0269] The results in Example 21 f) show that plants comprising foreign nucleic acid molecules coding for a glucosaminoglycan synthase and coding for a protein having the activity of a bacterial GFAT synthesize considerably higher amounts of glucosaminoglycan than plants having only foreign nucleic acid molecules coding for a glucosaminoglycan synthase.

[0270] Thus, it can be concluded that the amount of glucosaminoglycans synthesized in plants can be increased considerably by generating plants which, in addition to foreign nucleic acid molecules coding for a glucosaminoglycan synthase, additionally comprise foreign nucleic acid molecules coding for a protein having the activity of a bacterial GFAT.

[0271] All transformed plants whose results are shown in Examples 21 b) and d) have, in addition to nucleic acid molecules coding for different isoforms of a protein having the activity of a GFAT, also foreign nucleic acid molecules coding for a protein having the activity of a UDP-glucose dehydrogenase and foreign nucleic acid molecules coding for a glucosaminoglycan synthase. The essential difference between the transformed plants whose results are shown in

Examples 21 b) and d) accordingly consists in the different nucleic acid molecules coding for the different isoforms of a protein having the activity of a GFAT.

[0272] The results shown in Example 21 b) show that the content of glucosaminoglycans in plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT is increased considerably compared to plants having only the activity of a glucosaminoglycan synthase.

[0273] The results shown in Example 21 d) show that plants comprising a foreign nucleic acid molecule coding a protein having the activity of a GFAT-1 contain a slightly higher amount of glucosaminoglycans than plants having only the activity of a glucosaminoglycan synthase. In contrast, the content of glucosaminoglycans in plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 is considerably higher than in plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-1. Furthermore, it can be seen from Example 21 d) that individual plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a bacterial GFAT contain even higher amounts of glucosaminoglycans than plants having a foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2.

[0274] The results in Example 20 b) show that plants comprising a foreign nucleic acid molecule coding for a protein having the activity of a UDP-glucose dehydrogenase and a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase do not have any statistically significantly increased amounts of glucosaminoglycan compared to plants comprising only a foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase.

[0275] To conclude, the results shown indicate that the considerable increases in the amounts of glucosaminoglycans in plants comprising foreign nucleic acid molecules coding for a protein having the activity of a UDP-glucose dehydrogenase and coding for a protein having the activity of a glucosaminoglycan synthase and having the activity of a GFAT-2 or having the activity of a bacterial GFAT is not due to the presence of the foreign nucleic acid molecules having the activity of a UDP-glucose dehydrogenase but to the presence of nucleic acid molecules having the activity of a GFAT-2 or having the activity of a bacterial GFAT.

[0276] Since hyaluronan synthases used in an exemplary manner as proteins having the activity of a glucosaminoglycan synthase require, as substrates, both UDP-Glc-NAc and UDP-GlcA, it may also be concluded from the results shown that the increased amounts of hyaluronan (glucosaminoglycan) are due to increased amounts of N-acetylated glucosamine derivatives and not to increased amounts of UDP-GlcA in these plants.

SEQUENCE LISTING

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<130> BCS 06-5010 PCT

<150> EP05090279.0

<151> 2005-10-05

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<151> 2005-10-11

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	Glu	Thr	Ala	Asp	Thr	Leu	Met	Gly	Leu	Arg	Tyr	Cys	Lys	Glu	Arg	Gly	
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	Ala Thr His Gly Glu Pro Asn Pro Val Asn Ser His Pro Gln Arg Ser			
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10	Ser Glu Thr Asp Thr Glu Thr Ile Ala Lys Leu Val Lys Tyr Met Tyr	145	150	155	160
15	Asp Asn Trp Glu Ser Gln Asp Val Ser Phe Thr Thr Leu Val Glu Arg	165	170	175	
20	Val Ile Gln Gln Leu Glu Gly Ala Phe Ala Leu Val Phe Lys Ser Val	180	185	190	
25	His Phe Pro Gly Gln Ala Val Gly Thr Arg Arg Gly Ser Pro Leu Leu	195	200	205	
30	Ile Gly Val Arg Ser Glu His Lys Leu Ser Thr Asp His Ile Pro Ile	210	215	220	
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55	Leu Ser Ile His Arg Ile Lys Arg Thr Ala Gly Asp His Pro Gly Arg	290	295	300	
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50	Gln	Phe	Val	Ser	Leu	Val	Met	Phe	Ala	Leu	Met	Met	Cys	Asp	Asp	Arg	
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Leu Pro Asp Leu Ile Lys Glu Val Leu Ser Met Asp Asp Glu Ile Gln
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 Lys Leu Ala Thr Glu Leu Tyr His Gln Lys Ser Val Leu Ile Met Gly
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 Arg Gly Tyr His Tyr Ala Thr Cys Leu Glu Gly Ala Leu Lys Ile Lys
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 Glu Ile Thr Tyr Met His Ser Glu Gly Ile Leu Ala Gly Glu Leu Lys
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 His Gly Pro Leu Ala Leu Val Asp Lys Leu Met Pro Val Ile Met Ile
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    Lys Glu Ile Phe Glu Thr Leu Ile Arg Gly Leu Gln Arg Leu Glu Tyr
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    Arg Gly Tyr Asp Ser Ala Gly Val Ala Ile Asp Gly Asn Asn His Glu
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          50              55              60

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	Arg	Ser	Lys	Tyr	Lys	Leu	Ser	Thr	Glu	Gln	Ile	Pro	Val	Leu	Tyr	Pro	
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 Ala Leu Asp Glu Glu Leu Tyr Lys Gln Asp Ser Met Asp Leu Lys Val
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 Glu Phe Glu Thr His Phe Gly Ile Ala His Thr Arg Trp Ala Thr His
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 Gly Val Pro Asn Ala Val Asn Ser His Pro Gln Arg Ser Asp Lys Asp
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 Asn Glu Phe Val Val Ile His Asn Gly Ile Ile Thr Asn Tyr Lys Asp
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40	Gly	Arg	Gly	Tyr	Asn	Tyr	Ala	Thr	Cys	Leu	Glu	Gly	Ala	Leu	Lys	Ile	
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Asp Thr Glu Ser Ser Lys Phe Ala Tyr Lys Thr Ile Glu Leu Pro His
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	Pro Ser Glu Val Asn Ala His Pro His Val Ser Glu His Ile Val Val	
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	Ser Pro Leu Val Ile Gly Leu Gly Met Gly Glu Asn Phe Ile Ala Ser	
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	Val	Ala	Lys	Leu	Ser	Arg	Leu	Lys	Gly	Leu	Asp	Ala	Ser	Ile	Glu	His	
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35	tat gtc ttc gcc gat cag gat gcg ggt ttt gta agt agc gat aac atg Tyr Val Phe Ala Asp Gln Asp Ala Gly Phe Val Ser Ser Asp Asn Met 545 550 555 560			1680
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20	Leu Gly Lys Val Gln Met Leu Ala Gln Ala Ala Glu Glu His Pro Leu	50 55 60
25	His Gly Gly Thr Gly Ile Ala His Thr Arg Trp Ala Thr His Gly Glu	65 70 75 80
30	Pro Ser Glu Val Asn Ala His Pro His Val Ser Glu His Ile Val Val	85 90 95
35	Val His Asn Gly Ile Ile Glu Asn His Glu Pro Leu Arg Glu Glu Leu	100 105 110
40	Lys Ala Arg Gly Tyr Thr Phe Val Ser Glu Thr Asp Thr Glu Val Ile	115 120 125
45	Ala His Leu Val Asn Trp Glu Leu Lys Gln Gly Gly Thr Leu Arg Glu	130 135 140
50	Ala Val Leu Arg Ala Ile Pro Gln Leu Arg Gly Ala Tyr Gly Thr Val	145 150 155 160
55	Ile Met Asp Ser Arg His Pro Asp Thr Leu Leu Ala Ala Arg Ser Gly	165 170 175

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Ser Pro Leu Val Ile Gly Leu Gly Met Gly Glu Asn Phe Ile Ala Ser
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Asp Gln Leu Ala Leu Leu Pro Val Thr Arg Arg Phe Ile Phe Leu Glu
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Glu Gly Asp Ile Ala Glu Ile Thr Arg Arg Ser Val Asn Ile Phe Asp
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Lys Thr Gly Ala Glu Val Lys Arg Gln Asp Ile Glu Ser Asn Leu Gln
225 230 235 240

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Tyr Asp Ala Gly Asp Lys Gly Ile Tyr Arg His Tyr Met Gln Lys Glu
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Ile Tyr Glu Gln Pro Asn Ala Ile Lys Asn Thr Leu Thr Gly Arg Ile
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Ser His Gly Gln Val Asp Leu Ser Glu Leu Gly Pro Asn Ala Asp Glu
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Leu Leu Ser Lys Val Glu His Ile Gln Ile Leu Ala Cys Gly Thr Ser
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Tyr Asn Ser Gly Met Val Ser Arg Tyr Trp Phe Glu Ser Leu Ala Gly
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Ile Pro Cys Asp Val Glu Ile Ala Ser Glu Phe Arg Tyr Arg Lys Ser
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Ala Val Arg Arg Asn Ser Leu Met Ile Thr Leu Ser Gln Ser Gly Glu
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Thr Ala Asp Thr Leu Ala Gly Leu Arg Leu Ser Lys Glu Leu Gly Tyr
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15	Val Ala Lys Leu Ser Arg Leu Lys Gly Leu Asp Ala Ser Ile Glu His				
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20	Asp Ile Val His Gly Leu Gln Ala Leu Pro Ser Arg Ile Glu Gln Met				
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25	Leu Ser Gln Asp Lys Arg Ile Glu Ala Leu Ala Glu Asp Phe Ser Asp				
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30	Lys His His Ala Leu Phe Leu Gly Arg Gly Asp Gln Tyr Pro Ile Ala				
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35	Leu Glu Gly Ala Leu Lys Leu Lys Glu Ile Ser Tyr Ile His Ala Glu				
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40	Ala Tyr Ala Ala Gly Glu Leu Lys His Gly Pro Leu Ala Leu Ile Asp				
		500		505	510
45	Ala Asp Met Pro Val Ile Val Val Ala Pro Asn Asn Glu Leu Leu Glu				
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50	Lys Leu Lys Ser Asn Ile Glu Glu Val Arg Ala Arg Gly Gly Gln Leu				
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55	Tyr Val Phe Ala Asp Gln Asp Ala Gly Phe Val Ser Ser Asp Asn Met				
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	His Ile Ile Glu Met Pro His Val Glu Glu Val Ile Ala Pro Ile Phe				
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Tyr Thr Val Pro Leu Gln Leu Leu Ala Tyr His Val Ala Leu Ile Lys
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45	aag ctc gcc gct aaa gcg ggt tct gat aat ttt cgg agt tct gca att Lys Leu Ala Ala Lys Ala Gly Ser Asp Asn Phe Arg Ser Ser Ala Ile 305 310 315 320	1021		
50	gta gac ata atg gag cga ctt gca aac aag ggt tat cac att aag att Val Asp Ile Met Glu Arg Leu Ala Asn Lys Gly Tyr His Ile Lys Ile 325 330 335	1069		
55	ttc gaa cca act gtg gaa caa ttc gaa aac ttt gaa gtt gat aac aac Phe Glu Pro Thr Val Glu Gln Phe Glu Asn Phe Glu Val Asp Asn Asn 340 345 350	1117		
	ctg aca aca ttt gcg act gag agc gat gta att atc gca aac aga gtt Leu Thr Thr Phe Ala Thr Glu Ser Asp Val Ile Ile Ala Asn Arg Val 355 360 365	1165		

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ccc gtt gaa cat cgc att ctc ttt ggt aaa aaa tta atc aca cgt gat 1213
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30

Glu Asp Arg Val Gln Leu Ile Lys Asn Lys Lys Ser Pro Ile Glu Asp
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35

Lys Glu Ile Glu Glu Phe Leu Glu Thr Lys Asp Leu Asn Leu Thr Ala
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40

Thr Thr Asp Lys Val Leu Ala Tyr Glu Asn Ala Glu Phe Val Ile Ile
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45

Ala Thr Pro Thr Asp Tyr Asp Val Val Thr Arg Tyr Phe Asn Thr Lys
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50

Ser Val Glu Asn Val Ile Gly Asp Val Ile Lys Asn Thr Gln Thr His
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55

Pro Thr Ile Val Ile Lys Ser Thr Ile Pro Ile Gly Phe Val Asp Lys
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					165					170					175		
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35	Gly	Gln	Gly	Tyr	Ser	Asn	Pro	Ser	Phe	Gly	Tyr	Gly	Ala	Tyr	Cys	Phe	
				245						250					255		
40	Pro	Lys	Asp	Thr	Lys	Gln	Leu	Leu	Ala	Asn	Phe	Glu	Gly	Val	Pro	Gln	
				260					265					270			
45	Asp	Ile	Ile	Gly	Ala	Ile	Val	Glu	Ser	Asn	Glu	Thr	Arg	Lys	Glu	Val	
		275						280					285				
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	290						295					300					
55	Lys	Leu	Ala	Ala	Lys	Ala	Gly	Ser	Asp	Asn	Phe	Arg	Ser	Ser	Ala	Ile	
	305					310					315					320	
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	325	330	335
5	Phe Glu Pro Thr Val Glu Gln Phe Glu Asn Phe Glu Val Asp Asn Asn 340	345	350
10	Leu Thr Thr Phe Ala Thr Glu Ser Asp Val Ile Ile Ala Asn Arg Val 355	360	365
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Claims

1. A plant cell or plant containing a foreign nucleic acid molecule stably integrated into its genome, wherein the foreign nucleic acid molecule is coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase (GFAT), wherein the foreign nucleic acid molecule codes for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT) and wherein the plant cell or plant has a content of N-acetylated glucosamine derivatives of at least 2 μ mol per gram of fresh weight where the foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT is selected from the group consisting of

a) nucleic acid molecules coding for a protein having the amino acid sequence given under SEQ ID NO 7 (GFAT-2) or a protein having the amino acid sequence given under SEQ ID NO 9 (bacterial GFAT);

b) nucleic acid molecules coding for a protein whose sequence is at least 60%, identical to the amino acid sequence shown under SEQ ID NO 7 (GFAT-2) or under SEQ ID NO 9 (bacterial GFAT);

c) nucleic acid molecules comprising the nucleotide sequence shown under SEQ ID NO 6 (GFAT-2) or under SEQ ID NO 8 (bacterial GFAT) or under SEQ ID NO 10 (bacterial GFAT) or a sequence complementary thereto;

d) nucleic acid molecules which are at least 60% identical to the nucleic acid sequences shown under a) or c);

e) nucleic acid molecules which hybridize under stringent conditions with at least one strand of the nucleic acid sequences described under a) or c);

f) nucleic acid molecules whose nucleotide sequence differs from the sequence of the nucleic acid molecules mentioned under a) or c) owing to the degeneracy of the genetic code and

g) nucleic acid molecules which are fragments, allelic variants and/or derivatives of the nucleic acid molecules mentioned under a), b), c), d), e) or f).

2. A part of a plant containing plant cells according to claim 1 having a content of N-acetylated glucosamine derivatives of at least 2 μ mol per gram of fresh weight.

3. Propagation material of plants as claimed in claim 1 which contains plant cells according to claim 1, having a content of N-acetylated glucosamine derivatives of at least 2 μ mol per gram of fresh weight.

4. A process for producing a genetically modified plant which comprises the following steps:

a) introduction of a foreign nucleic acid molecule coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase into a plant cell where the foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT is selected from the group consisting of

i) nucleic acid molecules coding for a protein having the amino acid sequence given under SEQ ID NO 7 (GFAT-2) or a protein having the amino acid sequence given under SEQ ID NO 9 (bacterial GFAT);

ii) nucleic acid molecules coding for a protein whose sequence is at least 60%, identical to the amino acid sequence shown under SEQ ID NO 7 (GFAT-2) or under SEQ ID NO 9 (bacterial GFAT);

iii) nucleic acid molecules comprising the nucleotide sequence shown under SEQ ID NO 6 (GFAT-2) or under SEQ ID NO 8 (bacterial GFAT) or under SEQ ID NO 10 (bacterial GFAT) or a sequence complementary thereto;

iv) nucleic acid molecules which are at least 60% identical to the nucleic acid sequences shown under a) or c);

- v) nucleic acid molecules which hybridize under stringent conditions with at least one strand of the nucleic acid sequences described under a) or c);
- vi) nucleic acid molecules whose nucleotide sequence differs from the sequence of the nucleic acid molecules mentioned under a) or c) owing to the degeneracy of the genetic code and
- vii) nucleic acid molecules which are fragments, allelic variants and/or derivatives of the nucleic acid molecules mentioned under i), ii), iii), iv), v) or vi).

b) regeneration of a plant from plant cells obtained according to step a) wherein the plant has a content of N-acetylated glucosamine derivatives of at least 2 μmol per gram of fresh weight.

5. The use of nucleic acid molecules coding for a protein having the activity of a glutamine:fructose 6-phosphate amidotransferase of isoform II (GFAT-2) or coding for a protein having the activity of a bacterial glutamine:fructose 6-phosphate amidotransferase (bacterial GFAT) where the foreign nucleic acid molecule coding for a protein having the activity of a GFAT-2 or coding for a protein having the activity of a bacterial GFAT is selected from the group consisting of

- a) nucleic acid molecules coding for a protein having the amino acid sequence given under SEQ ID NO 7 (GFAT-2) or a protein having the amino acid sequence given under SEQ ID NO 9 (bacterial GFAT);
- b) nucleic acid molecules coding for a protein whose sequence is at least 60% identical to the amino acid sequence shown under SEQ ID NO 7 (GFAT-2) or under SEQ ID NO 9 (bacterial GFAT);
- c) nucleic acid molecules comprising the nucleotide sequence shown under SEQ ID NO 6 (GFAT-2) or under SEQ ID NO 8 (bacterial GFAT) or under SEQ ID NO 10 (bacterial GFAT) or a sequence complementary thereto;
- d) nucleic acid molecules which are at least 60% identical to the nucleic acid sequences shown under a) or c);
- e) nucleic acid molecules which hybridize under stringent conditions with at least one strand of the nucleic acid sequences described under a) or c);
- f) nucleic acid molecules whose nucleotide sequence differs from the sequence of the nucleic acid molecules mentioned under a) or c) owing to the degeneracy of the genetic code and
- g) nucleic acid molecules which are fragments, allelic variants and/or derivatives of the nucleic acid molecules mentioned under a), b), c), d), e) or f).

for preparing a genetically modified plant.

6. A composition comprising genetically modified plant cells as claimed in claim 1.

7. A process for producing flour which comprises the step of grinding parts of plants as claimed in claim 2 or propagation material as claimed in claim 3.

8. The use of plants as claimed in claim 1, of parts of plants as claimed in claim 2 or of propagation material as claimed in claim 3 for producing flour.

9. The method of claim 4 comprising a further step consisting of the generation of further plants carried out by vegetative propagation.

Patentansprüche

1. Pflanzenzelle oder Pflanze, die ein fremdes Nukleinsäuremolekül stabil in ihr Genom integriert enthält, wobei das fremde Nukleinsäuremolekül für ein Protein mit der Aktivität einer Glutamin:Fructose-6-phosphat-Amidotransferase (GFAT) codiert, wobei das fremde Nukleinsäuremolekül für ein Protein mit der Aktivität einer Glutamin:Fructose-6-phosphat-Amidotransferase der Isoform II (GFAT-2) oder ein Protein mit der Aktivität einer bakteriellen Glutamin:Fructose-6-phosphat-Amidotransferase (bakterielle GFAT) codiert und wobei die Pflanzenzelle bzw. Pflanze mindestens 2 μmol pro Gramm Frischgewicht an N-acetylierten Glucosaminderivaten enthält, wobei das für ein Protein mit der Aktivität einer GFAT-2 codierende oder für ein Protein mit der Aktivität einer bakteriellen GFAT codierende fremde Nukleinsäuremolekül ausgewählt ist aus der Gruppe bestehend aus

- a) Nukleinsäuremolekülen, die für ein Protein mit der unter SEQ ID NO 7 angegebenen Aminosäuresequenz (GFAT-2) oder ein Protein mit der unter SEQ ID NO 9 angegebenen Aminosäuresequenz (bakterielle GFAT) codieren;

- b) Nukleinsäuremolekülen, die für ein Protein, dessen Sequenz zu mindestens 60% identisch mit der unter SEQ ID NO 7 (GFAT-2) oder unter SEQ ID NO 9 (bakterielle GFAT) gezeigten Aminosäuresequenz ist, codieren;
- c) Nukleinsäuremolekülen, die die unter SEQ ID NO 6 (GFAT-2) oder unter SEQ ID NO 8 (bakterielle GFAT) oder unter SEQ ID NO 10 (bakterielle GFAT) gezeigte Nukleotidsequenz oder eine dazu komplementäre Sequenz umfassen;
- d) Nukleinsäuremolekülen, die zu mindestens 60% identisch mit den unter a) oder c) gezeigten Nukleinsäuresequenzen sind;
- e) Nukleinsäuremolekülen, die unter stringenten Bedingungen mit mindestens einem Strang der unter a) oder c) beschriebenen Nukleinsäuresequenzen hybridisieren;
- f) Nukleinsäuremolekülen, deren Nukleotidsequenz sich von der Sequenz der unter a) oder c) erwähnten Nukleinsäuremoleküle aufgrund der Degeneration des genetischen Codes unterscheidet, und
- g) Nukleinsäuremolekülen, bei denen es sich um Fragmente, Allelvarianten und/oder Derivate der unter a), b), c), d), e) oder f) erwähnten Nukleinsäuremoleküle handelt.

2. Teil einer Pflanzenzellen nach Anspruch 1 enthaltenden Pflanze mit einem Gehalt an N-acetylierten Glucosamin-derivaten von mindestens 2 μmol pro Gramm Frischgewicht.

3. Fortpflanzungsmaterial von Pflanzen nach Anspruch 1, welches Pflanzenzellen nach Anspruch 1 enthält, mit einem Gehalt an N-acetylierten Glucosamin-derivaten von mindestens 2 μmol pro Gramm Frischgewicht.

4. Verfahren zur Herstellung einer genetisch modifizierten Pflanze, welches die folgenden Schritte umfasst:

a) das Einführen eines für ein Protein mit der Aktivität einer Glutamin:Fructose-6-phosphat-Amidotransferase der Isoform II (GFAT-2) codierenden oder für ein Protein mit der Aktivität einer bakteriellen Glutamin-fructose-6-phosphat-amidotransferase codierenden fremden Nukleinsäuremoleküls in eine Pflanzenzelle, wobei das für ein Protein mit der Aktivität einer GFAT-2 codierende oder für ein Protein mit der Aktivität einer bakteriellen GFAT codierende fremde Nukleinsäuremolekül ausgewählt ist aus der Gruppe bestehend aus:

- i) Nukleinsäuremolekülen, die für ein Protein mit der unter SEQ ID NO 7 angegebenen Aminosäuresequenz (GFAT-2) oder ein Protein mit der unter SEQ ID NO 9 angegebenen Aminosäuresequenz (bakterielle GFAT) codieren;
- ii) Nukleinsäuremolekülen, die für ein Protein, dessen Sequenz zu mindestens 60% identisch mit der unter SEQ ID NO 7 (GFAT-2) oder unter SEQ ID NO 9 (bakterielle GFAT) gezeigten Aminosäuresequenz ist, codieren;
- iii) Nukleinsäuremolekülen, die die unter SEQ ID NO 6 (GFAT-2) oder unter SEQ ID NO 8 (bakterielle GFAT) oder unter SEQ ID NO 10 (bakterielle GFAT) gezeigte Nukleotidsequenz oder eine dazu komplementäre Sequenz umfassen;
- iv) Nukleinsäuremolekülen, die zu mindestens 60% identisch mit den unter i) oder iii) gezeigten Nukleinsäuresequenzen sind;
- v) Nukleinsäuremolekülen, die unter stringenten Bedingungen mit mindestens einem Strang der unter i) oder iii) beschriebenen Nukleinsäuresequenzen hybridisieren;
- vi) Nukleinsäuremolekülen, deren Nukleotidsequenz sich von der Sequenz der unter i) oder iii) erwähnten Nukleinsäuremoleküle aufgrund der Degeneration des genetischen Codes unterscheidet, und
- vii) Nukleinsäuremolekülen, bei denen es sich um Fragmente, Allelvarianten und/oder Derivate der unter i), ii), iii), iv), v) oder vi) erwähnten Nukleinsäuremoleküle handelt,

b) die Regeneration einer Pflanze aus gemäß Schritt a) erhaltenen Pflanzenzellen,

wobei die Pflanze einen Gehalt an N-acetylierten Glucosamin-derivaten von mindestens 2 μmol pro Gramm Frischgewicht hat.

5. Verwendung von für ein Protein mit der Aktivität einer Glutamin:Fructose-6-phosphat-Amidotransferase der Isoform II (GFAT-2) codierenden oder für ein Protein mit der Aktivität einer bakteriellen Glutamin:Fructose-6-phosphat-Amidotransferase (bakterieller GFAT) codierenden Nukleinsäuremolekülen zur Herstellung einer genetisch modifizierten Pflanze, wobei das für ein Protein mit der Aktivität einer GFAT-2 codierende oder für ein Protein mit der Aktivität einer bakteriellen GFAT codierende fremde Nukleinsäuremolekül ausgewählt ist aus der Gruppe bestehend aus

- a) Nukleinsäuremolekülen, die für ein Protein mit der unter SEQ ID NO 7 angegebenen Aminosäuresequenz (GFAT-2) oder ein Protein mit der unter SEQ ID NO 9 angegebenen Aminosäuresequenz (bakterielle GFAT) codieren;
- b) Nukleinsäuremolekülen, die für ein Protein, dessen Sequenz zu mindestens 60% identisch mit der unter SEQ ID NO 7 (GFAT-2) oder unter SEQ ID NO 9 (bakterielle GFAT) gezeigten Aminosäuresequenz ist, codieren;
- c) Nukleinsäuremolekülen, die die unter SEQ ID NO 6 (GFAT-2) oder unter SEQ ID NO 8 (bakterielle GFAT) oder unter SEQ ID NO 10 (bakterielle GFAT) gezeigte Nukleotidsequenz oder eine dazu komplementäre Sequenz umfassen;
- d) Nukleinsäuremolekülen, die zu mindestens 60% identisch mit den unter a) oder c) gezeigten Nukleinsäuresequenzen sind;
- e) Nukleinsäuremolekülen, die unter stringenten Bedingungen mit mindestens einem Strang der unter a) oder c) beschriebenen Nukleinsäuresequenzen hybridisieren;
- f) Nukleinsäuremolekülen, deren Nukleotidsequenz sich von der Sequenz der unter a) oder c) erwähnten Nukleinsäuremoleküle aufgrund der Degeneration des genetischen Codes unterscheidet, und
- g) Nukleinsäuremolekülen, bei denen es sich um Fragmente, Allelvarianten und/oder Derivate der unter a), b), c), d), e) oder f) erwähnten Nukleinsäuremoleküle handelt.

6. Zusammensetzung, umfassend genetisch modifizierte Pflanzenzellen nach Anspruch 1.

7. Verfahren zur Herstellung von Mehl, welches den Schritt des Mahlens von Pflanzenteilen nach Anspruch 2 oder Fortpflanzungsmaterial nach Anspruch 3 umfasst.

8. Verwendung von Pflanzen nach Anspruch 1 oder Pflanzenteilen nach Anspruch 2 oder Fortpflanzungsmaterial nach Anspruch 3 zur Herstellung von Mehl.

9. Verfahren nach Anspruch 4, welches einen weiteren, aus der Erzeugung weiterer Pflanzen durch vegetative Fortpflanzung bestehenden Schritt umfasst.

Revendications

1. Cellule végétale ou plante contenant une molécule d'acide nucléique étranger intégrée de manière stable dans son génome, dans laquelle la molécule d'acide nucléique étranger code pour une protéine ayant une activité de glutamine:fructose-6-phosphate amido-transférase (GFAT) où la molécule d'acide nucléique étranger code pour une protéine ayant une activité de glutamine:fructose-6-phosphate amidotransférase d'isoforme II (GFAT-2) ou une protéine ayant une activité de glutamine:fructose-6-phosphate amido-transférase bactérienne (GFAT bactérienne) et dans laquelle la cellule végétale ou la plante possède une teneur en dérivés de glucosamine N-acétylés d'au moins 2 μ mol par gramme de poids frais où la molécule d'acide nucléique étranger codant pour une protéine ayant une activité de GFAT-2 ou codant pour une protéine ayant une activité de GFAT bactérienne est choisie dans le groupe constitué par

- a) des molécules d'acide nucléique codant pour une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 7 (GFAT-2) ou une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 9 (GFAT bactérienne) ;
- b) des molécules d'acide nucléique codant pour une protéine dont la séquence est identique à au moins 60% par rapport à la séquence d'acides aminés illustrée sous SEQ ID n° 7 (GFAT-2) ou sous SEQ ID n° 9 (GFAT bactérienne) ;
- c) des molécules d'acide nucléique comprenant la séquence nucléotidique illustrée sous SEQ ID n° 6 (GFAT-2) ou sous SEQ ID n° 8 (GFAT bactérienne) ou sous SEQ ID n° 10 (GFAT bactérienne) ou une séquence en étant complémentaire ;
- d) des molécules d'acide nucléique qui sont identiques à au moins 60% par rapport aux séquences d'acide nucléique illustrées sous a) ou c) ;
- e) des molécules d'acide nucléique qui s'hybrident dans des conditions stringentes avec au moins un brin des séquences d'acide nucléique décrites sous a) ou c) ;
- f) des molécules d'acide nucléique dont la séquence nucléotidique diffère de la séquence des molécules d'acide nucléique mentionnées sous a) ou c) suite à la dégénérescence du code génétique ; et
- g) des molécules d'acide nucléique qui sont des fragments, des variants alléliques et/ou des dérivés des molécules d'acide nucléique mentionnées sous a), b), c), d), e) ou f).

2. Partie d'une plante contenant des cellules végétales selon la revendication 1, ayant une teneur en dérivés de glucosamine N-acétylés d'au moins 2 μmol par gramme de poids frais.

3. Matériel de propagation de plantes selon la revendication 1, contenant des cellules végétales selon la revendication 1, ayant une teneur en dérivés de glucosamine N-acétylés d'au moins 2 μmol par gramme de poids frais.

4. Procédé de production d'une plante génétiquement modifiée, comprenant les étapes suivantes :

a) l'introduction d'une molécule d'acide nucléique étranger codant pour une protéine ayant une activité de glutamine:fructose-6-phosphate amidotransférase d'isoforme II (GFAT-2) ou codant pour une protéine ayant une activité de glutamine:fructose-6-phosphate amidotransférase bactérienne dans une cellule végétale, où la molécule d'acide nucléique étranger codant pour une protéine ayant une activité de GFAT-2 ou codant pour une protéine ayant une activité de GFAT bactérienne est choisie dans le groupe constitué par

i) des molécules d'acide nucléique codant pour une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 7 (GFAT-2) ou une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 9 (GFAT bactérienne) ;

ii) des molécules d'acide nucléique codant pour une protéine dont la séquence est identique à au moins 60% par rapport à la séquence d'acides aminés illustrée sous SEQ ID n° 7 (GFAT-2) ou sous SEQ ID n° 9 (GFAT bactérienne) ;

iii) des molécules d'acide nucléique comprenant la séquence nucléotidique illustrée sous SEQ ID n° 6 (GFAT-2) ou sous SEQ ID n° 8 (GFAT bactérienne) ou sous SEQ ID n° 10 (GFAT bactérienne) ou une séquence en étant complémentaire ;

iv) des molécules d'acide nucléique qui sont identiques à au moins 60% par rapport aux séquences d'acide nucléique illustrées sous i) ou iii) ;

v) des molécules d'acide nucléique qui s'hybrident dans des conditions stringentes avec au moins un brin des séquences d'acide nucléique décrites sous i) ou iii) ;

vi) des molécules d'acide nucléique dont la séquence nucléotidique diffère de la séquence des molécules d'acide nucléique mentionnées sous i) ou iii) suite à la dégénérescence du code génétique ; et

vii) des molécules d'acide nucléique qui sont des fragments, des variants alléliques et/ou des dérivés des molécules d'acide nucléique mentionnées sous i), ii), iii), iv), v) ou vi) ;

b) la régénération d'une plante à partir des cellules végétales obtenues selon l'étape a)

dans lequel la plante possède une teneur en dérivés de glucosamine N-acétylés d'au moins 2 μmol par gramme de poids frais.

5. Utilisation des molécules d'acide nucléique étranger codant pour une protéine ayant une activité de glutamine:fructose-6-phosphate amidotransférase d'isoforme II (GFAT-2) ou codant pour une protéine ayant une activité de glutamine:fructose-6-phosphate amidotransférase bactérienne (GFAT bactérienne), où la molécule d'acide nucléique étranger codant pour une protéine ayant une activité de GFAT-2 ou codant pour une protéine ayant une activité de GFAT bactérienne est choisie dans le groupe constitué par

a) des molécules d'acide nucléique codant pour une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 7 (GFAT-2) ou une protéine ayant la séquence d'acides aminés donnée sous SEQ ID n° 9 (GFAT bactérienne) ;

b) des molécules d'acide nucléique codant pour une protéine dont la séquence est identique à au moins 60% par rapport à la séquence d'acides aminés illustrée sous SEQ ID n° 7 (GFAT-2) ou sous SEQ ID n° 9 (GFAT bactérienne) ;

c) des molécules d'acide nucléique comprenant la séquence nucléotidique illustrée sous SEQ ID n° 6 (GFAT-2) ou sous SEQ ID n° 8 (GFAT bactérienne) ou sous SEQ ID n° 10 (GFAT bactérienne) ou une séquence en étant complémentaire ;

d) des molécules d'acide nucléique qui sont identiques à au moins 60% par rapport aux séquences d'acide nucléique illustrées sous a) ou c) ;

e) des molécules d'acide nucléique qui s'hybrident dans des conditions stringentes avec au moins un brin des séquences d'acide nucléique décrites sous a) ou c) ;

f) des molécules d'acide nucléique dont la séquence nucléotidique diffère de la séquence des molécules d'acide nucléique mentionnées sous a) ou c) suite à la dégénérescence du code génétique ; et

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g) des molécules d'acide nucléique qui sont des fragments, des variants alléliques et/ou des dérivés des molécules d'acide nucléique mentionnées sous a), b), c), d), e) ou f) ;

pour la préparation d'une plante génétiquement modifiée.

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6. Composition comprenant des cellules végétales génétiquement modifiées selon la revendication 1.
7. Procédé de production de farine, comprenant l'étape consistant à broyer des parties de plantes selon la revendication 2 ou du matériel de propagation selon la revendication 3.
8. Utilisation de plantes selon la revendication 1, de parties de plantes selon la revendication 2 ou de matériel de propagation selon la revendication 3, pour la production de farine.
9. Méthode selon la revendication 4, comprenant une étape supplémentaire constituée de la génération de plantes supplémentaires effectuée par propagation végétative.

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Szabadalmi igénypontok

1. Növényi sejt vagy növény, amely idegen nukleinsav-molekulát tartalmaz stabilan integrálódva a genomjába, ahol az idegen nukleinsav-molekula olyan fehérjét kódol, amelynek glutamin:fruktóz-6-foszfát-amidotranszferáz (GFAT) aktivitása van, ahol az idegen nukleinsav-molekula glutamin:fruktóz-6-foszfát-amidotranszferáz II-es izoforma (GFAT-2) aktivitású fehérjét kódol vagy bakteriális glutamin:fruktóz-6-foszfát-amidotranszferáz (bakteriális GFAT) aktivitású fehérjét kódol, és ahol a növényi sejt vagy növény N-acetilált-glukózamin-származék tartalma legalább 2 μmol per gramm a friss tömegben, ahol a GFAT-2 aktivitású fehérjét kódoló vagy a bakteriális GFAT aktivitású fehérjét kódoló idegen nukleinsav-molekula a következők által alkotott csoportból van kiválasztva

a) olyan nukleinsav-molekulák, amelyek SEQ ID NO 7 szerinti aminosav-szekvenciájú fehérjét (GFAT-2) vagy SEQ ID NO 9 szerinti aminosav-szekvenciájú fehérjét (bakteriális GFAT) kódolnak;

b) olyan nukleinsav-molekulák, amelyek olyan fehérjét kódolnak, amelynek a szekvenciája legalább 60%-ban azonos a SEQ ID NO 7 szerinti (GFAT-2) vagy SEQ ID NO 9 szerinti (bakteriális GFAT) aminosav-szekvenciával;

c) olyan nukleinsav-molekulák, amelyek tartalmazzák a SEQ ID NO 6 szerinti (GFAT-2) vagy SEQ ID NO 8 szerinti (bakteriális GFAT) vagy SEQ ID NO 10 szerinti (bakteriális GFAT) nukleotid-szekvenciát vagy azzal komplementer szekvenciát;

d) olyan nukleinsav-molekulák, amelyek legalább 60%-ban azonosak az a) vagy c) alatti nukleinsav-szekvenciákkal;

e) olyan nukleinsav-molekulák, amelyek sztringens körülmények között hibridizálnak az a) vagy c) alatti nukleinsav-szekvenciák legalább egyik szálával;

f) olyan nukleinsav-molekulák, amelyek nukleotid-szekvenciája a genetikai kód degeneráltsága miatt tér el az a) vagy c) alatti nukleinsav-molekulák szekvenciájától és

g) olyan nukleinsav-molekulák, amelyek az a), b), c), d), e) vagy f) alatti nukleinsav-molekulák fragmensel, allelikus variánsai és/vagy származékai.

2. Az 1. igénypont szerinti növény vagy növényi sejt része, amelynek az N-acetilált-glukózamin-származék tartalma legalább 2 μmol per gramm a friss tömegben.

3. Az 1. igénypont szerinti növények szaporítóanyaga, amely 1. igénypont szerinti növényi sejteket tartalmaz, amelyeknek az N-acetilált-glukózamin-származék tartalma legalább 2 μmol per gramm a friss tömegben.

4. Eljárás genetikailag módosított növény előállítására, amely magában foglalja következő lépéseket:

a) olyan idegen nukleinsav-molekula bejuttatását növényi sejtbe, amely olyan fehérjét kódol, amelynek glutamin:fruktóz-6-foszfát-amidotranszferáz II-es izoforma (GFAT-2) aktivitása van vagy olyan fehérjét kódol, amelynek bakteriális glutamin:fruktóz-6-foszfát-amidotranszferáz aktivitása van, ahol a GFAT-2 aktivitású fehérjét kódoló vagy a bakteriális GFAT aktivitású fehérjét kódoló idegen nukleinsav-molekula a következők által alkotott csoportból van kiválasztva

i) olyan nukleinsav-molekulák, amelyek SEQ ID NO 7 szerinti aminosav-szekvenciájú fehérjét (GFAT-2) vagy SEQ ID NO 9 szerinti aminosav-szekvenciájú fehérjét (bakteriális GFAT) kódolnak;

ii) olyan nukleinsav-molekulák, amelyek olyan fehérjét kódolnak, amelynek a szekvenciája legalább 60%-ban azonos a SEQ ID NO 7 szerinti (GFAT-2) vagy SEQ ID NO 9 szerinti (bakteriális GFAT) aminosav-szekvenciával;

iii) olyan nukleinsav-molekulák, amelyek tartalmazzák a SEQ ID NO 6 szerinti (GFAT-2) vagy SEQ ID NO 8 szerinti (bakteriális GFAT) vagy SEQ ID NO 10 szerinti (bakteriális GFAT) nukleotid-szekvenciát vagy azzal komplementer szekvenciát;

iv) olyan nukleinsav-molekulák, amelyek legalább 60%-ban azonosak az a) vagy c) alatti nukleinsav-szekvenciákkal;

v) olyan nukleinsav-molekulák, amelyek sztringens körülmények között hibridizálnak az a) vagy c) alatti nukleinsav-szekvenciák legalább egyik szálával;

vi) olyan nukleinsav-molekulák, amelyek nukleotid-szekvenciája a genetikai kód degeneráltsága miatt tér el az a) vagy c) alatti nukleinsav-molekulák szekvenciájától és

vii) olyan nukleinsav-molekulák, amelyek az i), ii), iii), iv), v) vagy vi) alatti nukleinsav-molekulák fragmensei, allelikus variánsai és/vagy származékai.

b) növény regenerálása az a) lépés szerint kapott növényi sejtekből, ahol a növénynek az N-acetilált-glukózamin-származék tartalma legalább 2 μ mol per gramm a friss tömegben.

5. Nukleinsav-molekulák alkalmazása, amelyek olyan fehérjét kódolnak, amelynek glutamin:fruktóz-6-foszfát-amidotranszferáz II-es izoforma (GFAT-6) aktivitása van vagy olyan fehérjét kódolnak, amelynek bakteriális glutamin:fruktóz-2-foszfát-amidotranszferáz aktivitása van, ahol a GFAT-2 aktivitású fehérjét kódoló vagy a bakteriális GFAT aktivitású fehérjét kódoló idegen nukleinsav-molekula a következők által alkotott csoportból van kiválasztva

a) olyan nukleinsav-molekulák, amelyek SEQ ID NO 7 szerinti aminosav-szekvenciájú fehérjét (GFAT-2) vagy SEQ ID NO 9 szerinti aminosav-szekvenciájú fehérjét (bakteriális GFAT) kódolnak;

b) olyan nukleinsav-molekulák, amelyek olyan fehérjét kódolnak, amelynek a szekvenciája legalább 60%-ban azonos a SEQ ID NO 7 szerinti (GFAT-2) vagy SEQ ID NO 9 szerinti (bakteriális GFAT) aminosav-szekvenciával;

c) olyan nukleinsav-molekulák, amelyek tartalmazzák a SEQ ID NO 6 szerinti (GFAT-2) vagy SEQ ID NO 8 szerinti (bakteriális GFAT) vagy SEQ ID NO 10 szerinti (bakteriális GFAT) nukleotid-szekvenciát vagy azzal komplementer szekvenciát;

d) olyan nukleinsav-molekulák, amelyek legalább 60%-ban azonosak az a) vagy c) alatti nukleinsav-szekvenciákkal;

e) olyan nukleinsav-molekulák, amelyek sztringens körülmények között hibridizálnak az a) vagy c) alatti nukleinsav-szekvenciák legalább egyik szálával;

f) olyan nukleinsav-molekulák, amelyek nukleotid-szekvenciája a genetikai kód degeneráltsága miatt tér el az a) vagy c) alatti nukleinsav-molekulák szekvenciájától és

g) olyan nukleinsav-molekulák, amelyek az a), b), c), d), e) vagy f) alatti nukleinsav-molekulák fragmensei, allelikus variánsai és/vagy származékai,

genetikailag módosított növény előállítására.

6. Készítmény, amely 1. igénypont szerinti genetikailag módosított növényi sejteket tartalmaz.

7. Eljárás liszt előállítására, amely magában foglalja 2. igénypont szerinti növényi részek vagy 3. igénypont szerinti szaporítóanyag megőrlésének a lépését.

8. Az 1. igénypont szerinti növények, 2. igénypont szerinti növényi részek vagy 3. igénypont szerinti szaporítóanyag alkalmazása liszt előállítására.

9. A 4. igénypont szerinti eljárás, amely továbbá magában foglalja további növények vegetatív szaporítással történő előállításának lépését is.