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(54) **Titre : PLANTES QUI SYNTHETISENT DES QUANTITES ACCRUES DE GLUCOSAMINOGLYCANNES**
(54) **Title: PLANTS WHICH SYNTHESIZE INCREASED AMOUNTS OF GLUCOSAMINOGLYCANS**

(57) **Abrégé/Abstract:**

The present invention relates to plant cells and plants which synthesize an increased amount of glucosaminoglycans, and to methods for preparing such plants, and also to methods for preparing glucosaminoglycans with the aid of these plant cells or plants. Here, plant cells or genetically modified plants according to the invention have glucosaminoglycan synthase activity and additionally an increased glucosamine 6- phosphate acetyltransferase activity and an increased UDP-N-acetyl-glucosamine pyrophosphorylase activity compared to wild-type plant cells or wild-type plants. The present invention furthermore relates to compositions comprising plant cells having an increased glucosaminoglycan synthesis.

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(54) Title: PLANTS WHICH SYNTHESIZE INCREASED AMOUNTS OF GLUCOSAMINGLYCANS

(57) Abstract: The present invention relates to plant cells and plants which synthesize an increased amount of glucosaminoglycans, and to methods for preparing such plants, and also to methods for preparing glucosaminoglycans with the aid of these plant cells or plants. Here, plant cells or genetically modified plants according to the invention have glucosaminoglycan synthase activity and additionally an increased glucosamine 6- phosphate acetyltransferase activity and an increased UDP-N-acetyl-glucosamine pyrophosphorylase activity compared to wild-type plant cells or wild-type plants. The present invention furthermore relates to compositions comprising plant cells having an increased glucosaminoglycan synthesis.

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Plants which synthesize increased amounts of glucosaminoglycans

The present invention relates to plant cells and plants which synthesize an increased amount of glucosaminoglycans, and to methods for preparing such plants, and also
5 to methods for preparing glucosaminoglycans with the aid of these plant cells or plants. Here, plant cells or genetically modified plants according to the invention have glucosaminoglycan synthase activity and additionally an increased glucosamine 6-phosphate acetyltransferase activity and an increased UDP-N-acetyl-glucosamine pyrophosphorylase activity compared to wild-type plant cells or wild-type plants. The
10 present invention furthermore relates to compositions comprising plant cells having an increased glucosaminoglycan synthesis.

Proteoglycans, a class of glycoproteins, are inter alia an essential component of cartilage and have, attached to a core protein, glucosaminoglycans composed of
15 repetitive disaccharide units. The repetitive disaccharide units for their part are, via a characteristic carbohydrate binding sequence, attached covalently to the core protein. Depending on the composition of the disaccharide units, a distinction is made inter alia between the glucosaminoglycans heparan/heparin sulfate, keratan sulfate and chondroitin/dermatan sulfate whose disaccharide units each contain a
20 molecule which is glucosamine or a glucosamine derivative.

Hyaluronan, a further glucosaminoglycan, also has a derivative of glucosamine (N-acetyl-glucosamine) as one of the components of its disaccharide unit, but, in nature, is not attached to proteins. Except for hyaluronan, the glucosaminoglycans mentioned are naturally sulfated polymers. In these substances, sulfate groups are
25 introduced at various atoms or substituents of the disaccharide units so that the substances mentioned are not uniform polymers but groups of polymers summarized under the respective generic term. The individual molecules of the polymer groups in question may differ both in the degree of sulfatation and in the position of the monomers having sulfate groups.

30

Hyaluronan is a naturally occurring unbranched, linear mucopolysaccharide (glucosaminoglucan) which is constructed of alternating molecules of glucuronic acid (GlcA) and N-acetyl-glucosamine (GlcNAc). The basic building block of hyaluronan

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consists of the disaccharide glucuronic acid-beta-1,3-N-acetyl-glucosamine. In hyaluronan, these repeating units are attached to one another via beta-1,4 linkages. In pharmacy, use is frequently made of the term hyaluronic acid. Since hyaluronan is in most cases present as a polyanion and not as the free acid, hereinbelow, the term
5 hyaluronan is preferably used, but each term is to be understood as embracing both molecular forms.

Hyaluronan has unusual physical chemical properties, such as, for example, properties of polyelectrolytes, viscoelastic properties, a high capacity to bind water,
10 properties of gel formation, which, in addition to further properties of hyaluronan, are described in a review article by Lapcik et al. (1998, Chemical Reviews 98(8), 2663-2684).

Hyaluronan is a component of extracellular connective tissue and bodily fluids of vertebrates. In humans, hyaluronic acid is synthesized by the cell membrane of all
15 body cells, especially mesenchymal cells, and ubiquitously present in the body with a particularly high concentration in the connective tissues, the extracellular matrix, the umbilical cord, the joint fluid, the cartilaginous tissue, the skin and the vitreous body of the eye (Bernhard Gebauer, 1998, Inaugural-Dissertation, Virchow-Klinikum Medizinische Fakultät Charité der Humboldt Universität zu Berlin; Fraser et al., 1997,
20 Journal of Internal Medicine 242, 27-33).

Recently, hyaluronan was also found in animal non-vertebrate organisms (molluscs) (Volpi and Maccari, 2003, Biochimie 85, 619-625).

Furthermore, some human pathogenic gram-positive bacteria (*Streptococcus* group A and C) and gram-negative bacteria (*Pasteurella*) synthesize hyaluronan as
25 exopolysaccharides which protect these bacteria against attack by the immune system of their host, since hyaluronan is a non-immunogenic substance.

Viruses which infect single-cell green algae of the genus *Chlorella*, some of which are present as endosymbionts in *Paramecium* species, bestow upon the single-cell green algae the ability to synthesize hyaluronan after infection by the virus (Graves et al., 1999, Virology 257, 15-23). However, the ability to synthesize hyaluronan is not a
30 feature which characterizes the algae in question. The ability of the algae to synthesize hyaluronan is mediated by an infection with a virus whose genome has a sequence coding for hyaluronan synthase (DeAngelis, 1997, Science 278,

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1800-1803).

The catalysis of the hyaluronan synthesis is effected by a single membrane-integrated or membrane-associated enzyme, hyaluronan synthase.

- 5 The mechanism of the transfer of synthesized hyaluronan molecules across the cytoplasmic membrane into the medium surrounding the cell has not yet been fully elucidated. Earlier hypotheses assumed that transport across the cell membrane was effected by hyaluronan synthase itself. However, more recent results indicate that the transport of hyaluronan molecules across the cytoplasmic membrane takes place by
- 10 energy-dependent transport via transport proteins responsible for this action. Thus, *Streptococcus* strains were generated by mutation in which the synthesis of an active transport protein was inhibited. These strains synthesized less hyaluronan than corresponding wild-type bacteria strains (Ouskova et al., 2004, Glycobiology 14(10), 931-938). In human fibroblasts, it was possible to demonstrate, with the aid of agents
- 15 specifically inhibiting known transport proteins, that it is possible to reduce both the amount of hyaluronan produced and the activity of hyaluronan synthases (Prehm and Schumacher, 2004, Biochemical Pharmacology 68, 1401-1410). In which amount, if at all, transport proteins capable of transporting hyaluronan are present in plants is not known.

20

- The unusual properties of hyaluronan offer a wealth of possibilities for application in various fields, such as, for example, pharmacy, the cosmetics industry, in the production of food and feed, in technical applications (for example as lubricants), etc. The most important applications where hyaluronan is currently being used are in the
- 25 medicinal and cosmetics field (see, for example, Lapcik et al., 1998, Chemical Reviews 98(8), 2663-2684, Goa and Benfield, 1994, Drugs 47(3), 536-566).

- In the medical field, hyaluronan-containing products are currently used for the intraarticular treatment of arthrosis and in ophthalmics used for eye surgery. Hyaluronan is also used for treating joint disorders in racehorses. In addition,
- 30 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

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dermatics, hyaluronan-containing gel implants are used for correcting skin deformations in plastic surgery.

For pharmacological applications, preference is given to using hyaluronan having a high molecular weight.

- 5 In cosmetic medicine, hyaluronan preparations are among the most 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.

In cosmetic products, in particular in skin creams and lotions, hyaluronan is frequently used as moisturizer by virtue of its high water-binding capacity.

10

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.

- 15 Hyaluronan used for commercial purposes is currently isolated from animal tissues (cockscombs) or prepared fermentatively using bacterial cultures.

US 4,141,973 describes a process for isolating hyaluronan from cockscombs or alternatively from umbilical cords. In addition to hyaluronan, animal tissues (for example cockscombs, umbilical cords) also contain further mucopolysaccharides

- 20 related to hyaluronan, such as chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate and heparin. Furthermore, animal organisms contain proteins (hyaladherins) which bind specifically to hyaluronan and which are required for the most different functions in the organism, such as, for example, the degradation of hyaluronan in the liver, the function of hyaluronan as lead structure for cell migration, the regulation of endocytosis, the anchoring of hyaluronan on the cell surface or the formation of hyaluronan networks (Turley, 1991, Adv Drug Delivery Rev 7, 257 ff.; Laurent and Fraser, 1992, FASEB J. 6, 183 ff.; Stamenkovic and Aruffo, 1993, Methods Enzymol. 245, 195 ff; Knudson and Knudson, 1993, FASEB 7, 1233 ff.).

- 30 The *Streptococcus* strains used for the bacterial production of hyaluronan are exclusively bacteria pathogenic to humans. During cultivation, too, these bacteria produce (pyrogenic) exotoxins and hemolysins (streptolysin, in particular alpha- and beta-hemolysin) Kilian, M.: *Streptococcus and Enterococcus*. In: *Medical*

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Microbiology. Greenwood, D.; Slack, RCA; Peutherer, J.F. (Eds.). Chapter 16. Churchill Livingstone, Edinburgh, UK: pp. 174-188, 2002, ISBN 0443070776) which are released into the culture medium. This renders purification and isolation of the hyaluronan prepared with the aid of *Streptococcus* strains more difficult. In particular
5 for pharmaceutical application, the presence of exotoxins and hemolysins in the preparation is a problem.

US 4,801,539 describes the preparation of hyaluronan by fermentation of a mutagenized bacterial strain (*Streptococcus zooedemicus*). The mutagenized bacteria strain used no longer synthesizes beta-hemolysin. The yield achieved was
10 3.6 g of hyaluronan per liter of culture.

EP 0694616 describes a method for cultivating *Streptococcus zooedemicus* or *Streptococcus equi*, where, under the culture conditions employed, no streptolysin, but increased amounts of hyaluronan are synthesized. The yield achieved was 3.5 g of hyaluronan per liter of culture.

15 During cultivation, *Streptococcus* strains release the enzyme hyaluronidase into the culture medium, as a consequence of which, in this production system, too, the molecular weight is reduced during purification. The use of hyaluronidase-negative *Streptococcus* strains or of methods for the production of hyaluronan where the production of hyaluronidase during cultivation is inhibited are described in
20 US 4,782,046. The yield achieved was up to 2.5 g of hyaluronan per liter of culture, and the maximum mean molecular weight achieved was 3.8×10^6 Da, at a molecular weight distribution of from 2.4×10^6 to 4.0×10^6 .

US 20030175902 and WO 03 054163 describe the preparation of hyaluronan with the aid of heterologous expression of a hyaluronan synthase from *Streptococcus equisimilis* in *Bacillus subtilis*. To achieve the production of sufficient amounts of
25 hyaluronan, in addition to heterologous expression of a hyaluronan synthase, simultaneous expression of a UDP-glucose dehydrogenase in the *Bacillus* cells is also required. US 20030175902 and WO 03 054163 do not state the absolute amount of hyaluronan obtained in the production with the aid of *Bacillus subtilis*. The
30 maximum mean molecular weight achieved was about 4.2×10^6 . However, this mean molecular weight was only achieved for the recombinant *Bacillus* strain where a gene coding for the hyaluronan synthase gene from *Streptococcus equisimilis* and the gene coding for the UDP-glucose dehydrogenase from *Bacillus subtilis* were

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integrated into the *Bacillus subtilis* genome under the control of the *amyQ* promoter, where at the same time the *Bacillus subtilis*-endogenous *cypY* gene (which codes for a cytochrome P450 oxidase) was inactivated. Chien and Lee (2007, Biotechnol. Prog. Online publication, ASAP Article 10.1021/bp070036w, S8756-7938(07)00036-
5 7) describe various recombinant *Baciullus subtilis* strains. One strain, which had been transformed with a nucleic acid sequence coding for a hyaluronan synthase and a nucleic acid sequence coding for a UDP-glucose dehydrogenase, synthesized at most 1.14 g/l of hyaluronan. A strain which, in addition to the nucleic acid sequences just mentioned, had been transformed with a nucleic acid sequence
10 coding for *Vitreoscilla* hemoglobin synthesized 1.8 g/l hyaluronan.

WO 06 032538 describes transgenic plants transformed with nucleic acid molecules coding for hyaluronan synthases. The synthesis of hyaluronan in the plants in question was demonstrated unambiguously.

15

WO 05 012529 describes the production of transgenic tobacco plants which were transformed using nucleic acid molecules coding for hyaluronan synthases from *Chlorella*-infecting viruses. In WO 05 012529, use was made, on the one hand, of nucleic acid sequences coding for hyaluronan synthase of the *Chlorella* virus strain
20 CVH1 and, on the other hand, of the *Chlorella* virus strain CVKA1 for transforming tobacco plants. The synthesis of hyaluronan could only be demonstrated for a plant transformed with a nucleic acid coding for a hyaluronan synthase isolated from the *Chlorella* virus strain CVKA1. For tobacco plants transformed with a nucleic acid sequence coding for a hyaluronan synthase isolated from the *Chlorella* virus strain
25 CVH1, it was not possible to detect hyaluronan synthesis in the corresponding transgenic plants. The amount of hyaluronan synthesized by the only hyaluronan-producing transgenic tobacco plant in WO 05 012529 is stated as being about 4.2 µg of hyaluronan per ml of measured volume which, taking into account the description for carrying out the experiment in question, corresponds approximately to an amount
30 of at most 12 µg of hyaluronan produced per gram of fresh weight of plant material.

WO 2007 039314 describes transgenic plants having the activity of a hyaluronan synthase and additionally an increased glutamine:fructose 6-phosphate

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amidotransferase (GFAT) activity. These plants synthesize an increased amount of hyaluronan compared to plants having only the activity of a hyaluronan synthase. The maximum amount of hyaluronan synthesized by these tobacco plants is about 0.03% per gram of fresh weight of plant material used for the measurement (see FIG. 5 in WO 2007 039316).

WO 2007 039316 describes transgenic plants having the activity of a hyaluronan synthase and additionally an increased glutamine:fructose 6-phosphate amidotransferase (GFAT) activity and an increased UDP-Glucose dehydrogenase (UDP-Glc-DH) activity compared to wild-type plants. These plants synthesize an increased amount of hyaluronan compared to plants having the activity of a hyaluronan synthase and at the same time the activity of a protein having the activity of a GFAT. The maximum amount of hyaluronan synthesized by these tobacco plants is 0.2% per gram of fresh weight of plant material used for the measurement (see FIG. 6 in WO 2007 039316).

Furthermore, WO 2007 039316 contains a list of proteins which can be expressed in plants cells to increase further the amount of synthesized hyaluronan in plant cells. The proteins proposed in WO 2007 039316 are a random list of enzymes which, in various organisms, are involved in the synthesis of UDP-GlcNAc. The proteins listed in WO 2007 039316 have various enzymatic functions. WO 2007 039316 gives no indication whether and which of the enzymes listed may indeed increase the hyaluronan content when expressed in transgenic plants.

The synthesis of the disaccharide chain of the chondroitin/dermatan ([beta-1,4]-[glucuronic acid-beta-1,4-N-acetyl-galactosamine]_n) is catalyzed by a chondroitin synthase starting from UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetyl-galactosamine (UDP-GalNAc), an epimer of UDP-N-acetyl-glucosamine (UDP-GlcNAc) (Kitagawa et al., 2001, J Biol Chem 276(42), 38721-38726). By an epimerase, the glucuronic acid molecules of the chondroitin can be converted 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 at various positions of the disaccharide chain of the chondroitin or the

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dermatan is then catalyzed by other enzymes, resulting in the formation of chondroitin/dermatan sulfate. Here, the degree of sulfatation may differ from molecule to molecule.

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

The synthesis of the disaccharide chain of the heparin/heparan (heparosans) ([alpha-
10 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 starting from UDP-GlcA and UDP-GlcNAc (DeAngelis and White, 2004, J. Bacteriology 186(24), 8529-8532). The glucuronic acid molecules of the heparosan can be converted by an epimerase into iduronic acid. The introduction of the sulfate groups at various
15 positions of the disaccharide chain of the heparosan is then catalyzed by other enzymes, resulting in the formation of heparin 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 heparan sulfate the glucuronic acid molecules predominate (Gallagher et al., 1992, Int. J. Biochem 24,
20 553-560). As in the case of chondroitin/dermatan sulfate, in the case of heparin/heparan sulfate, too, the degree of sulfatation may differ from molecule to molecule.

Heparin sulfate is used inter alia as an anticoagulant, for example for preventing and treating thromboses.

25

Currently, chondroitin/dermatan sulfat and heparin/heparan sulfate are prepared by isolation from animal tissue. Chondroitin sulfate is isolated mainly from bovine cartilage or shark cartilage, heparin/heparan sulfate is isolated from porcine intestine or bovine lungs. Since the disaccharide chains of chondroitin/dermatan
30 sulfate and heparin/heparan sulfate have no uniform sulfatation pattern, it is difficult to obtain a uniform specific product. Accordingly, the product is always a mixture of molecules having various degrees of sulfatation.

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As already described, glucosaminoglycans, such as, for example, chondroitin sulfate or heparin/heparan sulfate are currently isolated from animal tissues. In addition to the desired substances, these tissues also contain other glucosaminoglycans. The separation of the individual glucosaminoglycans, if possible at all, is difficult and expensive. Furthermore, the potential contamination of animal tissues by pathogenic microorganisms and/or by other substance such as, for example, BSE or the bird flu pathogen, which may lead to diseases in man, poses a problem when using glucosaminoglycans isolated from animal tissue. In patients, the use of medicinal preparations contaminated by animal proteins can 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.

In addition, substances prepared from animal raw materials are unacceptable for some ways of life such as, for example, vegans or for kosher food preparation.

A further problem in the isolation of glucosaminoglycans from animal tissues consists in effect that frequently the molecular weight of glucosaminoglycans is reduced during purification since animal tissues also contain glucosaminoglycan-degrading enzymes.

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

The production of glucosaminoglycans by fermentation of bacteria strains is associated with high costs, since the bacteria have to be fermented in sealed sterile containers under expensive controlled culture conditions (see, for example, for hyaluronan, US 4,897,349). Furthermore, the amount of glucosaminoglycans which can be produced by fermentation of bacteria strains is limited by the production facilities present in each case. Here, it also has to be taken into account that fermenters, as a consequence of physical laws, cannot be built for excessively large culture volumes. Particular mention may be made here of homogeneous mixing of the substances fed in from the outside (for example essential nutrient sources for bacteria, reagents for regulating the pH, oxygen) with the culture medium required for efficient production, which, in large fermenters, can be ensured only with great technical expenditure, if at all.

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Plants do not naturally produce glucosaminoglycans such as, for example, hyaluronan, heparan/heparin sulfate, keratan sulfate or chondroitin/dermatan sulfate. Naturally occurring plants themselves do not have any nucleic acids in their genome
5 which code for proteins catalyzing the synthesis of glucosaminoglycans, and, although a large number of plant carbohydrates have been described and characterized, it has hitherto not been possible to detect any of the glucosaminoglycans mentioned in non-infected natural plant cells (Graves et al., 1999, Virology 257, 15-23).

10

WO 98 35047 (US 6,444,878) describes a metabolic path for the synthesis of GlcNAc in plant cells where glucosamine is converted via a plurality of successive enzymatically catalyzed reaction steps with formation of the metabolites GlcNAc, N-acetyl-glucosamine 6-phosphate and N-acetyl-glucosamine 1-phosphate into UDP-
15 GlcNAc. In higher concentrations, glucosamine 6-phosphate is toxic for plant cells (WO 98 35047).

An alternative metabolic path described for plants comprises the reaction of fructose 6-phosphate and glutamine giving glucosamine 6-phosphate which is subsequently converted by a number of successive enzymatically catalyzed reaction steps with
20 formation of the metabolites glucosamine 1-phosphate and N-acetyl-glucosamine 1-phosphate into UDP-GlcNAc (Mayer et al., 1968, Plant Physiol. 43, 1097-1107).

To date, it is still not clear which protein activities have to be modified in the metabolic path for the synthesis of UDP-GlcNAc in the plant for the plants to
25 synthesize increased amounts of glucosaminoglycans.

Accordingly, it is the object of the present invention to provide alternative means and processes for preparing efficient amounts of glucosaminoglycans in plants.

30 This object is achieved by the embodiments referred to in the claims.

Surprisingly, it has been found that genetically modified plant cells or genetically modified plants comprising a nucleic acid molecule coding for a glucosaminoglycan

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synthase and additionally comprising a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase and a foreign nucleic acid molecule coding for a protein comprising the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase produce significantly
5 higher amounts of glucosaminoglycan than plant cells or plants having (only) the activity of a glucosaminoglycan synthase.

Thus, the present invention relates to genetically modified plant cells or genetically modified plants comprising a foreign nucleic acid molecule coding for a
10 glucosaminoglycan synthase, characterized in that said genetically modified plant cells or said genetically modified plants additionally comprise a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.

15

The production of glucosaminoglycans by fermentation of bacteria strains is associated with high costs, since the bacteria have to be fermented in sealed sterile containers under expensive controlled culture conditions (see, for example, US 4,897,349). Furthermore, the amount of glucosaminoglycans which can be
20 produced by fermentation of bacteria strains is limited by the production facilities present in each case. The high price of, for example, hyaluronan which is currently commercially available means that this glucosaminoglycan, in spite of its special properties (for example viscoelastic properties, a high capacity to bind water), is not available for industrial applications.

25 Thus, compared to known means for producing glucosaminoglycans, plant cells according to the invention and plants according to the invention offer the advantage that they synthesize increased amounts of glucosaminoglycans (for example hyaluronan) compared to plant cells or plants comprising only the activity of a glucosaminoglycan synthase.

30

Here, the genetic modification of genetically modified plant cells according to the invention or genetically modified plants according to the invention can be any genetic modification resulting in an integration of a foreign nucleic acid molecule encoding for

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a glucosamine synthase and an integration of a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase and an integration of a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase into a
5 plant cell or a plant.

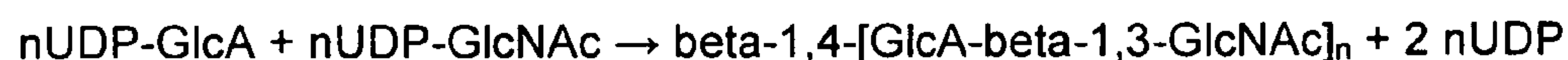
In the context of the present invention, the term "glucosaminoglycan synthase" is to be understood as meaning a protein which synthesizes glucosaminoglycan from the substrates UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetyl-aldohexsamine
10 (UDP-AldohexNAc). The catalysis of the glucosaminoglycans takes place according to the general reaction scheme below:



15 Preferably, the UDP-N-acetyl-aldohexsamine prepared in the above reaction sequence is UDP-N-acetyl-glucosamine or UDP-N-acetyl-galactosamine.

In a preferred embodiment, the present invention relates to plant cells according to the invention or plants according to the invention where the foreign nucleic acid
20 molecule coding for a glucosaminoglycan synthase is coding for a hyaluronan synthase or a chondroitin synthase or a heparin/heparosan synthase.

In the context of the present invention, the term "hyaluronan synthase" (EC 2.4.1.212) is to be understood as meaning a protein which synthesizes
25 hyaluronan from the substrates UDP-glucuronic acid (UDP-GlcA) and UDPN-acetyl-glucosamine (UDP-GlcNAc). The synthesis of hyaluronan is catalyzed according to the reaction scheme below:



30

The hyaluronan synthases which have hitherto been studied can be classified into two groups: hyaluronan synthases of Class I and hyaluronan synthases of Class II (DeAngelis, 1999, CMLS, Cellular and Molecular Life Sciences 56, 670-682).

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The hyaluronan synthases from vertebrates are distinguished further by the isoenzymes identified. The various isoenzymes are referred to by Arab numerals in the order of their identification (for example hsHAS1, hsHAS2, hsHAS3).

- 5 Nucleic acid molecules and corresponding protein sequences coding for hyaluronan synthases have been described, inter alia, for the following organisms: rabbit (*Oryctolagus cuniculus*) ocHas2 (EMBL AB055978.1, US 20030235893), ocHas3 (EMBL AB055979.1, US 20030235893); baboon (*Papio anubis*) paHas1 (EMBL AY463695.1); frog (*Xenopus laevis*) xlHas1 (EMBL M22249.1, US 20030235893),
 10 xlHas2 (DG42) (EMBL AF168465.1), xlHas3 (EMBL AY302252.1); human (*Homo sapiens*) hsHAS1 (EMBL D84424.1, US 20030235893), hsHAS2 (EMBL U54804.1, US 20030235893), hsHAS3 (EMBL AF232772.1, US 20030235893); mouse (*Mus musculus*), mmHas1 (EMBL D82964.1, US 20030235893), mmHAS2 (EMBL U52524.2, US 20030235893), mmHas3 (EMBL U86408.2, US 20030235893); cattle
 15 (*Bos taurus*) btHas2 (EMBL AJ004951.1, US 20030235893); chicken (*Gallus gallus*) ggHas2 (EMBL AF106940.1, US 20030235893); rat (*Rattus norvegicus*) rnHas 1 (EMBL AB097568.1, Itano et al., 2004, J. Biol. Chem. 279(18) 18679-18678), rnHas2 (EMBL AF008201.1); rnHas 3 (NCBI NM_172319.1, Itano et al., 2004, J. Biol. Chem. 279(18) 18679-18678), horse (*Equus caballus*) ecHAS2 (EMBL AY056582.1,
 20 GI:23428486), pig (*Sus scrofa*) sscHAS2 (NCBI NM_214053.1, GI:47522921), sscHas 3 (EMBL AB159675), zebra fish (*Danio rerio*) brHas1 (EMBL AY437407), brHas2 (EMBL AF190742.1) brHas3 (EMBL AF190743.1); *Pasteurella multocida* pmHas (EMBL AF036004.2); *Streptococcus pyogenes* spHas (EMBL, L20853.1, L21187.1, US 6,455,304, US 20030235893); *Streptococcus equis* seHas (EMBL
 25 AF347022.1, AY173078.1), *Streptococcus uberis* suHasA (EMBL AJ242946.2, US 20030235893), *Streptococcus equisimilis* seqHas (EMBL AF023876.1, US 20030235893); *Sulfolobus solfataricus* ssHAS (US 20030235893), *Sulfolobus tokodaii* stHas (AP000988.1), *Paramecium bursaria* Chlorella virus 1, cvHAS (EMBL U42580.3, PB42580, US 20030235893).

30

In a preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where the foreign nucleic acid molecule coding for a glucosaminoglycan

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synthase is characterized in that it codes for a hyaluronan synthase. The foreign nucleic acid molecule in question coding for a hyaluronan synthase is with preference a foreign nucleic acid molecule coding for a viral hyaluronan synthase. Preferably, the foreign nucleic acid molecule coding for a hyaluronan synthase codes for a
5 hyaluronan synthase of a virus which infects algae.

With respect to an algae-infecting virus, the foreign nucleic acid molecule which codes for a hyaluronan synthase preferably codes for a hyaluronan synthase of a, *Chlorella*-infecting virus, particularly preferably a hyaluronan synthase of a *Paramecium bursaria Chlorella* virus 1 and especially preferably a hyaluronan
10 synthase of a *Paramecium bursaria Chlorella* virus of an H1 strain.

Preferably, the foreign nucleic acid molecule coding for a hyaluronan synthase is characterized in that it codes for a hyaluronan synthase whose amino acid sequence is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the amino
15 acid sequence shown under SEQ ID NO 2. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for a hyaluronan synthase is characterized in that it codes for a hyaluronan synthase which has the amino acid sequence shown under SEQ ID No 2.

In a further embodiment, the foreign nucleic acid molecule coding for a hyaluronan
20 synthase is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence shown under SEQ ID NO 1 or SEQ ID NO 3. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for a hyaluronan synthase is characterized in that it has the nucleic acid sequence shown
25 under SEQ ID No 3 or that the sequence of the foreign nucleic acid molecule differs from the nucleic acid sequence shown under SEQ ID No 1 or 3 owing to the degeneration of the genetic code.

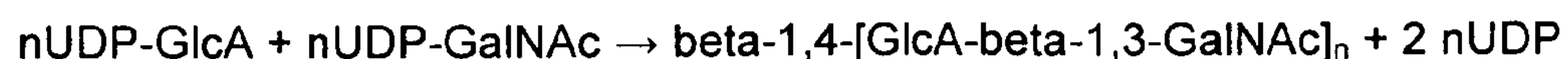
On 8. 25. 2004, the plasmid IC 341-222, comprising a synthetic nucleic acid molecule
30 coding for a *Paramecium bursaria Chlorella* virus hyaluronan synthase was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Brunswick, Germany, under the number DSM16664, in accordance with the Budapest treaty. The amino acid sequence shown under SEQ

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ID NO 2 can be derived from the coding region of the nucleic acid sequence integrated into the plasmid IC 341-222 and codes for a *Paramecium bursaria Chlorella* virus hyaluronan synthase.

- 5 Accordingly, the present invention also relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where the nucleic acid molecule which codes for the hyaluronan synthase is characterized in that it codes for a protein whose amino acid sequence can be derived from the coding region of the nucleic acid sequence inserted into plasmid
10 DSM16664 or that it codes for a protein whose amino acid sequence is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the amino acid sequence which can be derived from the coding region of the nucleic acid sequence inserted into plasmid DSM16664.
- 15 The present invention also relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where the foreign nucleic acid molecule coding for hyaluronan synthase is characterized in that it is the hyaluronan-synthase-encoding nucleic acid sequence integrated into plasmid DSM16664 or that it is at least 70%, with preference at least 80%, preferably at least
20 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence integrated into plasmid DSM16664.

In the context of the present invention, the term "chondroitin synthase" (EC 2.4.1.175, EC 1.4.1.226) is to be understood as meaning a protein or a protein complex,
25 consisting of two proteins which synthesizes chondroitin from the substrates UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetyl-galactosamine (UDP-GalNAc). The synthesis of chondroitin is catalyzed according to the reaction scheme below:



30

In some organisms, the elongation of the chondroitin molecule attached to a proteoglycan is catalyzed by a chondroitin synthase enzyme complex which consists of two different proteins. One of the two proteins, N-acetylgalactosaminyltransferase

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II (EC 2.4.1.175), adds N-acetyl-galactosamine monomers via a beta-1,4-attachment, the second protein, N-acetylgalactosaminyl-proteoglycan 3-beta-glucuronosyltransferase (EC 2.4.1.226), adds glucuronate monomers via a beta-1,3-attachment to the chondroitin molecule. However, the person skilled in the art is also
5 familiar with bifunctional proteins where a single protein adds both N-acetyl-galactosamine monomers and glucuronate monomers to the chondroitin molecule.

In a further preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants
10 according to the invention where the foreign nucleic acid molecule coding for glucosaminoglycan synthase is characterized in that it codes for a chondroitin synthase.

A preferred embodiment of the present invention relates to plant cells according to the invention or plants according to the invention where the foreign nucleic acid
15 molecule coding for a chondroitin synthase codes for a bifunctional chondroitin synthase which attaches both N-acetyl-galactosamine monomers and glucuronate monomers to the chondroitin molecule.

In the context of the present invention, the term "bifunctional chondroitin synthase" is
20 to be understood as meaning a protein where the activity of an N-acetylgalactosaminyltransferase II (EC 2.4.1.175) and the activity of an acetylgalactosaminyl-proteoglycan 3-beta-glucuronosyltransferase (EC 2.4.1.226) are present in one molecule.

25 Nucleic acid molecules and amino acid sequences derived therefrom coding for monofunctional chondroitin synthases have been described, for example, from bacteria (for example *Escherichia coli*, US2003109693, EP 1283259).

Nucleic acid molecules and amino acid sequences derived therefrom, coding for bifunctional chondroitin synthases have been described, for example, from mammals
30 (for example *Homo sapiens*, WO 03 012099, US 2005048604, US 2006052335, NCBI acc. No: BC046247.1, BC023531.2; Kitagawa et al., 2001, J. Biol. Chem. 276(42), 38721-38726) or *Pasteurella multocida* (US 2003104601, EMBL acc. No: AF195517, DeAngelis and Padgett-McCue, 2000, J. Biol. Chem. 275(31), 24124-

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24129).

The foreign nucleic acid molecule coding for a chondroitin synthase is with preference a foreign nucleic acid molecule coding for a bacterial chondroitin synthase, preferably coding for a chondroitin synthase from *Pasteurella*, particularly preferably coding for a chondroitin synthase from *Pasteurella multocida*.

Preferably, the foreign nucleic acid molecule coding for a chondroitin synthase is characterized in that it codes for a chondroitin synthase whose amino acid sequence is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the amino acid sequence shown under SEQ ID NO 5. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for a chondroitin synthase is characterized in that it codes for a chondroitin synthase which has the amino acid sequence shown under SEQ ID No 5.

In a further embodiment, the foreign nucleic acid molecule coding for a chondroitin synthase is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence shown under SEQ ID NO 4. In a particularly preferred embodiment, the nucleic acid molecule coding for the chondroitin synthase is characterized in that it has the nucleic acid sequence shown under SEQ ID No 4 or that the sequence of the foreign nucleic acid molecule differs from the nucleic acid sequence shown under SEQ ID No 4 owing to the degeneration of the genetic code.

In the context of the present invention, the term "heparin/heparosan synthase" or „heparosan synthase“ (EC 2.4.1.224, EC 2.4.1.225) is to be understood as meaning a protein or an enzyme complex consisting of two proteins which synthesizes heparin/heparan from the substrates UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetyl-glucosamine (UDP-GlcNAc). The synthesis of heparin/heparan is catalyzed according to the reaction scheme below:

30



Nucleic acid molecules and amino acid sequences derived therefrom, coding for a

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heparin/heparosan synthase have been described, for example, from bacteria (*Pasteurella multocida* EMBL acc. Nos: AF425591, AF439804, AY292199, AY292200, US 20030099967, *Escherichia coli* EMBL acc. No: X77617.1) or humans (NCBI acc. Nos: BC001174.1, NM_207122.1).

5

In some organisms, the elongation of the heparin/heparosan molecule attached to a proteoglycan is catalyzed by a heparin/heparosan synthase enzyme complex which consists of two different proteins. One of the two proteins, glucuronosyl-N-acetylglucosaminyl-proteoglycan 4-alpha-N-glucosaminyltransferase (EC 2.4.1.224),
10 adds N-acetyl-glucosamine monomers via a beta-1,4-attachment, the second protein, N-acetylglucosaminyl-proteoglycan 4-beta-glucuronosyltransferase (EC 2.4.1.225), adds glucuronate monomers via a beta-1,3-attachment to the heparin/heparan molecule. However, the person skilled in the art is also familiar with bifunctional proteins where a single protein adds both N-acetyl-glucosamine monomers and
15 glucuronate monomers to the heparin/heparosan molecule. Such bifunctional heparin/heparosan synthases have been described, for example, from humans (Busse and Kusche-Gullberg, 2003, J. Biol. Chem. 278(42), 41333-41337) or from *Pasteurella* (DeAngelis and White, 2004, J. Bacteriology 186(24), 8529-8532). Bifunctional proteins having the activity of a heparin/heparosan synthase have both
20 the activity of an enzyme classified under EC number 2.4.1.224 and the activity of an enzyme classified under EC number 2.4.1.225.

In a further preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants
25 according to the invention where the foreign nucleic acid molecule coding for a glucosaminoglycan synthase is characterized in that it codes for a heparin/heparosan synthase.

A preferred embodiment of the present invention relates to plant cells according to the invention or plants according to the invention where the foreign nucleic acid
30 molecule coding for a heparin/heparosan synthase codes for a bifunctional heparin/heparan synthase which attaches both N-acetyl-glucosamine monomers and glucuronate monomers to the heparin/heparan molecule.

The foreign nucleic acid molecule coding for a heparin/heparosan synthase is with

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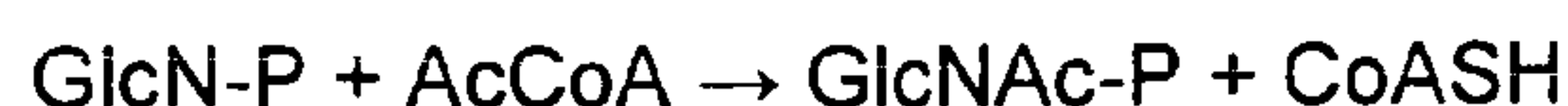
preference a foreign nucleic acid molecule coding for a bacterial heparin/heparosan synthase, preferably coding for a heparin/heparosan synthase from *Pasteurella*, particularly preferably coding for a heparin/heparosan synthase from *Pasteurella multocida*.

5

Preferably, the foreign nucleic acid molecule coding for heparin/heparosan synthase is characterized in that it codes for a heparin/heparosan synthase whose amino acid sequence is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to
10 the amino acid sequence shown under SEQ ID NO 7. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for the heparin/heparosan synthase is characterized in that it codes for a heparin/heparosan synthase which has the amino acid sequence shown under SEQ ID No 7.

In a further embodiment, the foreign nucleic acid molecule coding for a
15 heparin/heparosan synthase is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence shown under SEQ ID NO 6. In a particularly preferred embodiment, the nucleic acid molecule coding for a heparin/heparosan synthase is characterized in that it has the nucleic acid sequence
20 shown under SEQ ID No 6 or that the sequence of the foreign nucleic acid molecule differs from the nucleic acid sequence shown under SEQ ID No 6 owing to the degeneration of the genetic code.

In the context of the present invention, the term "glucosamine phosphate N-
25 acetyltransferase (acetyl-CoA:D-glucosamine phosphate N-acetyltransferase or GlcN-P acetyltransferase)" (EC 2.3.1.4) is to be understood as meaning a protein which synthesizes N-acetyl-D-glucosamine phosphate (GlcNAc-P) from the substrates D-glucosamine phosphate (GlcN-P) and acetyl-CoA (AcCoA). The synthesis of N-acetyl-glucosamine 6-phosphate is catalyzed according to the reaction
30 scheme below:



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In the reaction equation shown, the substrate GlcN-P may be either glucosamine 1-phosphate (GlcN-1-P) or glucosamine 6-phosphate (GlcN-6-P).

In the metabolic pathways in question for the synthesis of UDP-N-acetyl-glucosamine, an essential difference between the bacterial and eukaryotic organisms studied is that different intermediates of the metabolic pathway in question are used as substrate for the acetylation reaction. In bacterial organisms, the acetylation of GlcN-1-P is carried out by a protein having the activity of a GlcN-1-P acetyltransferase (EC 2.3.1.157) (Gehring et al., 1996, Biochemistry 35, 579-585), whereas in eukaryotic animals or fungi the acetylation of GlcN-6-P is catalyzed by a protein having the activity of a glucosamine 6-phosphate acetyltransferase (EC 2.3.1.4) (Milewski et al., 2006, Yeast 23, 1-14, published online in Wiley InterScience, DOI: 10.1002./yea.1337). Accordingly, in the different organisms, both different substrates and different proteins are used for the synthesis of UDP-GlcNAc.

Surprisingly, it has been found that, in contrast to the disclosure in the prior art (WO 2007 023682), it is not possible to increase the amount of glucosaminoglycans synthesized in plant cells by introducing any nucleic acid molecule coding for a protein having the activity of a GlcN-P acetyltransferase into plant cells. Rather, it has been found that the introduction of a foreign nucleic acid molecule coding for a protein having the activity of a GlcN-P acetyltransferase which acetylates GlcN-1-P (for example *glmu* from *Escherichia coli*) does not lead to an increase in the amount of glucosaminoglycans synthesized by plant cells or plants. Accordingly, it is essential for plant cells according to the invention or plants according to the invention that the foreign nucleic acid molecule codes for a protein having the activity of a GlcN-P acetyltransferase which uses GlcN-6-P as substrate for the acetylation reaction and is thus a protein having the activity of a GlcN-6-P acetyltransferase (EC 2.3.1.4). In contrast, foreign nucleic acid molecules coding for proteins having the activity of a GlcN-1-P acetyltransferase (EC 2.3.1.157), which use GlcN-1-P for the acetylation reaction are not suitable for producing plant cells according to the invention or plants according to the invention.

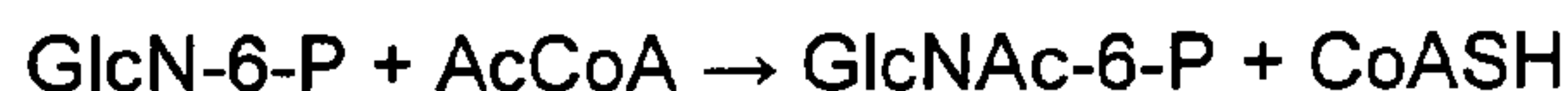
Furthermore, it has been found that plant cells or plants having a foreign nucleic acid molecule coding for a glucosamine 6-phosphate mutase (GlcN-6-P mutase), which catalyzes the isomerization of GlcN-6-P to GlcN-1-P, do not synthesize significantly

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higher amounts of glucosaminoglycan.

In the context of the present invention, the term "glucosamine 6-phosphate acetyltransferase (acetyl-CoA:D-glucosamine 6-phosphate N-acetyltransferase or
5 GlcN-6-P acetyltransferase)" (EC 2.3.1.4) is meant to be understood as a protein which synthesizes N-acetyl-D-glucosamine 6-phosphate (GlcNAc-6-P) from the substrates D-glucosamine 6-phosphate (GlcN-6-P) and acetyl-CoA (AcCoA). The synthesis of N-acetyl-glucosamine 6-phosphate is catalyzed according to the reaction scheme below:

10



The functional form of a protein having the activity of a GlcN-6-P acetyltransferase is a homodimer. The tertiary structure of the monomer has a central core region. This
15 core region consists of a beta-sheet structure having five antiparallel strands (beta strands 1 – 5) which are surrounded by four alpha-helices and a sixth beta strand (beta-6 strand). During the formation of the homodimer, there is an interaction of a beta-6 strand of a subunit with the corresponding beta-6 strand of the respective other subunit.

20 The amino acid sequence shown under SEQ ID No 9 (EMBL acc. No: AB017626.1) codes for a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae*. In the amino acid sequence shown under SEQ ID No 9, the amino acids 7 – 11 form the beta-1 strand, the amino acids 13 - 26 form the alpha-1 strand, the amino acids 37 – 47 form the alpha-2 strand, the amino acids 62
25 – 69 form the beta-2 strand, the amino acids 74 – 86 form the beta-3 strand, the amino acids 92 – 103 form the beta-4 strand, the amino acids 111 – 125 form the alpha-3 strand, the amino acids 130 – 136 form the beta-5 strand, the amino acids 139 – 146 form the alpha-3 strand and the amino acids 154 – 159 form the beta-6 strand. The amino acids Glu (position 98), Asp (position 99) and Ile (position 100)
30 present in the beta-4 strand in the sequence shown under SEQ ID No 9 interact with the substrate AcCoA, they polarize its carbonyl bond and they stabilize the negative charge of the oxygen atom of the AcCoA in the tetraedric reaction intermediate consisting of AcCoA and GlcN-6-P and GlcN-6-P acetyltransferase. The amino acid

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Tyr (position 143) in the sequence shown under SEQ ID No 9 stabilizes the thiolate anion of the CoA molecule to be cleaved off. These interactions during the catalysis of the reaction are supported by the amino acid Leu (position 133) in the sequence shown under SEQ ID No 9. During the catalysis of the reaction, GlcN-6-P is bound in
 5 a pocket formed between the monomers of the homodimer, with amino acid residues of the beta-6 strand participating in its formation. During the catalysis of the reaction, the amino acid Asp (position 134) in the sequence shown under SEQ ID No 9 increases the nucleophilicity of the amino group of GlcN-6-P (Milewski et al., 2006, published online in Wiley InterScience, Corporate Headquarters, 111 River Street, Hoboken, NJ 07030-5774, USA
 10 DOI:10.1002/yea.1337). Further amino acids of a protein having the activity of a GlcN-6-P acetyltransferase which are involved in the catalysis of the reaction in question are described in Peneff et al. (2004, J. Biological Chemistry 276(19), 16328-16334, Fig. 1).

The amino acids which are involved here in an exemplary manner for the amino acid
 15 sequence of *Saccharomyces cerevisiae* in the catalysis of the reaction can also be identified in amino acid sequences coding for proteins having the activity of a GlcN-6-P acetyltransferase from other organisms. These are, for example, the amino acids Glu88, Asp80, Ile90, Asp124 and Tyr133 in the amino acid sequence coding for a protein having the activity of a GlcN-6-P acetyltransferase from *Candida albicans*
 20 (EMBL acc. No: AB017627.1).

Nucleic acid molecules and corresponding protein sequences coding for proteins having the activity of a GlcN-6-P acetyltransferase have been described, inter alia, for the following organisms: *Saccharomyces cerevisiae* (EMBL acc. No: AB017626.1), *Schizosaccharomyces pombe* (EMBL acc. No: AB017629.1), *Candida albicans* (EMBL acc. No: AB017627.1), *Aspergillus oryzae* (EMBL CDS acc. No: BAE62756.1), *Caenorhabditis elegans* (NCBI acc. No: NM_073253.4, EMBL CDS acc. No: BAA63497.1, CAA044531.1), *Drosophila melanogaster* (EMBL CDS acc. No: AAL13916.1), *Xenopus traopicalis* (EMBL acc. No: CR760021.2), *Mus musculus*
 25 (EMBL CDS acc. No: BAE39886.1), *Homo sapiens* (EMBL CDS acc. No: BAC03482.1), *Pongo pygmaeus* (EMBL CDS acc. No: CR858996.1), *Acanthamoeba polyphaga* mimivirus (EMBL CDS acc. No: AAV50586.1). Although, as already described, the amino acid residues involved in the catalysis of the reaction are
 30

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conserved in proteins originating from various organisms having the activity of a GlcN-6-P acetyltransferase, in some cases their sequences have very low identity to one another. Thus, the amino acid sequence coding for a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae* (EMBL acc. No: AB017626.1) is only 44% identical to the corresponding sequence from *Candida albicans* (EMBL acc. No: AB017627.1) and even has only 25% identity to that from *Schizosaccharomyces pombe* (EMBL acc. No: AB017629.1) (Milewski et al., 2006, published online in Wiley InterScience, Corporate Headquarters, 111 River Street, Hoboken, NJ 07030-5774, USA DOI:10.1002/yea.1337). In spite of the low identity of the amino acid sequences in question to one another, all abovementioned sequences coding for a protein having the activity of a GlcN-6-P-acetyltransferase are suitable for producing plant cells according to the invention or plants according to the invention.

According to the invention, the foreign nucleic acid molecule coding for a protein having the enzymatic activity of a GlcN-6-P acetyltransferase may originate from any organism; preferably, said nucleic acid molecule originates from fungi, animals or plants, particularly preferably from fungi and especially preferably from *Saccharomyces cerevisiae*.

Preferably, the foreign nucleic acid molecule coding for GlcN-6-P acetyltransferase is characterized in that it codes for a GlcN-6-P acetyltransferase whose amino acid sequence is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the amino acid sequence shown under SEQ ID NO 9. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for a protein having the activity of a GlcN-6-P acetyltransferase is characterized in that it codes for a protein having the activity of a GlcN-6-P acetyltransferase which has the amino acid sequence shown under SEQ ID No 9.

In a further embodiment, the foreign nucleic acid molecule coding for a protein having the activity of a GlcN-6-P acetyltransferase is at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence shown under SEQ ID NO 8. In a particularly preferred embodiment, the nucleic acid molecule coding for a GlcN-6-P acetyltransferase is characterized in that it has the nucleic acid sequence

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shown under SEQ ID No 8 or that the sequence of the foreign nucleic acid molecule differs from the nucleic acid sequence shown under SEQ ID No 8 owing to the degeneration of the genetic code.

- 5 In the context of the present invention, the term "UDP-GlcNAc pyrophosphorylase (2-acetamido-2-deoxy-d-glucose 1-phosphate uridyltransferase) (EC 2.7.7.23)" is to be understood as meaning a protein which synthesizes UDP-N-acetyl-glucosamine (UDP-GlcNAc) from the substrates uridine triphosphate (UTP) and N-acetyl-D-glucosamine 1-phosphat (GlcNAc-1-P). The synthesis of UDP-GlcNAc is catalyzed
10 according to the reaction scheme below:



- Procaryotic proteins having the activity of a UDP-GlcNAc pyrophosphorylase are
15 generally bifunctional proteins which, in addition to the reaction shown above (EC 2.7.7.23), have the function of a glucosamine 1-phosphate acetyltransferase (GlcN-1-P acetyltransferase, EC 2.3.1.157), i.e. they catalyze an N-acetylation of glucosamine 1-phosphate (GlcN-1-P) to N-acetyl-glucosamine 1-phosphate (GlcNAc-1-P) ($\text{GlcN-1-P} + \text{AcCoA} \rightarrow \text{GlcNAc-1-P} + \text{CoASH}$) (Gehring et al., 1996,
20 Biochemistry 35, 579-585). In contrast, eukaryotic proteins having the activity of a UDP-GlcNAc pyrophosphorylase are monofunctional proteins which only catalyze the reaction described above ($\text{UTP} + \text{GlcNAc-1-P} \rightleftharpoons \text{P-P} + \text{UDP-GlcNAc}$) (Mio et al., 1998, J. Biol. Chem. 273 (23), 14392-14397).

- 25 In the context of the present invention, the term "monofunctional proteins having the activity of a UDP-GlcNAc pyrophosphorylase" is to be understood as meaning a protein which catalyzes the reaction shown above for a protein having the activity of a UDP-GlcNAc pyrophosphorylase ($\text{UTP} + \text{GlcNAc-1-P} \rightleftharpoons \text{P-P} + \text{UDP-GlcNAc}$). A monofunctional protein having the activity of a UDP-GlcNAc pyrophosphorylase has
30 no (additional) activity which catalyzes an acetylation of GlcN-1-P to GlcNAc-1-P. Accordingly, monofunctional proteins having the activity of a GlcNAc pyrophosphorylase are therefore proteins classified under the EC number 2.7.7.23, whereas bifunctional proteins having the activity of a UDP-GlcNAc

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pyrophosphorylase have both the activity of an enzyme classified under the EC number 2.7.7.23 and the activity of an enzyme classified under the EC number 2.3.1.157.

5 Surprisingly, it has been found that, in contrast to the disclosure in the prior art (for example WO 2007 023682), that plant cells according to the invention or plants according to the invention having a foreign nucleic acid molecule coding for a bifunctional protein having the activity of a UDP-GlcNAc pyrophosphorylase and the activity of a GlcN-1-P acetyltransferase (for example *glmU* aus *E. coli*, EC 2.7.7.23
10 and EC 2.3.1.157) do not synthesize increased amounts of glucosaminoglycans. Accordingly, it is essential for plant cells according to the invention or plants according to the invention that the foreign nucleic acid molecule coding for a UDP-GlcNAc pyrophosphorylase codes for a monofunctional protein having the activity of a UDP-GlcNAc pyrophosphorylase (EC 2.7.7.23). Accordingly, the foreign nucleic
15 acid molecule coding for a UDP-GlcNAc pyrophosphorylase should not code for a protein which, in addition to the just mentioned activity of a UDP-GlcNAc pyrophosphorylase has the additional activity of a GlcN-1-P acetyltransferase (EC 2.3.1.157). Thus, it is preferably a foreign nucleic acid molecule of eukaryotic origin. Furthermore, it has surprisingly been found that, in contrast to the disclosure in the
20 prior art (for example WO 2007 023682), the expression of a phosphoacetylglucosamine mutase (GlcNAc-P mutase, EC 5.4.2.3) in addition to the expression of a protein having the activity of a GlcN-6-P acetyltransferase and the expression of a protein having the activity of a UDP-GlcNAc pyrophosphorylase does not lead to a further increase in the amount of glucosaminoglycans in plant cells or
25 plants.

Amino acid sequences coding for monofunctional proteins having the activity of a UDP-GlcNAc pyrophosphorylase comprise amino acid residues which are highly conserved between the proteins. Amino acid sequences coding for eukaryotic
30 proteins having the activity of a UDP-GlcNAc pyrophosphorylase have in each case three domains which are conserved between the proteins. The consensus sequence of the first domain is GlyGlyGlnXxxThrArgLeuGlyXxxXxxXxxProLysGly (amino acids 111-124 in the sequence shown under SEQ ID No 11), that of the second domain is

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Pro(Asp or Asn)GlyAsn(Gly or Ala)GlyXxxXxxXxxAla (amino acids 219-228 in the sequence shown under SEQ ID No 11) and that of the third domain is LysXxxGluXxxPheXxxPheAspXxxPhe (amino acids 377-386 in the sequence shown under SEQ ID No 11), where Xxx is any amino acid. Prokaryotic proteins having the activity of a UDP-GlcNAc pyrophosphorylase (for example glmU aus *Escherichia coli*, EMBL acc. No: EAY46949.1) have a conserved domain (GlyXxxGlyThr(Arg or Ser)XxxXxxXxxXxxProLys) which is similar to the first domain of corresponding proteins from eukaryotes. For the domains two and three of the eukaryotic proteins, no corresponding domains are found in the prokaryotic proteins. (Mok and Edwards, 2000, J. Biol. Chem. 280(47), 39363-39372)

The amino acids Gly (position 112), Gly (position 114), Thr (position 115), Arg (position 116), Pro (position 122) and Lys (position 123) in the amino acid sequence shown under SEQ ID No 11 are conserved in the primary sequences coding for proteins having the activity of a UDP-GlcNAc pyrophosphorylase. An exchange of the amino acids Gly (position 112), Arg (position 116), or Lys (position 123) in the amino acid sequence shown under SEQ ID No 11 leads to virtually inactive proteins. In contrast, an exchange of the amino acids Gly (position 114), Thr (position 115) or Pro (position 122) in the amino acid sequence shown under SEQ ID No 11 shows only a reduction of the activity of the protein in question. Accordingly, the amino acids Gly (position 112), Arg (position 116) and Lys (position 123) in the amino acid sequence shown under SEQ ID No 11 are amino acids which have a catalytic function in proteins having the activity of a UDP-GlcNAc pyrophosphorylase (Mio et al., 1998, J. Biol. Chem. 273(23), 14392-14397).

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In the amino acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Giardia intestinales* (EMBL acc. No: AAM54702.1), the amino acid Gly (position 108) corresponds to the amino acid Gly (position 112) of the the sequence shown under SEQ ID No 11. The exchange of the amino acid Gly (position 108) in the amino acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Giardia intestinales* via the amino acid Ala also results in an almost complete reduction of the activity of the protein (Mok and Edwards, 2005, J. Biol. Chem. 280(47), 39363-39372). The exchange of the amino

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acid Gly (position 111) in the amino acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Homo sapiens* (EMBL acc. No: BAA31202.1), which corresponds to the amino acid Gly (position 112) in the sequence shown under SEQ ID No 11, also leads to almost complete reduction of the activity (Wang-Gillam et al., 2000, J. Biol. Chem. 275(2), 1433-1438).

An exchange of the amino acid Gly (position 222) in the protein coding for a UDP-GlcNAc pyrophosphorylase from *Homo sapiens* (EMBL acc. No: BAA31202) and the corresponding amino acid Gly (position 210) of a corresponding protein from *Giardia intestinales* (EMBL acc. No: AAM54702.1) results in both cases likewise in an almost complete loss of activity, which indicates that the amino acids mentioned are likewise amino acids involved in the catalysis (Mok and Edwards, 2005, J. Biol. Chem. 280(47), 39363-39372). An exchange of the amino acid Gly (position 224) in the protein coding for a UDP-GlcNAc pyrophosphorylase from *Homo sapiens* (EMBL acc. No: BAA31202) lead to a considerable, but not complete, loss of the activity of the protein, and an exchange of the amino acid Pro (position 222) resulted in only a slight reduction of activity. From this, it was concluded that the amino acids Gly (position 221) and Gly (position 223) of the sequence shown under SEQ ID No 11 take part in the recognition of the UTP and amino acids Gly (position 111) and Gly (position 112), conserved in the respective primary sequences, of the sequence shown under SEQ ID No 11 are involved in binding GlcNAc-1-P (Wang-Gillam et al., 2000, J. Biol. Chem. 275(2), 1433-1438).

Nucleic acid molecules and corresponding protein sequences coding for proteins having the monofunctional activity of a UDP-GlcNAc pyrophosphorylase having the properties mentioned above have been described inter alia for the following organisms: *Giardia intestinales* (EMBL acc. No: AAM54702.1), *Saccharomyces cerevisiae* (EMBL acc. No: X79380.1, NCBI protein ID: accession No: CAA557927), *Candida albicans* (NCBI acc. No: XM_715480.1), *Pichia stipitis* (NCBI acc. No: XM_001385151.1), *Mus musculus* (NCBI acc. No: NM_133806.4), *Canis lupus* (NCBI acc. No: XM_844774.1); *Bos taurus* (NCBI acc. No: NM_001046404.1), *Xenopus tropicalis* (NM_001011142.1), *Xenopus laevis* (NCBI acc. No: BC077836.1), *Arabidopsis thaliana* (NCBI acc. No: NM_102845.4), *Danio rerio* (NCBI acc. No: NM_212621.1), *Homo sapiens* (NCBI acc. No: NM_003115.3, EMBL acc.No.:

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BAA31202.1).

According to the invention, the foreign nucleic acid molecule coding for a protein having the enzymatic activity of a UDP-GlcNAc pyrophosphorylase may originate
5 from any eukaryotic organism; preferably, said nucleic acid molecule originates from fungi, animals or plants, particularly preferably from fungi, especially preferably from *Saccharomyces cerevisiae*.

Preferably, the foreign nucleic acid molecule coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase is characterized in that it codes for a UDP-
10 GlcNAc pyrophosphorylase whose amino acid sequence at least 70%, with preference at least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the amino acid sequence shown under SEQ ID NO 11. In a particularly preferred embodiment, the foreign nucleic acid molecule coding for a protein having the activity of a UDP-GlcNAc
15 pyrophosphorylase is characterized in that it codes for a protein having the activity of a UDP-GlcNAc pyrophosphorylase having the amino acid sequence shown under SEQ ID No 11.

In a further embodiment, the foreign nucleic acid molecule coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase is at least 70%, with preference at
20 least 80%, preferably at least 90%, particularly preferably at least 95% and especially preferably at least 98% identical to the nucleic acid sequence shown under SEQ ID NO 10. In a particularly preferred embodiment, the nucleic acid molecule coding for a UDP-GlcNAc pyrophosphorylase is characterized in that it has the nucleic acid sequence shown under SEQ ID No 10 or that the sequence of the foreign nucleic
25 acid molecule differs from the nucleic acid sequence shown under SEQ ID No 10 owing to the degeneration of the genetic code.

In the context of the present invention, the term "foreign nucleic acid molecule" is to be understood as meaning a molecule which either does not naturally occur in the
30 corresponding wild-type plant cells or which does not naturally occur in the concrete 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.

Preferably, the foreign nucleic acid molecule is a recombinant molecule comprising

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various elements whose combination or specific spatial arrangement does not naturally occur in plant cells.

In the context of the present invention, the term "recombinant nucleic acid molecule" is to be understood as meaning a nucleic acid molecule which comprises various nucleic acid molecules which are not naturally present in a combination like that present in a recombinant nucleic acid molecule. Thus, recombinant nucleic acid molecules may, in addition to nucleic acid molecules coding for a glucosaminoglycan synthase and/or a protein having the activity of a GlcN-6-P acetyltransferase and/or a protein having the activity of a UDP-GlcNAc pyrophosphorylase, additionally comprise nucleic acid sequences which are not naturally present in combination with the nucleic acid molecules mentioned. The additional nucleic acid sequences mentioned which are present on a recombinant nucleic acid molecule in combination with a nucleic acid molecule coding for a glucosaminoglycan synthase and/or a protein having the activity of a GlcN-6-P acetyltransferase and/or a protein having the activity of a UDP-GlcNAc pyrophosphorylase may be any sequences. For example, they may be genomic plant nucleic acid sequences. The additional nucleic acid sequences are preferably regulatory sequences (promoters, termination signals, enhancers), particularly preferably regulatory sequences which are active in plant tissue, especially preferably tissue-specific regulatory sequences which are active in plant tissue. Methods for generating recombinant nucleic acid molecules are known to the person skilled in the art and comprise genetic engineering methods, such as, for example, linking of nucleic acid molecules by ligation, genetic recombination or the *de novo* 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).

A preferred embodiment of the present invention relates to plant cells according to the invention or plants according to the invention in which the foreign nucleic acid molecules are stably integrated into the genome of the plant cell or plant.

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Genetically modified plant cells and genetically modified plants having a foreign nucleic acid molecule (stably) integrated into their genome or a plurality of foreign nucleic acid molecules integrated into their genome which code for a glucosaminoglycan synthase and/or a protein having the activity of a GlcN-6-P acetyltransferase and/or a protein having the activity of a UDP-GlcNAc pyrophosphorylase can be distinguished from wild-type plant cells and wild-type plants inter alia by the fact that they comprise a foreign nucleic acid molecule which does not naturally occur in wild-type plant cells and wild-type plants, respectively, or in that such a molecule is integrated at a site in the genome of the genetically modified plant cell according to the invention or in the genome of the genetically modified plant according to the invention where it does not occur in wild-type plant cells and wild-type plants, respectively, i.e. in a different genomic environment. Furthermore, such genetically modified plant cells according to the invention and genetically modified plants according to the invention can be distinguished from not genetically modified wild-type plant cells and not genetically modified wild-type plants, respectively, in that they comprise at least one copy of the foreign nucleic acid molecule integrated into their genome, if appropriate in addition to copies of such a molecule naturally present in the wild-type plant cells or wild-type plants. If the foreign nucleic acid molecule(s) introduced into the genetically modified plant cells according to the invention or the genetically modified plant according to the invention are additional copies of molecules already naturally present in the wild-type plant cells or the wild-type plants, the genetically modified plant cells according to the invention and the genetically modified plants according to the invention can be distinguished from wild-type plant cells and wild-type plants, respectively, in particular by the fact that this additional copy/these additional copies is/are localized at sites in the genome where it/they is/are not present in wild-type plant cells and wild-type plants, respectively.

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 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

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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 of the RFLP analysis (Restriction Fragment Length Polymorphism) (Nam et al., 1989, The Plant Cell 1, 699-705; 5 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 10 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).

15 In the context of the present invention, the term "wild-type plant cell" is to be understood as meaning plant cells which served as starting material for the production of the genetically modified plant cells according to the invention, i.e. their genetic information, apart from the genetic modifications introduced and resulting in an integration of a nucleic acid molecule coding for a glucosaminoglycan synthase 20 and/or a protein having the activity of a GlcN-6-P acetyltransferase and/or a protein having the activity of a UDP-GlcNAc pyrophosphorylase corresponds to that of a genetically modified plant cell according to the invention.

In the context of the present invention, the term "wild-type plant" is to be understood 25 as meaning plants which served as starting material for the production of the genetically modified plants according to the invention, i.e. their genetic information, apart from the genetic modifications introduced and resulting in an integration of a nucleic acid molecule encoding for a glucosaminoglycan synthase and/or a protein having the activity of a GlcN-6-P acetyltransferase and/or a protein having the activity 30 of a UDP-GlcNAc pyrophosphorylase corresponds to that of a genetically modified plant according to the invention.

In the context of the present invention, the term "genome" is to be understood as

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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.

- 5 A large number of techniques for (stably) integrating nucleic acid molecules into a plant host cell 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",
10 Leandro ed., Humana Press 2004, ISBN 1-59259-827-7).

The use of agrobacterium-mediated transformation of plant cells has been subject to in-depth studies and has been described exhaustively in EP 120516; Hoekema, IN: The Binary Plant Vector System Offsetdrukkerij Kanters B.V. Alblasterdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant Sci. 4, 1-46 and in An et al. EMBO J. 4,
15 (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.

The transformation of monocotyledonous plants using vectors based on
20 *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). An alternative
25 system for transforming monocotyledonous plants is 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, the introduction of
30 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

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(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*, Somleva et al., 2002 Crop Science 42: 2080 - 2087; Richards et al., 2001, Plant Cell Reporters 20, 48-54) of sugar cane (Bower and Birch, 1992, Plant Journal 5 2(3), 409-416; Bower et al., 1996 Molecular Breeding 2, 239-249; Arencibia et al., 1998, Transgenic Research 7, 213-222) or millet (Casas et al., 1993, PNAS 90, 11212-11216; US 6,369,298) has also been described.

The successful transformation of other cereal species has also been described, for example for barley (Wan and Lemaux, s.o.; Ritala et al., s.o.; Krens et al., Nature 10 296, (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.

Compared to the prior art, genetically modified plant cells according to the invention 15 or genetically modified plants according to the invention offer the advantage that they produce higher amounts of glucosaminoglycan (for example hyaluronan) than plants having only the activity of a glucosamino-glycan synthase. This allows glucosaminoglycan to be produced at little expense since the isolation of glucosaminoglycan from plants having a higher glucosaminoglycan content is less 20 complicated and more cost efficient. Furthermore, compared to the plants described in the prior art, smaller cultivation areas are required to produce glucosaminoglycan using the genetically modified plants according to the invention. This leads to the possibility to provide glucosaminoglycan in sufficient amounts even for industrial application where it is currently not used owing to its scarcity and the high price. 25 Virus-infected plant organisms of the genus *Chlorella* are unsuitable for producing relatively large amounts of glucosaminoglycan (hyaluronan). In the production of glucosaminoglycan (hyaluronan), virus-infected algae have the disadvantage that the genes required for glucosaminoglycan synthase are not stably integrated into their genome (Van Etten and Meints, 1999, Annu. Rev. Microbiol. 53, 447-494), so that, 30 for producing glucosaminoglycan (hyaluronan), the virus infection has to be repeated. Accordingly, it is not possible to isolate individual *Chlorella* cells which synthesize continuously the desired quality and quantity of glucosaminoglycan (hyaluronan). Furthermore, in virus-infected *Chlorella* algae, glucosaminoglycan (hyaluronan) is

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only produced for a limited period of time, and as a result of the lysis caused by the virus, the algae are killed only about 8 hours after the infection (Van Etten et al., 2002, Arch Virol 147, 1479-1516). In contrast, the present invention offers the advantage that the genetically modified plant cells according to the invention and the
5 genetically modified plants according to the invention can be propagated in an unlimited manner vegetatively or sexually and that they produce glucosaminoglycan (hyaluronan) continuously.

The transgenic plants described in WO 05 012529, which have a nucleic acid molecule coding for a hyaluronan synthase, synthesize a relatively small amount of
10 glucosaminoglycan (hyaluronan). In contrast, the present invention offers the advantage that genetically modified plant cells according to the invention and genetically modified plants according to the invention synthesize considerably higher amounts of glucosaminoglycan.

15 Accordingly, the present invention also provides genetically modified plant cells according to the invention or genetically modified plants according to the invention which synthesize glucosaminoglycan.

In a preferred embodiment, plant cells according to the invention or plants according to the invention synthesize glucosaminoglycans selected from the group consisting of
20 chondroitin, heparin/heparosan and hyaluronan.

To determine the glucosaminoglycan content with respect to the fresh weight in genetically modified plants according to the invention, use is preferably made of the entire above-ground material of the plants, i.e. all plant parts except for the root.

25 Genetically modified plant cells according to the invention or genetically modified plants according to the invention which synthesize glucosaminoglycan can be identified by isolating the glucosaminoglycan synthesized by them and confirming its structure. Since plant tissue has the advantage that it does not contain any glucosaminoglycans, a simple and quick isolation method can be used for
30 demonstrating the presence of glucosaminoglycans in genetically modified plant cells according to the invention or genetically modified plants according to the invention. Since plant tissue furthermore has the advantage that it does not contain any glucosaminoglycan-degrading enzymes, a simple and quick isolation method can be

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used for demonstrating the presence of glucosaminoglycans in genetically modified plant cells according to the invention or genetically modified plants according to the invention. To this end, water is added to the plant tissue to be examined, and the plant tissue is then comminuted mechanically (for example with the aid of a bead mill,
5 a Warring Blender, a juice extractor, etc.). If required, more water may then be added to the suspension, and cell debris and water-insoluble components are then removed by centrifugation. The presence of glucosaminoglycans (for example hyaluronan) in the supernatant obtained after centrifugation can then be demonstrated using, for example, a protein which binds specifically to the relevant glucosaminoglycan (for
10 example hyaluronan).

Such test kits based on immunological reagents (ELISA) for various glucosaminoglycans are known to the person skilled in the art and commercially available (for example test kit for heparin: Lifespan Technologies, 2401 Foothill Drive, Salt Lake City, UT 84109-1405, Prod. No.: K-2100).

15 A method for the detection of hyaluronan with the aid of a protein which specifically binds to hyaluronan is described, for example, in US 5,019,498. Test kits for carrying out the method described in US 5,019,498 are commercially available (for example the hyaluronic acid (HA) test kit from Corgenix, Inc., Colorado, USA, Prod. No. 029-001; see also General Methods item 4).

20 Chondroitin can be detected, for example, with the aid of immunological methods (Mizuguchi et al., 2003, Nature 423, 443-448).

The presence of glucosaminoglycans in the centrifugation supernatant can furthermore also be confirmed using other analysis methods, such as, for example, IR, NMR or mass spectroscopy.

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Since it has been observed that, over the time of the development of plants according to the invention, glucosaminoglycans accumulate in plant tissue, the amount of glucosaminoglycan with respect to the fresh weight in genetically modified plants according to the invention is particularly preferably determined at the time of
30 harvest or (one or two) days before harvesting of the plants in question.

In a preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the

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invention, characterized in that they produce an increased amount of glucosaminoglycan compared to genetically modified plant cells or compared to genetically modified plants having only a foreign nucleic acid molecule coding for a glucosaminoglycan synthase or compared to genetically modified plant cells or
5 compared to genetically modified plants having a foreign nucleic acid molecule, coding for a glucosaminoglycan synthase and no foreign nucleic acid molecules, coding for proteins having the activity of a UDP-GlcNAc acetyltransferase and no foreign nucleic acid molecules coding for proteins having the activity of a UDP-GlcNAc pyrophosphorylase.

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Preferably, the amount of glucosaminoglycan produced with respect to the fresh weight of the plant material in genetically modified plant cells according to the invention or in genetically modified plants according to the invention is increased by a factor of at least 1.2, with preference by a factor of at least 1.4, particularly preferably
15 by a factor of at least 1.6 and especially preferably by a factor of at least 1.8 compared to corresponding genetically modified plant cells or compared to corresponding genetically modified plants having (only) the activity of a glucosaminoglycan synthase. To determine the increase of the glucosaminoglycan content with respect to the fresh weight of the plant material in genetically modified
20 plant cells according to the invention or in genetically modified plants according to the invention, use will preferably be made of a comparison between genetically modified plant cells according to the invention or genetically modified plants according to the invention with corresponding plant cells and plants, respectively, which (only) have glucosaminoglycan synthase activity, where equivalent material (for example leaf,
25 tuber) of plant cells or plants is to be compared where the plant cells or plants from which this material is taken have been cultivated under identical conditions and where the glucosaminoglycan content of plant material having a comparable age (development stage) is to be compared. For example, young leaves of a plant should not be compared to old leaves of a different plant. The same applies to the
30 determination of the glucosaminoglycan content of entire above-ground parts of plants. The plants to be compared should have been cultivated under comparable conditions and have the same development stage.

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In a preferred embodiment, the present invention relates to plant cells according to the invention or plants according to the invention synthesizing at least 160 µg, preferably at least 180 µg, particularly preferably at least 200 µg, especially preferably at least 225 µg and most preferably at least 250 µg of glucosaminoglycan per g of fresh weight (FW) of plant material.

In a further embodiment, plant cells according to the invention or plants according to the invention synthesize at most 450 µg, preferably at most 400 µg, particularly preferably at most 300 µg, especially preferably at most 280 µg and most preferably at most 260 µg of glucosaminoglycan per g of fresh weight (FW) of plant material.

In a further embodiment of the present invention, the genetically modified plant cells according to the invention or the genetically modified plants according to the invention are plant cells of a green terrestrial plant or green terrestrial plants, respectively, which synthesize glucosaminoglycans.

In the context of the present invention, the term "green terrestrial plant (Embryophyta)" is to be understood as defined in Strasburger, "Lehrbuch der Botanik" [Textbook of Botany], 34th ed., Spektrum Akad. Verl., 1999, (ISBN 3-8274-0779-6).

A preferred embodiment of the present invention relates to genetically modified plant cells according to the invention of multicellular plants or genetically modified plants according to the invention which are multicellular organisms. Accordingly, this embodiment relates to plant cells or plants which do not originate from single-cell plants (protists) or which are not protists.

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 (for example corn, rice, wheat, alfalfa, rye, oats, barley, manioc, potato, tomato, switchgrass (*Panicum virgatum*), sago, mung beans,

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- peas, sorghum, carrots, aubergine, radish, oilseed rape, soybeans, peanuts, cucumbers, pumpkins, melons, leek, garlic, cabbage, spinach, sweet potato, asparagus, courgettes, lettuce, artichokes, sweetcorn, parsnip, scorzonera, jerusalem artichoke, banana, sugarbeet, sugarcane, beetroot, broccoli, cabbage,
- 5 onion, yellow beet, dandelion, strawberry, apple, apricot, plum, peach, grapevines, cauliflower, celery, bell peppers, swede, rhubarb). Particularly preferred are corn, sugar cane, sweet potato or sugar millet, very particularly preferred are tomato or potato plants.
- 10 In a preferred embodiment, the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where the foreign nucleic acid molecule coding for proteins are characterized in that the codons of the foreign nucleic acid molecule are modified compared to the codons of the nucleic acid molecule coding for the respective
- 15 protein of the original organism. With particular preference, the codons of the foreign nucleic acid molecules have been modified such that they are adapted to the frequency of the use of the codons of the plant cell or the plant into whose genome they are integrated or to be integrated.
- 20 Owing to the degeneration of the genetic code, amino acids can be coded by one or more codons. In different organisms, the codons coding for an amino acid are used at different frequencies. Adapting the codon 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
- 25 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
- 30 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. Suitable computer programs are publicly accessible and provided for free inter alia on the internet (for example Dr. Thomas Schödl, University of Regensburg, Universitätsstraße 31, 93040, Regensburg, Germany; Kazusa DNA Research Institute, 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan; Entelechon GmbH, Industriestr. 1, 93077 Bad Abbach, Germany).

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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).

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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 in identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90% and especially preferably of at least 95%. 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. Preferably, the identity with respect to a protein having the activity of a hyaluronan synthase is determined by comparison with the amino acid sequence given under SEQ ID NO 2 and the identity with respect to a nucleic acid molecule coding for a protein having the activity of a hyaluronan synthase is determined by comparison with the nucleic acid sequence given under SEQ ID NO 1 or SEQ ID No 3, the identity with respect to a protein having the activity of a chondroitin synthase is determined by comparison with the amino acid sequence shown under SEQ ID NO 5 or the identity with respect to a nucleic acid molecule coding for a protein having the activity of a chondroitin synthase is determined by comparison with the nucleic acid sequence shown under SEQ ID NO 4, the identity with respect to a protein having the activity of a heparin/heparosan synthase is determined by comparison with the

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amino acid sequence shown under SEQ ID NO 7 or the identity with respect to a nucleic acid molecule coding for a protein having the activity of a heparin/heparosan synthase is determined by comparison with the nucleic acid sequence shown under SEQ ID NO 6, the identity with respect to a protein having the activity of a GlcNAc-6-P acetyltransferase is determined by comparison with the amino acid sequence shown under SEQ ID NO 9 or the identity with respect to a nucleic acid molecule coding for a protein having the activity of a GlcNAc-6-P acetyltransferase is determined by comparison with the nucleic acid sequence shown under SEQ ID NO 8, the identity with respect to a protein having the activity of a UDP-GlcNAc pyrophosphorylase is determined by comparison with the amino acid sequence given under SEQ ID NO 11 or the identity with respect to a nucleic acid molecule coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase is determined by comparison with the nucleic acid sequence shown under 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 lengths, the identity is to be determined by determining the identity in percent of the number of amino acids 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 Génétique 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).

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.

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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.

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. 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 targeted 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 owing to the degeneration of the genetic code.

Proteins encoded by different nucleic acid molecule derivatives have certain common characteristics.

These may, for example, be biological activity, substrate specificity, molecular weight, immunological reactivity, conformation, etc.

The present invention furthermore provides genetically modified plant cells according to the invention or genetically modified plants according to the invention characterized in that the foreign nucleic acid molecules integrated into the genome of the plant cell or the plant coding for a glucosaminoglycan synthase and coding for a protein having the activity of a GlcNAc-6-P acetyltransferase and/or coding for a protein having the activity of a UDP-GlcNAc acetyltransferase are linked to regulatory

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elements initiating the transcription in plant cells (promoters). These may be homologous or heterologous promoters. The promoters can be constitutive, tissue-specific, development-specific or regulated by external factors (for example after application of chemical substances, by action of abiotic factors, such as heat and/or cold, drought, disease, etc.). Here, nucleic acid molecules coding for a glucosaminoglycan synthase or a protein having the activity of a GlcNAc-6-P acetyltransferase or a protein having the activity of a UDP-GlcNAc pyrophosphorylase which are integrated into the genome of a genetically modified plant cell according to the invention or a genetically modified plant according to the invention may in each case be attached to the same promoter, or different promoters may be attached to the individual sequences. Here, two or three different promoters may be present in any combination, in each case attached to a relevant foreign nucleic acid molecule coding for a glucosaminoglycan synthase or to a protein having the activity of a GlcNAc-6-P acetyltransferase or to a protein having the activity of a UDP-GlcNAc pyrophosphorylase in a genetically modified plant cell according to the invention or a genetically modified plant according to the invention.

A preferred embodiment of the present invention relates to genetically modified plant cells according to the invention or genetically modified plants according to the invention where at least one foreign nucleic acid molecule, particularly preferably at least two foreign nucleic acid molecules, especially preferably three foreign nucleic acid molecules selected from the group consisting of nucleic acid molecules coding for a glucosaminoglycan synthase or a protein having the activity of a GlcNAc-6-P acetyltransferase or a protein having the activity of a UDP-GlcNAc pyrophosphorylase is (are) linked to a tissue-specific promoter. Preferred tissue-specific promoters are promoters which initiate the initiation of the transcription specifically in plant tuber, fruit or seed cells or leaves.

In general, each promoter which is active in plant cells is suitable for the expression of nucleic acid molecules coding for a glucosaminoglycan synthase or a protein having the activity of a GlcNAc-6-P acetyltransferase or a protein having the activity of a UDP-GlcNAc pyrophosphorylase.

Here, the promoter may be chosen such that expression is constitutively or only in a

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certain tissue, at a certain point of the development of the plant or at a point of time determined by external factors. Both in respect to the plant and in respect of the nucleic acid molecule to be expressed, the promoter may be homologous or heterologous.

- 5 Suitable promoters are, for example, the promoter of 35S RNS of the cauliflower mosaic virus or the ubiquitin promoter from corn Christensen and Quail, 1996, Transgenic Research 5(3), 213-218), the kafirin promoter from millet (De Rose et al., 1996, Plant Molecular Biology 32 1029-1035; Mishra et al., 2007, Molecular Biology Reports online: 2 February 2007, DOI: 10.1007/s11033-007-9056-8) or the *Cestrum*
- 10 YLCV promoter (Yellow Leaf Curling Virus; WO 01 73087; Stavelone 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,
- 15 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;
- 20 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), the glutelin promoter (Leisy et al., Plant Mol. Biol. 14 (1990), 41-50; Zheng et al., Plant J. 4 (1993), 357-
- 25 366; Yoshihara et al., FEBS Lett. 383 (1996), 213-218) or the 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
- 30 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).

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The use of promoters present in the genome of algae-infecting viruses are 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).

5

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.

10 In the context of the present invention, the terms "tuber, fruit or seed cell" are to be understood as meaning all cells present in a tuber, a fruit or in a seed.

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
15 used for the production of genetically modified plant cells according to the invention or genetically modified plants according to the invention (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 sequence was isolated (homologous with respect to the nucleic acid molecule to be expressed).

20

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 production of genetically modified plant cells according to the invention or genetically modified plants according to the invention (heterologous with
25 respect to the plant cell or plant) or as meaning a promoter which is, in the organism from which a nucleic acid sequence to be expressed was isolated, not naturally present for regulating the expression of said nucleic acid sequence (heterologous with respect to the gene to be expressed).

30 Also present may be a termination sequence (polyadenylation signal) which serves to add a poly-A tail to the mRNA transcript of the nucleic acid molecule. 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

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desired.

It is also possible for intron sequences to be present between the promoter and the coding region or in the foreign nucleic acid molecule, coding for a protein. Such intron
5 sequences may lead to stability of expression and in 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).
10 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*.

The present invention also relates to plants comprising genetically modified plant
15 cells according to the invention. Such plants may be produced by regeneration from genetically modified plant cells according to the invention.

In a further embodiment, the present invention relates to harvestable plant parts of genetically modified plants according to the invention, such as fruits, storage and
20 other roots, flowers, buds, shoots, leaves or stalks, preferably seeds, fruits or tubers, these harvestable parts comprising genetically modified plant cells according to the invention.

In a preferred embodiment of the present invention, the harvestable plant parts
25 according to the invention are processable or consumable parts of genetically modified plants according to the invention comprising genetically modified plant cells according to the invention.

In the context of the present invention, the term "processable parts" is to be
30 understood as meaning plant parts which are used for preparing foodstuff or feedstuff, which are used as a raw material source for industrial processes, as a raw material source for the production of pharmaceutical products or as a raw material source for the production of cosmetic products.

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

5

The present invention also relates to a propagation material of genetically modified plants according to the invention comprising a genetically modified plant cell according to the invention.

- 10 Here, the term "propagation material" comprises those components of the plant which are suitable for generating progeny via the vegetative or sexual route. Suitable for vegetative propagation are, for example, cuttings, callus cultures, rhizomes or tubers but also e.g. protoplasts and cell cultures. Propagation material produced by means of sexual processes includes, for example, fruits, seeds, seedlings, etc. The
- 15 propagation material preferably takes the form of tubers, fruits or seeds.

- Preferably, the present invention relates to propagation material according to the invention or harvestable parts of plants according to the invention comprising glucosaminoglycan (for example hyaluronan). Particularly preferably, the propagation
- 20 material according to the invention or the harvestable parts of plants according to the invention is/are propagation material according to the invention or harvestable parts of plants according to the invention synthesizing glucosaminoglycan. Preferably, said glucosaminoglycan is chondroitin, heparin/heparan or hyaluronan, particularly preferably hyaluronan.

25

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*.

- 30 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*.

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A further advantage of the present invention is that harvestable parts, propagation material, processible parts or consumable parts of genetically modified plants according to the invention comprise more glucosaminoglycan (for example hyaluronan) than plants comprising only a foreign nucleic acid molecule coding for a glucosamine synthase. Accordingly, genetically modified plants according to the invention are not only particularly suitable for use as raw material from which glucosaminoglycan (for example hyaluronan) may be isolated but can also be used directly as foodstuff/feedstuff or for preparing foodstuff/feedstuff having a prophylactic or therapeutic character (for example for osteoarthritis prophylaxis, US 6,607,745).

Since genetically modified plants according to the invention have a higher glucosaminoglycan content than plants having only a foreign nucleic acid molecule, coding for a glucosaminoglycan synthase, the production of such foodstuff/feedstuff requires lower amounts of harvestable parts, propagation material, processible parts or consumable parts of genetically modified plants according to the invention. If consumable parts of genetically modified plants according to the invention are consumed, for example, directly as a so-called "nutraceutical", it is possible to achieve a positive effect even by ingesting relatively small amounts of substance. This may be of particular significance inter alia in the production of animal feed, since animal feed having too high a content of plant components is unsuitable as feedstuff for various animal species.

By virtue of the high capacity of glucosaminoglycans, in particular hyaluronan, to bind water, harvestable parts, propagation material, processible parts or consumable parts of genetically modified plants according to the invention furthermore have the advantage that less thickeners are required when solidified foodstuff/feedstuff is produced. Thus, for example, the production of jelly requires less sugar, which is associated with an additional positive effect on health. In the production of foodstuff/feedstuff requiring the dehydration of the crude plant material, the advantage of using harvestable parts, propagation material, processible parts or consumable parts of genetically modified plants according to the invention consists in the fact that less water has to be removed from the plant material in question, resulting in lower production costs and, owing to more gentle preparation methods (for example lower and/or shorter input of heat), an elevated nutritional value of the foodstuff/feedstuff in question. Thus, for example, in the production of tomato

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ketchup less energy has to be introduced in order to achieve the desired consistency.

The present invention furthermore provides a process for producing a plant, which comprises

5 a) genetically modifying a plant cell, where the genetic modification comprises steps i to iii below

- i) introduction of a foreign nucleic acid molecule coding for a glucosaminoglycan synthase into a plant cell
- 10 ii) introduction of a foreign nucleic acid molecule coding for a glucosamine 6-phosphate acetyltransferase into a plant cell
- iii) introduction of a foreign nucleic acid molecule coding for a UDP-N-acetyl-glucosamine pyrophosphorylase into a plant cell

where steps i to iii can be carried out in any order, individually or any combinations of steps i to iii may be carried out simultaneously

- 15 b) regenerating a plant from plant cells from step a) i and/or a)ii and/or a) iii;
- c) generating, if appropriate, further plants using the plants according to step b), where, if appropriate, plant cells are isolated from plants according to step b) or c) and the process steps a) to c) are repeated until a plant is generated which has a foreign nucleic acid molecule coding for a glucosaminoglycan synthase and a foreign
- 20 nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase and a foreign nucleic acid molecule coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase.

A preferred embodiment of the process according to the invention for producing a plant relates to a process for preparing a plant, which comprises

25 a) genetically modifying a plant cell, where the genetic modification comprises steps i to iii below in any order or any combinations of the following steps i to iii carried out individually or simultaneously

- 30 i) introduction of a foreign nucleic acid molecule coding for a glucosaminoglycan synthase into the plant cell
- ii) introduction of a foreign nucleic acid molecule coding for a glucosamine 6-phosphate acetyltransferase into a plant cell
- iii) introduction of a foreign nucleic acid molecule coding for a UDP-

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GlcNAc pyrophosphorylase into a plant cell

- b) regenerating a plant from plant cells comprising the genetic modification according to steps
- i) a) i
 - 5 ii) a) ii
 - iii) a) iii
 - iv) a) i and a) ii,
 - v) a) i and a) iii,
 - vi) a) ii and a) iii, or
 - 10 vii) a) i and a) ii and a) iii
- c) introducing into plant cells of plants according to step
- i) b) i a genetic modification according to step a) ii,
 - ii) b) i a genetic modification according to step a) iii,
 - 15 iii) b) i a genetic modification according to step a) ii and at the same time a genetic modification according to step a) iii,
 - iv) b) ii a genetic modification according to step a) i,
 - v) b) ii a genetic modification according to step a) iii,
 - vi) b) ii a genetic modification according to step a) i and at the same time a genetic modification according to step a) iii,
 - 20 vii) b) iii a genetic modification according to step a) i,
 - viii) b) iii a genetic modification according to step a) ii,
 - ix) b) iii a genetic modification according to step a) i and at the same time a genetic modification according to step a) ii,
 - x) b) iv a genetic modification according to step a) iii,
 - 25 xi) b) v a genetic modification according to step a) ii, or
 - xii) b) vi a genetic modification according to step a) i
- and regenerating a plant
- d) introducing into plant cells of plants according to step
- i) c) i a genetic modification according to step a) iii,
 - 30 ii) c) ii a genetic modification according to step a) ii,
 - iii) c) iv a genetic modification according to step a) iii,
 - iv) c) v a genetic modification according to step a) ii,
 - v) c) vii a genetic modification according to step a) ii,

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vi) c) vii a genetic modification according to step a) i, or

vii) c) ix a genetic modification according to step a) ii

and regenerating a plant

5 e) if appropriate generating further plants with the aid of the plants according to
any of steps b) vii c) iii, c) vi, c) x, c) xi, c) xii or according to any of steps d) i to
d) vii.

For introducing foreign nucleic acid molecules according to step a) of the process
according to the invention for producing a plant, any available method may be used.
10 Various processes for the transformation of plant cells have already been described
above and can be applied here in a corresponding manner. If the process steps
according to step a) of the process according to the invention for producing a plant
are not carried out simultaneously but in succession, identical or different methods
may be used for the individual transformation steps.

15

The regeneration of the plants according to step b) and, if appropriate, steps c) and
d) of the processes according to the invention for producing a plant can be carried
out by 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-
20 89603-549-2).

The generation of further plants (according to step c) or step e), depending on the
process) of the processes according to the invention for producing a plant may take
place, for example, by vegetative propagation (for example via cuttings, tubers or via
25 callus cultivation and regeneration of entire plants) or by sexual propagation. Here,
sexual propagation is preferably carried out in a controlled manner, i.e. selected
plants with certain properties are crossed with one another and propagated.
Selection is preferably carried out such that the further plants (which, depending on
the process, are generated according to step c) or step e)) have the foreign nucleic
30 acid molecules introduced in the preceding steps.

In the processes according to the invention for producing plants, the genetic
modifications for generating the genetically modified plant cells according to the

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invention can be carried out simultaneously or in successive steps and in any combination. It is possible to use both wild-type plants and wild-type plant cells into which a foreign nucleic acid molecule has not yet been introduced, or it is possible to use plant cells or plants which are already genetically modified and into which one or
5 more foreign nucleic acid molecules have already been introduced.

In the genetic modification of introducing the foreign nucleic acid molecules into the plant cell or the plant, in step a) of the process according to the invention for producing a plant the foreign nucleic acid molecules may be a single nucleic acid
10 molecule or a plurality of nucleic acid molecules. Thus, the foreign nucleic acid molecules coding for a glucosaminoglycan synthase or coding for a protein having the enzymatic activity of a GlcNAc-6-P acetyltransferase or coding for a protein having the enzymatic activity of a UDP-GlcNAc pyrophosphorylase can be present together on a single nucleic acid molecule, or two of the foreign nucleic acid
15 molecules mentioned may be present together on a single nucleic acid molecule and the third foreign nucleic acid molecule may be present on another nucleic acid molecule in any possible combination, or all three of the foreign nucleic acid molecules mentioned may each be present on individual separate nucleic acid molecules.

20

Preferred properties of foreign nucleic acid molecules or recombinant nucleic acid molecules have already been described above in connection with plant cells according to the invention or plants according to the invention, and they can be applied correspondingly in the practice of processes according to the invention for
25 producing a plant.

30

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

The present invention also provides plants obtainable by a process according to the invention for producing a plant which synthesizes hyaluronan.

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The present invention furthermore relates to a process for producing glucosaminoglycans (for example hyaluronan) which comprises the step of extracting glucosaminoglycans from genetically modified plant cells according to the invention, from genetically modified plants according to the invention, from propagation material
5 according to the invention, from harvestable plant parts according to the invention or from plants or parts of these plants obtainable by a process according to the invention for producing plants. 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
10 according to the invention, the harvestable plant parts according to the invention, the processible plant parts according to the invention prior to extracting the glucosaminoglycan (for example hyaluronan) and particularly preferably furthermore the step of cultivating genetically modified plant cells according to the invention or genetically modified plants according to the invention prior to harvesting.

15

In contrast to bacterial or animal tissues, plant tissues have no glucosaminoglycan-degrading enzymes (for example hyaluronidases). Accordingly, as already described above, extraction of glucosaminoglycans from plant tissues is possible using relatively simple methods. If required, the aqueous extracts, described above, of
20 plant cells or tissues containing glucosaminoglycans 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 hyaluronan is described under General Methods item 3.

25 The processes already described for extracting glucosaminoglycans from genetically modified plant cells according to the invention or genetically modified plants according to the invention are also suitable for isolating glucosaminoglycans (for example hyaluronan) from propagation material according to the invention, from harvestable plant parts according to the invention or from plants or parts of these
30 plants obtainable by a process according to the invention for preparing plants which synthesize hyaluronan.

The present invention also provides the use of genetically modified plant cells

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according to the invention, genetically modified plants according to the invention, propagation material according to the invention, harvestable plant parts according to the invention or plants obtainable by a process according to the invention for producing a plant for preparing glucosaminoglycans.

5

The present invention furthermore relates to compositions comprising genetically modified plant cells according to the invention. 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 or feedstuff, pharmaceutical or cosmetic products.

10

The present invention preferably provides compositions comprising components of genetically modified plant cells according to the invention, of genetically modified plants according to the invention, of propagation material according to the invention, of harvestable plant parts according to the invention or of plants obtainable by a process according to the invention and comprising recombinant nucleic acid molecules, where the recombinant nucleic acid molecules are characterized in that they comprise nucleic acid molecules coding for a glucosaminoglycan synthase and proteins having the enzymatic activity of a GlcNAc-6-P acetyltransferase and proteins having the enzymatic activity of a UDP-GlcNAc pyrophosphorylase.

15

20

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 a plant cell or plant by genomic plant nucleic acid sequences.

25

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.

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.

30

The recombinant nucleic acid molecules present in the compositions according to the invention may be individual or different recombinant nucleic acid molecules, in which

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nucleic acid molecules coding for a glucosaminoglycan synthase (for example hyaluronan synthase) and proteins having the activity of a GlcN-6-P acetyltransferase and proteins having the activity of a UDP-GlcNAc pyrophosphorylase are present in one nucleic acid molecule, or those where the

5 nucleic acid molecules mentioned are present in separate nucleic acid molecules. Nucleic acid molecules coding for a glucosaminoglycan synthase (for example hyaluronan synthase) or coding for a protein having the activity of a GlcN-6-P acetyltransferase or coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase may be present together on a single recombinant nucleic acid

10 molecule, or two of the nucleic acid molecules mentioned may be present together on a single recombinant nucleic acid molecule and the third nucleic acid molecule may be present on another recombinant nucleic acid molecule in any possible combination, or all three nucleic acid molecules mentioned may in each case be present on individual separate recombinant nucleic acid molecules. Depending on

15 how the nucleic acid molecules coding for a glucosaminoglycan synthase (for example hyaluronan synthase) or coding for a protein having the activity of a GlcN-6-P acetyltransferase or coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase are present in a composition according to the invention, they may be flanked by identical or different genomic plant nucleic acid sequences.

20

That compositions according to the invention comprise recombinant nucleic acid molecules may be demonstrated using methods known to the person skilled in the art, such as, for example, methods based on hybridization or, preferably, using methods based on PCR (polymerase chain reaction).

25

As already mentioned above, it is possible to use genetically modified plant cells according to the invention, genetically modified plants according to the invention, propagation material according to the invention, harvestable plant parts according to the invention, or plants obtainable by a process according to the invention to prepare

30 foodstuff or feedstuff. However, use as raw materials for industrial applications is also possible, without glucosaminoglycans (for example hyaluronan) having to be isolated. Thus, for example, genetically modified plants according to the invention or parts of genetically modified plants according to the invention can be applied to areas

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under agricultural cultivation to achieve increased water binding of the soil. Furthermore, genetically modified plants according to the invention or genetically modified plant cells according to the invention can be used for preparing drying agents (for example for use when shipping moisture-sensitive items) or as absorbers
5 of liquids (for example in diapers or for absorbing spilt aqueous liquids). For such applications, it is possible to use entire genetically modified plants according to the invention, parts of genetically modified plants according to the invention or comminuted (for example ground) genetically modified plants according to the invention or plant parts according to the invention, as required. Suitable for
10 applications in which ground plants or plant parts are used are in particular plant parts containing glucosaminoglycans (for example hyaluronan), but only a low proportion of water. These are preferably grains of cereal plants (corn, rice, wheat, rye, oats, barley, sago or sorghum). Since genetically modified plant cells according to the invention and genetically modified plants according to the invention have a
15 higher glucosaminoglycan (for example hyaluronan) content than plants which have only one foreign nucleic acid molecule coding for glucosaminoglycan synthase, compared to these less material has to be used for industrial applications when use is made of genetically modified plant cells according to the invention or genetically modified plants according to the invention.

20

The present invention also provides processes for preparing a composition according to the invention, where genetically modified plant cells according to the invention, genetically modified plants according to the invention, propagation material according to the invention, harvestable plant parts according to the invention, or plants
25 obtainable by a process according to the invention for producing a plant are used. The processes for preparing a composition according to the invention are preferably processes for preparing foodstuff or feedstuff, processes for preparing a pharmaceutical product or processes for preparing a cosmetic product.

30 Process for preparing foodstuff or feedstuff are known to the person skilled in the art. Processes for using genetically modified plants according to the invention or plant parts according to the invention in industrial areas are also known to the person skilled in the art and include inter alia comminuting or grinding of genetically modified

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plants according to the invention or plant parts according to the invention; however, they are not exclusively limited thereto. Some of the advantages resulting from using subject-matters according to the invention for preparing foodstuff/feedstuff or for use in industrial areas have already been described above.

5

A process according to the invention for preparing a composition is particularly preferably a process for preparing a composition which comprises glucosaminoglycan (for example hyaluronan).

- 10 Compositions obtainable by a process for preparing a composition according to the invention are likewise provided by the present invention.

The present invention also relates to the use of genetically modified plant cells according to the invention, genetically modified plants according to the invention, 15 propagation material according to the invention, harvestable plant parts according to the invention, or plants obtainable by a process according to the invention for producing a plant for preparing a composition according to the invention. Preference is given to the use of genetically modified plant cells according to the invention, genetically modified plants according to the invention, propagation material according 20 to the invention, harvestable plant parts according to the invention, or of plants obtainable by a process according to the invention for producing a plant for preparing foodstuff or feedstuff, for preparing a pharmaceutical or for preparing a cosmetic product.

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Accordingly, the present invention as claimed relates to:

- 1) A genetically modified plant cell comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase,
5 and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;
- 2) The cell of clause 1 that is a cell of a crop plant;
- 3) The cell of clause 1 or 2 that is a seed cell;
- 4) The cell of clause 1 or 2 that is a leaf cell;
- 10 5) A process for producing a plant, which comprises a) genetically modifying a plant cell, wherein the genetic modification comprises steps i), ii) and iii):
i) introduction of a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase into the plant cell, ii) introduction of a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase
15 into the plant cell, and iii) introduction of a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase into the plant cell, wherein individual steps i), ii) and iii) are carried out in any order; or any combination of steps i), ii) and iii) are carried out simultaneously; b) regenerating a plant from the plant cell produced in step a); and c) optionally, generating further plants
20 using the plant obtained in step b);
- 6) The process of clause 5, further comprising the step of isolating cells from the plant produced in step b) or c); and repeating steps a) to c) until a plant is generated which has a foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a
25 protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;

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7) Use of the cell of any of the clauses 1 to 4, for producing a plant comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;

8) Use of a plant comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for producing progeny plants or seeds;

9) A process for producing glucosaminoglycans, which comprises the step of extracting glucosaminoglycans from a genetically modified plant comprising the cell of any of the clauses 1 to 4, or from a plant that is obtained by the process of clause 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;

10) Use of a genetically modified plant comprising the cell of any of the clauses 1 to 4, or a plant that is obtained by the process of clause 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for producing glucosaminoglycans;

11) A composition comprising the genetically modified plant cell of any of the clauses 1 to 4, a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;

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- 12) A process for preparing a composition comprising glucosaminoglycans, which comprises comminuting a genetically modified plant comprising the cell of any of the clauses 1 to 4 or a plant that is obtained by the process of clause 5 or 6 and which comprises a nucleic acid molecule coding for a
- 5 glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase;
- 13) Use of a genetically modified plant comprising the cell of any of the
- 10 clauses 1 to 4, or a plant that is obtained by the process of clause 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for preparing the
- 15 composition of clause 11.

Description of the sequences

SEQ ID NO 1: Nucleic acid sequence coding for a hyaluronan synthase from *Paramecium bursaria Chlorella virus 1*.

20 SEQ ID NO 2: Amino acid sequence of a hyaluronan synthase from *Paramecium bursaria Chlorella virus 1*. The amino acid sequence shown can be derived from SEQ ID NO 1.

SEQ ID NO 3: Synthetic nucleic acid sequence coding for a hyaluronan synthase from *Paramecium bursaria Chlorella virus 1*. The synthesis of the codons of the sequence shown was performed in a manner that they were

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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.

5 SEQ ID NO 4: Nucleic acid sequence coding for a protein having the activity of a chondroitin synthase from *Pasteurella multocida*.

SEQ ID NO 5: Amino acid sequence of a protein having the activity of a chondroitin synthase from *Pasteurella multocida*. The amino acid sequence shown can be derived from SEQ ID NO 4.

10 SEQ ID NO 6: Nucleic acid sequence coding for a protein having the activity of a heparosan synthase from *Pasteurella multocida*.

SEQ ID NO 7: Amino acid sequence of a protein having the activity of a heparosan synthase from *Pasteurella multocida*. The amino acid sequence shown can be derived from SEQ ID NO 6.

15 SEQ ID NO 8: Nucleic acid sequence coding for a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae*.

SEQ ID NO 9: Amino acid sequence of a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae*. The amino acid sequence shown can be derived from SEQ ID NO 8.

20 SEQ ID NO 10: Nucleic acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Saccharomyces cerevisiae*.

SEQ ID NO 11: Amino acid sequence of a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Saccharomyces cerevisiae*. The amino acid sequence shown can be derived from SEQ ID NO 10.

25 SEQ ID NO 12: Nucleic acid sequence of an expression cassette comprising the YLCV promoter, restriction sites, the polyadenylation signal sequence of the ocs terminator from *Agrobacterium* and the polyadenylation signal sequence of the nos terminators from *Agrobacterium*.

SEQ ID NO 13: Synthetic oligonucleotide for preparing an MCS ("Multiple Cloning Site").

30 SEQ ID NO 14: Synthetic oligonucleotide for preparing an MCS ("Multiple

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Cloning Site").

- SEQ ID NO 15: Primer used for PCR reactions.
- SEQ ID NO 16: Primer used for PCR reactions.
- SEQ ID NO 17: Primer used for PCR reactions.
- 5 SEQ ID NO 18: Primer used for PCR reactions.
- SEQ ID NO 19: Primer used for PCR reactions.
- SEQ ID NO 20: Primer used for PCR reactions.
- SEQ ID NO 21: Nucleic acid sequence coding for a protein having the activity of a GlcN-1-P mutase from *Escherichia coli*.
- 10 SEQ ID NO 22: Amino acid sequence of a protein having the activity of a GlcN-1-P mutase from *Escherichia coli*. The amino acid sequence shown can be derived from SEQ ID NO 21.
- SEQ ID NO 23: Synthetic Oligonucleotide used as PCR primer.
- SEQ ID NO 24: Synthetic Oligonucleotide used as PCR primer.
- 15 SEQ ID NO 25: Nucleic acid sequence coding for a protein having the bifunctional activities of a glucosamine 1-phosphate acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli* (*glmu*).
- SEQ ID NO 26: Amino acid sequence of a protein having the bifunctional activities of a glucosamine 1-phosphate acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli*. The amino acid sequence shown can be derived from SEQ ID NO 25.
- 20 SEQ ID NO 27: Primer used for PCR reactions.
- SEQ ID NO 28: Primer used for PCR reactions.
- SEQ ID NO 29: Nucleic acid sequence coding for a protein having the activity of a phosphoacetylglucosamine (GlcN-P) mutase from *Saccharomyces cerevisiae*.
- 25 SEQ ID NO: 30 Amino acid sequence of a protein having the activity of a phosphoacetylglucosamine mutase from *Saccharomyces cerevisiae*. The amino acid sequence shown can be derived from SEQ ID NO 30.

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General methods

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.

1. Transformation of potato plants

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

2. Isolation of glucosaminoglycan using the example of hyaluronan from plant tissue

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 plant 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 ≥ 18 MΩ) was 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 xg for 5 minutes. To determine the amount of hyaluronan in the entire above-ground parts of plants, the above-ground parts of the plants were cut off about 1 cm to 3 cm above the culture substrate, cut into small pieces and then comminuted using a Warring blender as described under General Methods item 3. To determine the hyaluronan content, an aliquot may then be removed from the centrifugation supernatant obtained (see General Methods item 3).

3. Purification of glucosaminoglycan using the example of hyaluronan

Comminuted plant material or the entire above-ground part of a plant was, after addition of water (about 100 ml of water, demineralized, conductivity ≥ 18 MΩ, in each case 100 g of plant material) comminuted in a Warring blender at maximum speed for about 30 seconds. The cell debris was then removed using a tea sieve. The cell

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debris that had been removed was resuspended in 300 ml of water (demineralized, conductivity $\geq 18 \text{ M}\Omega$) and again removed using a tea sieve. The two suspensions obtained (100 ml + 300 ml) were combined and centrifuged at 13 000 xg for 15 minutes. NaCl was added to the centrifugation supernatant obtained until a final
5 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 xg for 15 minutes. The sedimented precipitate obtained after this centrifugation was dissolved in 100 ml of buffer (50 mM TrisHCl, pH 8, 1mM CaCl_2)
10 and proteinase K was then added to a final concentration of 100 $\mu\text{g/ml}$ 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,
15 thorough mixing and incubation at -20°C for about 96 hours. This was followed by 15 minutes of centrifugation at 13 000 xg. The sedimented precipitate obtained after this centrifugation was dissolved in 30 ml of water (demineralized, conductivity $\geq 18 \text{ M}\Omega$), 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
20 precipitation was carried out. The precipitate obtained after subsequent centrifugation at 13 000 xg for 15 minutes was dissolved in 20 ml of water (demineralized, conductivity $\geq 18 \text{ M}\Omega$).

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,
25 pore width 10 000 NMWL, Prod. No. UCF8 010 96), and the sample was centrifuged at 2200 xg until only about 3 ml of the solution above the filter remained. Two more times, in each case 3 ml of water (demineralized, conductivity $\geq 18 \text{ M}\Omega$) 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
30 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 $\geq 18 \text{ M}\Omega$). All solutions which were still present above the membrane and the solutions obtained from rinsing were

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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 xg for 15 minutes was dissolved in 4 ml of water
5 (demineralized, conductivity ≥ 18 M Ω) and then freeze-dried (24 hours under a pressure of 0.37 mbar, freeze drying apparatus Christ Alpha 1-4 from Christ, Osterode, Germany).

4. Detection of hyaluronan and determination of the hyaluronan content

Hyaluronan was detected using a commercial test (hyaluronic acid (HA) test kit from
10 Corgenix, Inc., Colorado, USA, Prod. No. 029-001) according to the instructions of the manufacturer.

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. The
15 hyaluronan values are determined with the aid of a calibration curve using defined amounts of hyaluronan which are included with the test kit. 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
20 plant tissue, depending on whether a limit was exceeded or not reached).

In parallel batches, aliquots of the samples to be determined were initially subjected to hyaluronidase digestion and then measured using the commercial test (hyaluronic acid (HA) test kit from Corgenix, Inc., Colorado, USA, Prod. No. 029-001). Hyaluronidase digestion was carried out using 400 μ l of plant extract in
25 hyaluronidase buffer (0.1 M potassium phosphate buffer, pH 5.3; 150 mM NaCl) by adding 5 μ g (~3 units) of hyaluronidase (hyaluronidase type III from Sigma, Prod. No. H 2251) and incubating at 37°C for 30 min.

In each case in a dilution of 1:10, all samples were then used for determining the hyaluronan content.

30 5. Determination of the activity of a GFAT

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

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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.

Examples

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

5 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 fragment of a length of 529 bp which comprised 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
10 ligated between the *EcoR* I and *Kpn* I restriction sites of the polylinker of pUC18. In this manner, the plasmid pUC18-35S was formed. Using the restriction endonucleases *Hind* III and *Pvu* II, a fragment of a length of 192 bp which included the polyadenylation signal (3' terminus) of the *Octopine Synthase* gene (gene 3) of the T-DNA of the Ti plasmid pTiACH5 (Gielen et al, 1984, EMBO Journal 3, 835-846)
15 (nucleotides 11 749-11 939) was isolated from the plasmid pAGV40 (Herrera-Estrella et al, 1983 Nature, 303, 209-213). Following 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. Here, the entire polylinker comprising the 35S promoter and Ocs terminator was removed using *EcoR* I and
20 *Hind* III and ligated into the appropriately cleaved vector pBin19. This gave the plant expression vector pBinAR (Höfgen and Willmitzer, 1990, Plant Science 66, 221-230). The promoter of the patatin gene B33 from *Solanum tuberosum* (Rocha-Sosa et al., 1989, EMBO J. 8, 23-29) was, as *Dra* I fragment (nucleotides -1512 - +14), ligated into the *Sst* I-cleaved vector pUC19 whose ends had been blunted using T4-DNA
25 polymerase. This gave the plasmid pUC19-B33. From this plasmid, the B33 promoter was removed using *EcoR* I and *Sma* I and ligated into the appropriately restricted vector pBinAR. This gave the plant expression vector pBinB33.

To facilitate further cloning steps, the MCS (Multiple Cloning Site) was extended. To this end, two complementary oligonucleotides were synthesized, heated at 95°C for 5
30 minutes, slowly cooled to room temperature to allow good annealing and cloned into the *Sal* I and *Kpn* I restriction sites of pBinB33. The oligonucleotides used for this purpose are shown under SEQ ID NO 13 and SEQ ID NO 14. The plasmid obtained

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was named IR 47-71.

2. Preparation of the plant expression vector pBinARHyg

The fragment comprising the 35S promoter, the Ocs terminator and the entire Multiple Cloning Site was removed from pA7 using the restriction endonucleases
5 *EcoR* I and *Hind* III and cloned into the vector pBIBHyg (Becker, 1990, Nucleic Acids Res. 18, 203) which had been cut using the same restriction endonucleases. The plasmid obtained was named pBinARHyg.

3. Preparation of the cloning vector IC 317-204

Using the restriction endonucleases *Xho* I and *Hind* III, nucleic acid fragments
10 comprising the ocs terminator were isolated from the plasmid IR 47-71 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.

Using the restriction endonucleases *Bam* HI and *Eco* RI, nucleic acid fragments
15 comprising the B33 promoter were isolated from the plasmid IR 47-71 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
20 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.

4. Synthesis of nucleic acid molecules

a) Synthesis of nucleic acid molecules coding for a hyaluronan synthase from
25 *Paramecium bursaria Chlorella* virus 1

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
30 HAS protein from *Paramecium bursaria Chlorella* virus 1, is shown under SEQ ID NO 3. The corresponding nucleic acid sequence originally isolated from the

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Paramecium bursaria Chlorella virus 1 is shown under SEQ ID NO 1.

- b) Synthesis of the nucleic acid sequences comprising a YLCV promoter and an MCS, an *nos* terminator and an *ocs* terminator

The nucleic acid sequence comprising a YLCV promoter (Stavolone et al., Plant Molecular Biology 53: 703–713, 2003) and an MCS ("Multiple Cloning Site") containing the restriction sites *Sac* I and *Sma* I, an *nos* terminator and an *ocs* terminator was synthesized by Entelechon GmbH and cloned into the vector pCR4Topo from Invitrogen (Prod. No. K4510-20). The plasmid obtained was named IC 389-337. The synthetic nucleic acid sequence is shown under SEQ ID NO 12.

10 5. Isolation of nucleic acid molecules

- a) Isolation and cloning of a coding nucleic acid sequence for a protein having the activity of a GlcN-6-P acetyltransferase

The nucleic acid sequence coding for a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae* (*gna1*) was isolated by PCR and cloned into the vector pCR 2.1 from Invitrogen (Prod. No. K4510-20). The reaction conditions for the PCR were as follows:

1. step: 5 min 94 °C,
2. step: 45 sec, 94 °C
3. step: 45 sec, 59 °C
20 4. step: 45 sec, 72 °C
5. step: 10 min, 72 °C
6. step: 4°C

Steps 2 to 4 were repeated 35 times, and the procedure was then continued with step 5.

25 The 50 µl reaction batch contained buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 3 mM MgSO₄), in each case 500 nM of amplification primer, shown under SEQ ID NO 15 and SEQ ID NO 16, 10 µl of Q-solution (contained in Qiagen, Prod. No. 206143), in each case 0.2 mM of deoxyribonucleotide, 0.5 µl of Taq DNA polymerase (Invitrogen, Prod. No.: 11304-011) and 250 ng of genomic yeast DNA as template.
30 The PCR was carried out using the Mastercycler from Eppendorf (Prod. NR. 5331 000.010).

The isolated nucleic acid sequence coding for the protein having the activity of a

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GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae* is shown under SEQ ID NO 8.

After cloning of the fragment obtained into the vector pCR 2.1 and confirmation of the sequence, the nucleic acid sequence in question coding for a protein having the
5 activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae* was isolated using the restriction endonucleases *Kpn* I and *Xba* I and cloned into the vector pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 298-204.

b) Isolation and cloning of a nucleic acid sequence coding for a protein having the
10 activity of a UDP-GlcNAc pyrophosphorylase

The nucleic acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Saccharomyces cerevisiae* (*qri*) was isolated by PCR and cloned into the vector pCR 2.1 from Invitrogen (Prod. No. K2000-01). The reaction conditions for the PCR were as follows:

- 15 1. step: 5 min 94 °C,
2. step: 45 sec, 94 °C
3. step: 45 sec, 59 °C
4. step: 45 sec, 72 °C
5. step: 30 min, 72 °C
20 6. step: 4°C

Steps 2 to 4 were repeated 35 times, and the procedure was then continued with step 5.

The 50 µl reaction batch contained buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 3 mM MgSO₄), in each case 500 nM of amplification primer, shown under SEQ ID NO
25 17 and SEQ ID NO 18, 10 µl of Q-solution (contained in Qiagen, Prod. No.: 206143), in each case 0.2 mM of deoxyribonucleotide, 0.5 µl of Taq DNA polymerase (Invitrogen, Prod. No.: 11304-011) and 250 ng of genomic yeast DNA (Invitrogen Prod. No. 40802) as template. The PCR was carried out using the Mastercycler from Eppendorf (Prod. No. 5331 000.010).

30 The isolated nucleic acid sequence coding for the protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Saccharomyces cerevisiae* is shown under SEQ ID NO 10.

After cloning of the fragment obtained into the vector pCR 2.1 and confirmation of the

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sequence, the nucleic acid sequence coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase from *Saccharomyces cerevisiae* was isolated using the restriction endonucleases *Kpn* I and *Xba* I and cloned into the vector pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was
5 named IC 303-204.

c) Isolation and cloning of a nucleic acid sequence coding for a protein having the activity of a GlcNAc-P mutase

The nucleic acid sequence coding for a protein having the activity of a phosphoacetylglucosamine mutase from *Saccharomyces cerevisiae* (*pcm* I, EC
10 5.4.2.3) was isolated by PCR and cloned into the vector pCR 2.1 from Invitrogen (Prod. No. K2000-01). The reaction conditions for the PCR were as follows:

1. step: 5 min 94 °C,
2. step: 45 sec, 94 °C
3. step: 45 sec, 59 °C
- 15 4. step: 45 sec, 72 °C
5. step: 30 min, 72 °C
6. step: 4°C

Steps 2 to 4 were repeated 35 times, and the procedure was then continued with step 5.

20 The 50 µl reaction batch contained buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 3 mM MgSO₄), in each case 500 nM of amplification primer, shown under SEQ ID NO 27 and SEQ ID NO 28, 10 µl of Q-solution (contained in Qiagen, Prod. No.: 206143), in each case 0.2 mM of deoxyribonucleotide, 0.5 µl of Taq DNA polymerase (Invitrogen, Prod. No.: 11304-011) and 250 ng of genomic yeast DNA (Invitrogen
25 Prod. No. 40802) as template. The PCR was carried out using the Mastercycler from Eppendorf (Prod. No. 5331 000.010).

After cloning of the fragment obtained into the vector pCR 2.1 and confirmation of the sequence, the nucleic acid sequence coding for a protein having the activity of a phosphoacetylglucosamine mutase from *Saccharomyces cerevisiae* was isolated
30 using the restriction endonucleases *Kpn* I and *Xba* I and cloned into the vector pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 304-204.

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- d) Isolation and cloning of a nucleic acid sequence coding for a protein having the activity of a GlcN-1-P mutase from *Escherichia coli*

The nucleic acid sequence coding for a protein having the activity of a glucosamine 1-phosphate mutase (GlcN-1-P mutase) from *E. coli* (*glmm*) was isolated by PCR
5 and cloned into the vector pCR 2.1 from Invitrogen (Prod. No. K2000-01). The reaction conditions for the PCR were as follows:

1. step: 5 min 94 °C,
2. step: 45 sec, 94 °C
3. step: 45 sec, 59 °C
- 10 4. step: 45 sec, 72 °C
5. step: 30 min, 72 °C
6. step: 4°C

Steps 2 to 4 were repeated 35 times, and the procedure was then continued with step 5.

- 15 The 50 µl reaction batch contained buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 3 mM MgSO₄), in each case 500 nM of amplification primer, shown under SEQ ID NO 19 and SEQ ID NO 20, 10 µl of Q-solution (contained in Qiagen, Prod. No.: 206143), in each case 0.2 mM of deoxyribonucleotide, 0.5 µl of Taq DNA polymerase (Invitrogen, Prod. No.: 11304-011) and 250 ng of genomic *E. coli* DNA as template.
20 The PCR was carried out using the Mastercycler from Eppendorf (Prod. No. 5331 000.010).

The isolated nucleic acid sequence coding for the protein having the activity of a glucosamine 1-phosphate mutase protein from *E. coli* (*glmm*) is shown under SEQ ID NO 21.

- 25 After cloning of the fragment obtained into the vector pCR 2.1 and confirmation of the sequence, the nucleic acid sequence coding for a protein having the activity of a GlcN-1-P mutase from *E. coli* was isolated using the restriction endonucleases *Kpn* I and *Xba* I and cloned into the vector pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 300-204.

- 30 e) Isolation and cloning of a nucleic acid sequence coding for a protein having the bifunctional activity of a GlcN-1-P acetyltransferase and a UDP-GlcNAc-1-P pyrophosphorylase from *Escherichia coli*

The nucleic acid sequence coding for a bifunctional protein having the activity of a

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glucosamine 1-phosphate acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli* (*glmu*) was isolated by PCR and cloned into the vector pCR2.1 from Invitrogen (Prod. No. K2000-01). The reaction conditions for the PCR were as follows:

- 5 1. step: 5 min 94 °C,
2. step: 45 sec, 94 °C
3. step: 45 sec, 59 °C
4. step: 45 sec, 72 °C
5. step: 30 min, 72 °C
- 10 6. step: 4°C

Steps 2 to 4 were repeated 35 times, and the procedure was then continued with step 5.

- The 50 µl reaction batch contained buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 3 mM MgSO₄), in each case 500 nM of amplification primer, shown under SEQ ID NO
- 15 23 and SEQ ID NO 24, 10 µl of Q-solution (contained in Qiagen, Prod. No.: 206143), in each case 0.2 mM of deoxyribonucleotide, 0.5 µl of Taq DNA polymerase (Invitrogen, Prod. No.: 11304-011) and 250 ng of genomic *E. coli* DNA as template. The PCR was carried out using the Mastercycler from Eppendorf (Prod. No. 5331 000.010).

- 20 The isolated nucleic acid sequence coding for a protein having the bifunctional activities of a glucosamine 1-phosphate acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli* (*glmu*) is shown under SEQ ID NO 25.

- After cloning of the fragment obtained into the vector pCR 21 and confirmation of the sequence, the nucleic acid sequence coding for a bifunctional protein having the
- 25 activity of a GlcN-1-P acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli* (*glmu*) was isolated using the restriction endonucleases *Kpn* I and *Xba* I and cloned into the vector pA7, which had been cut with the same restriction endonucleases. The plasmid obtained was named IC 299-204.

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

Nucleic acid molecules comprising the coding sequence of the hyaluronan synthase, were isolated from the plasmid IC 323-215 by restriction digestion with *Bam*H I and

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Xho I and cloned into the *Bam*H I and *Xho* I restriction sites of the plasmid IR 47-71. The plant expression vector obtained was named IC 341-222.

7. Preparation of the plant expression vector IC 351-222 comprising a coding nucleic acid sequence for a protein having the activity of a GlcN-6-P acetyltransferase from *Saccharomyces cerevisiae*

Starting plasmid is the above-described plant expression vector pUBI bar (WO 97 44472) into whose *Eco*R I and *Sda* I restriction sites the coding sequence of the *gna* gene from yeast was cloned. The coding sequence of the *gna* gene from yeast was isolated by *Eco*R I and *Sda* I restriction digestion from the plasmid IC 298-204. The vector obtained was named IC 351-222.

8. Preparation of the plant expression vector IC 392-337 containing coding nucleic acid sequences for a protein having the activity of a GlcN-6-P acetyltransferase and a protein having the activity of a UDP-GlcNAc pyrophosphorylase

Starting plasmid is the plasmid IC 351-222 described further above into whose *Eco* RI restriction site the cassette of YLCV promoter and NOS terminator and OCS terminator, isolated using the restriction endonuclease *Eco* RI from the plasmid IC 389-337, was cloned. The vector obtained was named IC 390-337.

From the above-described plasmid IC 303-204, the coding sequence of the *qri* gene was isolated by *Sac* I and *Eco* RV restriction digestion and ligated into the *Sac* I and *Sma* I restriction sites of the vector IC 390-337. The vector obtained was named IC 391-337.

To remove the redundant OCS terminator, the vector IC 391-337 was digested with *Aat* II and then religated. The plant expression vector obtained was named IC 392-337.

9. Preparation of the plant expression vector IC 360-237 comprising a coding nucleic acid sequence for a protein having the activity of a GlcN-1-P mutase and a bifunctional protein having the bifunctional activity of a GlcN-1-P acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *Escherichia coli*

The starting plasmid for the introduction of the nucleic acid sequence coding for a protein having the bifunctional activity of a GlcN-1-P acetyltransferase and a UDP-GlcNAc-1-P pyrophosphorylase from *Escherichia coli* was the plasmid IC 299-204,

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described further above, whose coding sequence was isolated by *Eco* RI restriction digestion and cloned into the *Eco* RI restriction site of the pMCS5 vector (MoBiTec GmbH, Prod.No.: pMCS5). The vector obtained was named IC 307-204. In the next step, the vector IC 307-204 was digested with *Pme* I and *Sma* I restriction endonucleases and religated. The vector obtained was named IC 311-204. The nucleic acid sequence coding for a protein having the activity of a GLMU was then isolated from the plasmid IC 311-204 by restriction digestion with *Bam* HI and *Kpn* I and ligated into the restriction sites *Bam* HI and *Kpn* I of the vector IC 312-204. The vector obtained was named IC 315-204. The vector IC 312-204 was prepared by simultaneous ligation of three fragments composed of a 35S promoter fragment, isolated by *Eco* RI and *Sal* I restriction digestion from the plasmid pA7, an *ocs* fragment isolated by *Hind* III and *Sal* I restriction digestion from IC 309-204 and the vector IC310-204, which had been opened by *Eco* RI restriction digestion. The plasmid IC310-204 is a pUC 18 vector, part of whose MCS has been removed by *Hind* III and *Ecl* 135II restriction digestion and subsequent religation. IC 309-204 was prepared by isolating the *ocs* fragment from pA7 using *Hind* III and *Sal* I and cloning it into the pBS KS vector, digested with *Hind* III and *Sal* I.

From the plasmid IC 315-204, the 35S promoter, the nucleic acid sequence coding for a protein having the bifunctional activity of a glucosamine 1-phosphate acetyltransferase and a UDP-GlcNAc pyrophosphorylase from *E. coli* (*qlmu*) and the *ocs* terminator were isolated by *Eco* RI restriction digestion and cloned into the *Eco* RI restriction site of the Ubi Bar vector (WO 97 44472). The vector obtained was named IC 359-237.

The starting plasmid for the introduction of the nucleic acid sequence coding for a protein having the activity of a GlcN-1-P mutase is the plasmid IC 299-204 described further above, whose coding sequence was isolated by *Sda* I and *Sma* I restriction digestion and ligated into the *Sda* I and *Hpa* I restriction sites of the Ubi bar vector. The vector obtained was named IC 355-222.

From the plasmid IC 355-222, the coding sequence of the *glmm* gene was isolated by *Spe* I and *Dra* I restriction digestion and cloned into the *Spe* I and *Pme* I restriction sites of the IC 359-237 plasmids. The vector obtained was named IC 360-237 .

10. Preparation of the plant expression vector IC 393-337 comprising coding nucleic acid sequences for a protein having the activity of a GlcN-6-P

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acetyltransferase, a protein having the activity of a UDP-GlcNAc pyrophosphorylase and a protein having the activity of a GlcNAc-P mutase from *Saccharomyces cerevisiae*

The starting plasmid for the introduction of the nucleic acid sequence coding for
5 GlcNAc-P mutase from *Saccharomyces cerevisiae* is the plasmid IC 304-204 described further above, whose coding sequence was isolated by *Eco* RI restriction digestion and cloned into the *Eco* RI restriction site of the pMCS5 vector (MoBiTec GmbH, Prod. No.: pMCS5). The vector obtained was named IC 313-204. In the next step, the nucleic acid sequence coding for GlcNAc-P mutase from *Saccharomyces*
10 *cerevisiae* was isolated from the vector IC 313-204 by *Pme* I and *Pac* I restriction digestion and cloned into the vector IC 393-337, which had been digested with *Pme* I and *Pac* I. The vector obtained was named IC 394-337.

Starting vector for the preparation of the plasmid IC 393-337 is the plasmid IC 391-337 described further above, which already contained the nucleic acid sequences for
15 a protein having the activity of a GlcN-6-P acetyltransferase and a protein having the activity of a UDP-GlcNAc pyrophosphorylase. To this end, the B33 promoter described further above was isolated by *Pac* I and *Avr* II restriction digestion and cloned into the vector IC 391-337, which had been digested with *Pac* I and *Avr* II. The plant expression vector obtained was named IC 393-337.

20 11. Transformation of potato plants

Potato plants were transformed with the plant expression vector IC 341-222, comprising a coding nucleic acid sequence for 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,
25 23-29) according to the method described in General Methods item 1.

The potato plants obtained, transformed with the plasmid IC 341-222, were named 365 ES X, where X denotes plants independently obtained from the transformation.

The cultivars obtained after the transformation, named 365 ES X, were analyzed for the amount of the hyaluronan synthesized by the plants in question (see also WO
30 2006 032538). The cultivars 365 ES 13 and 365 ES 74 were chosen for the transformations described below.

Potato plants of the cultivars 365 ES 13 and 365 ES 74 were transformed with the

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plant expression vector IC 392-337 or IC 360-237 or IC 394-337 according to the method described in General Methods item 1.

The transgenic potato plants obtained of the cultivar 365 ES 13 transformed with the plasmid IC 392-337 were named 437 ES X, where X denotes plants independently
5 obtained from the transformation.

The transgenic potato plants obtained of the cultivar 365 ES 74 transformed with the plasmid IC 392-337 were named 438 ES X, where X denotes plants independently obtained from the transformation.

The transgenic potato plants obtained of the cultivar 365 ES 13 transformed with the
10 plasmid IC 360-237 were named 397 ES X, where X denotes plants independently obtained from the transformation.

The transgenic potato plants obtained of the cultivar 365 ES 74 transformed with the plasmid IC 360-237 were named 398 ES X, where X denotes plants independently obtained from the transformation.

15 The potato plants obtained of the cultivar 365 ES 13 transformed with the plasmid IC 393-337 were named 444 ES X, where X denotes plants independently obtained from the transformation.

The potato plants obtained of the cultivar 365 ES 74 transformed with the plasmid IC 393-337 were named 445 ES X, where X denotes plants independently obtained
20 from the transformation.

12. Analysis of potato plants containing foreign nucleic acid molecules coding for a hyaluronan synthase and coding for a protein having the activity of a GlcNAc-6-P acetyltransferase and coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase

25 In a greenhouse, individual plants of the cultivars 365 ES 13, 365 ES 74, 437 ES X, 438 ES X, 397 ES X, 398 ES X, 444 ES X and 445 ES X were cultivated in 6 cm pots in soil. The entire above-ground part of the plant was harvested from 7- to 9-week-old plants and processed according to the method described in General Methods item 3. The amount of hyaluronan in the plant extracts in question was determined by
30 measuring the hyaluronan contained in an aliquot of the plant extracts in question using the method described in General Methods item 4 and with the aid of a calibration curve. For the determination of the hyaluronan content, the supernatant obtained after centrifugation was used in a dilution of 1:10. For selected plants, the

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following results were obtained:

Name of the plant	HA [$\mu\text{g/g fw}$]
365ES 74-67	120.9
365ES 74-68	125.1
365ES 74-71	129.2
365ES 74-72	118.2
365ES 74-79	129.0
365ES 74-80	140.2
365ES 74-81	92.7
365ES 74-82	100.9
wt Desiree 1	0.2
wt Desiree 2	-0.2
wt Desiree 3	2.1

Name of the plant	HA [$\mu\text{g/g fw}$]
365ES 13-221	90.6
365ES 13-222	57.9
365ES 13-223	59.0
365ES 13-224	95.3
365ES 13-225	84.0
365ES 13-226	91.7
365ES 13-227	69.1
365ES 13-228	76.2
265ES 13-231	84.3
365ES 13-232	75.5

Table 1: Amount of hyaluronan (in μg of hyaluronan per g of fresh weight) produced by independent selected transgenic plants of cultivars 365 ES 13 and 365 ES 74 which only contain a foreign nucleic acid molecule coding for a hyaluronan synthase, in the entire above-ground parts of the plant in question. Column 1 shows the name of the plant from which material was harvested ("wt Desiree" refers to untransformed wild-type plants of the cultivar Désirée). Column 2 shows the amount of haluronan based on the fresh weight employed.

10

Name of the plant	HA [$\mu\text{g/g fw}$]
438ES 1	183.0
438ES 5	192.5
438ES 10	189.5
438ES 11	168.9
438ES 13	195.5
438ES 14	184.3
438ES 16	168.9
438ES 23	231.2
438ES 25	169.2

Name of the plant	HA [$\mu\text{g/g fw}$]
437ES 2	105.6
437ES 3	97.6
437ES 6	129.4
437ES 12	103.2
437ES 13	144.2
437ES 14	163.7
437ES 15	128.2
437ES 16	100.3
437ES 17	186.3

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Name of the plant	HA [$\mu\text{g/g}$ fw]
438ES 32	178.6
438ES 33	173.4
438ES 41	178.7
438ES 57	239.4
438ES 62	172.5
438ES 64	199.5
438ES 80	235.4
438ES 84	189.2
438ES 85	168.1
438ES 88	167.4
438ES 97	164.4
438ES 102	160.4
438ES 108	209.8
438ES 112	185.9

Name of the plant	HA [$\mu\text{g/g}$ fw]
437ES 21	100.7
437ES 23	114.5
437ES 26	105.1
437ES 31	102.0
437ES 34	178.9
437ES 35	104.4
437ES 39	98.2
437ES 40	116.8
437ES 48	125.1
437ES 66	146.8
437ES 69	106.8
437ES 70	115.4
437ES 75	100.3
437ES 76	125.5
437ES 79	102.0
437ES 80	125.7
437ES 82	135.7
437ES 95	100.3
437ES 105	108.8

Table 2: Amount of hyaluronan (in μg of hyaluronan per g of fresh weight) produced by independent selected transgenic plants of cultivar 437 ES or 438 ES in the entire above-ground parts of the plant in question. Column 1 shows the name of the plant from which material was harvested. Column 2 shows the amount of haluronan based on the fresh weight employed.

The results shown illustrate that plants containing simultaneously foreign nucleic acid molecules coding for a hyaluronan synthase and coding for a protein having the activity of a GlcN-6-P acetyltransferase and coding for a protein having the activity of a UDP-GlcNAc pyrophosphorylase synthesize considerably higher amounts of hyaluronan than plants containing only a foreign nucleic acid molecule coding for a hyaluronan synthase.

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Name of the plant	HA [$\mu\text{g/g fw}$]
397 ES 1	4.51
397 ES 2	22.54
397 ES 3	16.27
397 ES 5	10.13
397 ES 6	13.60
397 ES 7	33.62
397 ES 8	19.87
397 ES 9	70.37
397 ES 10	51.91
397 ES 11	78.71
397 ES 12	9.76
397 ES 13	6.62
397 ES 14	32.74
397 ES 16	21.34
397 ES 17	81.90
397 ES 18	33.16
397 ES 19	32.18
397 ES 20	26.67
397 ES 21	63.21
397 ES 22	2.66
397 ES 24	1.41
397 ES 25	37.27
397 ES 26	20.34
397 ES 27	32.89
397 ES 28	9.89
397 ES 29	8.31
397 ES 30	85.77
397 ES 31	47.44
397 ES 32	53.47
397 ES 33	5.25

Name of the plant	HA [$\mu\text{g/g fw}$]
398 ES 1	53.04
398 ES 2	43.64
398 ES 3	130.01
398 ES 4	89.26
398 ES 5	74.35
398 ES 6	55.39
398 ES 7	99.61
398 ES 8	90.82
398 ES 9	41.80
398 ES 10	79.66
398 ES 11	9.57
398 ES 12	41.24
398 ES 13	89.05
398 ES 14	77.19
398 ES 15	96.96
398 ES 16	84.24
398 ES 17	124.63
398 ES 18	76.19
398 ES 19	71.45
398 ES 20	46.17
398 ES 21	80.96
398 ES 22	31.25
398 ES 23	87.35
398 ES 24	62.31
398 ES 25	58.98
398 ES 26	71.14
398 ES 27	18.48
398 ES 28	105.85
398 ES 29	12.24
398 ES 30	95.82

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Name of the plant	HA [$\mu\text{g/g}$ fw]
397 ES 34	17.10
397 ES 35	16.80
397 ES 36	17.53
397 ES 37	25.90
397 ES 38	7.68
397 ES 39	0.49
397 ES 40	0.85
397 ES 41	14.65
397 ES 42	35.36
397 ES 43	49.96
397 ES 44	28.78
397 ES 45	18.95
397 ES 46	7.93
397 ES 47	28.28
397 ES 48	13.94
397 ES 49	60.16
397 ES 50	29.77

Name of the plant	HA [$\mu\text{g/g}$ fw]
398 ES 31	18.43
398 ES 32	109.41
398 ES 33	87.10
398 ES 34	88.30
398 ES 35	97.85
398 ES 36	58.51
398 ES 37	78.51
398 ES 38	99.67
398 ES 39	11.28
398 ES 40	29.12
398 ES 41	74.15
398 ES 42	22.75
398 ES 43	77.05
398 ES 44	101.34
398 ES 45	57.16
398 ES 46	54.19
398 ES 47	64.35
398 ES 48	60.04
398 ES 49	77.36
398 ES 50	50.84

Table 3: Amount of hyaluronan (in μg of hyaluronan per g of fresh weight) produced by independent selected transgenic plants of cultivar 497 ES or 498 ES in the entire above-ground parts of the plant in question. Column 1 shows the name of the plant from which material was harvested. Column 2 shows the amount of haluronan based on the fresh weight employed.

The results shown illustrate that plants containing simultaneously foreign nucleic acid molecules coding for a hyaluronan synthase and coding for a protein having the activity of a GlcN-P mutase and a protein having the bifunctional activity of a GlcN-1-P acetyltransferase and a UDP-GlcNAc pyrophosphorylase do not synthesize significantly higher amounts of hyaluronan than plants containing only a foreign nucleic acid molecule coding for a hyaluronan synthase.

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Name of the plant	HA [$\mu\text{g/g fw}$]		Name of the plant	HA [$\mu\text{g/g fw}$]
444ES 6	81.06		445ES 1	102.53
444ES 12	95.89		445ES 6	99.65
444ES 14	90.76		445ES 7	152.66
444ES 17	83.20		445ES 8	82.24
444ES 19	69.18		445ES 9	119.74
444ES 23	59.45		445ES 12	82.68
444ES 27	58.93		445ES 16	102.24
444ES 35	58.45		445ES 18	86.47
444ES 37	67.34		445ES 19	103.37
444ES 41	63.27		445ES 20	96.06
444ES 43	60.82		445ES 23	116.64
			445ES 24	95.13
			445ES 34	87.24
			445ES 40	81.74
			445ES 42	98.72
			445ES 47	84.41
			445ES 60	86.71
			445ES 135	94.13

Table 4: Amount of hyaluronan (in μg of hyaluronan per g of fresh weight) produced by independent selected transgenic plants of cultivar 444 ES or 445 ES in the entire above-ground parts of the plant in question. Column 1 shows the name of the plant from which material was harvested. Column 2 shows the amount of haluronan based on the fresh weight employed.

The results shown illustrate that plants containing simultaneously foreign nucleic acid molecules coding for a hyaluronan synthase and coding for a protein having the activity of a GlcN-6-P mutase and a protein having the activity of a GlcN-6-P acetyltransferase and a protein having the activity of a UDP-GlcNAc pyrophosphorylase do not synthesize significantly higher amounts of hyaluronan than plants containing only a foreign nucleic acid molecule coding for a hyaluronan synthase and a protein having the activity of a GlcN-6-P acetyltransferase and a

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protein having the activity of a UDP-GlcNAc pyrophosphorylase.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 30517-459 Seq 02-03-10 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Bayer CropScience AG

<120> Plants which synthesize increased amounts of glucosaminoglycans

<130> BCS 07-5007 PCT

<150> EP07116174.9

<151> 2007-09-12

<150> US60/933,575

<151> 2007-09-13

<160> 30

<170> PatentIn version 3.3

<210> 1

<211> 1707

<212> DNA

<213> Paramecium bursaria Chlorella Virus 1

<220>

<221> CDS

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<300>

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<309> 1995-12-24

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Ser	Asn	Leu	Ile	Ala	Val	Gly	Gly	Ala	Ser	Leu	Ile	Leu	Ala	Pro	Ala	
				20				25					30			

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Gly Val Ser Ala Tyr Gly Ile Phe Val Phe Gly Phe Phe Leu Ala Gln	
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Val Leu Phe Ser Glu Leu Asn Arg Lys Arg Leu Arg Lys Trp Ile Ser	
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Leu Arg Pro Lys Gly Trp Asn Asp Val Arg Leu Ala Val Ile Ile Ala	
85 90 95	
gga tat cgc gag gat cct tat atg ttc cag aag tgc ctc gag tct gta	336
Gly Tyr Arg Glu Asp Pro Tyr Met Phe Gln Lys Cys Leu Glu Ser Val	
100 105 110	
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Arg Asp Ser Asp Tyr Gly Asn Val Ala Arg Leu Ile Cys Val Ile Asp	
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Tyr Asn Asp Asn Ile Lys Lys Pro Glu Phe Val Leu Cys Glu Ser Asp	
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agg att act gca atg atg acg ctt tgg gac att ggc tgg ggt act cgc Arg Ile Thr Ala Met Met Thr Leu Trp Asp Ile Gly Trp Gly Thr Arg 450 455 460	1392
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 Val Leu Phe Ser Glu Leu Asn Arg Lys Arg Leu Arg Lys Trp Ile Ser
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 Gln Leu Ala Lys Met Asp Pro Ser Val Asn Ala Val Val Leu Ile Asp
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 Ser Asp Thr Val Leu Glu Lys Asp Ala Ile Leu Glu Val Val Tyr Pro
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 Leu Ala Cys Asp Pro Glu Ile Gln Ala Val Ala Gly Glu Cys Lys Ile
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 Trp Asn Thr Asp Thr Leu Leu Ser Leu Leu Val Ala Trp Arg Tyr Tyr
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 Ser Ala Phe Cys Val Glu Arg Ser Ala Gln Ser Phe Phe Arg Thr Val
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 Gln Cys Val Gly Gly Pro Leu Gly Ala Tyr Lys Ile Asp Ile Ile Lys
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Lys Ile Val Glu Phe Gln Ile Ile Lys Cys Lys Glu Lys Leu Ser Thr
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Leu Asp Ile Ala Thr Gln Leu Leu Leu Ser Asn Val Lys Lys Leu Thr
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cta tcc gaa tca gaa aaa aac agt tta aaa aat aaa tgg aaa tct atc 288
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Asp Thr His Asn Ile Thr Ala Glu Gln Phe Leu Asn Asp Pro Tyr Leu	
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Leu Thr Asp Gly Phe Asn Glu Asn Ile Glu Asn Ala Val Asp Tyr Asp	
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Gln	Arg	Cys	Val	Asp	Ser	Ala	Leu	Asn	Gln	Thr	Val	Val	Asp	Leu	Glu	
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Val	Cys	Ile	Cys	Asn	Asp	Gly	Ser	Thr	Asp	Asn	Thr	Leu	Glu	Val	Ile	
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Asn	Lys	Leu	Tyr	Gly	Asn	Asn	Pro	Arg	Val	Arg	Ile	Met	Ser	Lys	Pro	
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Asn	Gly	Gly	Ile	Ala	Ser	Ala	Ser	Asn	Ala	Ala	Val	Ser	Phe	Ala	Lys	
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Gly	Tyr	Tyr	Ile	Gly	Gln	Leu	Asp	Ser	Asp	Asp	Tyr	Leu	Glu	Pro	Asp	
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Ala	Val	Glu	Leu	Cys	Leu	Lys	Glu	Phe	Leu	Lys	Asp	Lys	Thr	Leu	Ala	
	530					535					540					
Cys	Val	Tyr	Thr	Thr	Asn	Arg	Asn	Val	Asn	Pro	Asp	Gly	Ser	Leu	Ile	
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Ala	Asn	Gly	Tyr	Asn	Trp	Pro	Glu	Phe	Ser	Arg	Glu	Lys	Leu	Thr	Thr	
			565						570					575		

Ala	Met	Ile	Ala	His	His	Phe	Arg	Met	Phe	Thr	Ile	Arg	Ala	Trp	His	580	585	590
Leu	Thr	Asp	Gly	Phe	Asn	Glu	Asn	Ile	Glu	Asn	Ala	Val	Asp	Tyr	Asp	595	600	605
Met	Phe	Leu	Lys	Leu	Ser	Glu	Val	Gly	Lys	Phe	Lys	His	Leu	Asn	Lys	610	615	620
Ile	Cys	Tyr	Asn	Arg	Val	Leu	His	Gly	Asp	Asn	Thr	Ser	Ile	Lys	Lys	625	630	635
Leu	Gly	Ile	Gln	Lys	Lys	Asn	His	Phe	Val	Val	Val	Asn	Gln	Ser	Leu	645	650	655
Asn	Arg	Gln	Gly	Ile	Asn	Tyr	Tyr	Asn	Tyr	Asp	Lys	Phe	Asp	Asp	Leu	660	665	670
Asp	Glu	Ser	Arg	Lys	Tyr	Ile	Phe	Asn	Lys	Thr	Ala	Glu	Tyr	Gln	Glu	675	680	685
Glu	Met	Asp	Ile	Leu	Lys	Asp	Leu	Lys	Leu	Ile	Gln	Asn	Lys	Asp	Ala	690	695	700
Lys	Ile	Ala	Val	Ser	Ile	Phe	Tyr	Pro	Asn	Thr	Leu	Asn	Gly	Leu	Val	705	710	715
Lys	Lys	Leu	Asn	Asn	Ile	Ile	Glu	Tyr	Asn	Lys	Asn	Ile	Phe	Val	Ile	725	730	735
Ile	Leu	His	Val	Asp	Lys	Asn	His	Leu	Thr	Pro	Asp	Ile	Lys	Lys	Glu	740	745	750
Ile	Leu	Ala	Phe	Tyr	His	Lys	His	Gln	Val	Asn	Ile	Leu	Leu	Asn	Asn	755	760	765
Asp	Ile	Ser	Tyr	Tyr	Thr	Ser	Asn	Arg	Leu	Ile	Lys	Thr	Glu	Ala	His	770	775	780
Leu	Ser	Asn	Ile	Asn	Lys	Leu	Ser	Gln	Leu	Asn	Leu	Asn	Cys	Glu	Tyr	785	790	795
Ile	Ile	Phe	Asp	Asn	His	Asp	Ser	Leu	Phe	Val	Lys	Asn	Asp	Ser	Tyr	805	810	815
Ala	Tyr	Met	Lys	Lys	Tyr	Asp	Val	Gly	Met	Asn	Phe	Ser	Ala	Leu	Thr	820	825	830
His	Asp	Trp	Ile	Glu	Lys	Ile	Asn	Ala	His	Pro	Pro	Phe	Lys	Lys	Leu	835	840	845
Ile	Lys	Thr	Tyr	Phe	Asn	Asp	Asn	Asp	Leu	Arg	Ser	Met	Asn	Val	Lys	850	855	860
Gly	Ala	Ser	Gln	Gly	Met	Phe	Met	Lys	Tyr	Ala	Leu	Pro	His	Glu	Leu	865	870	875
Leu	Thr	Ile	Ile	Lys	Glu	Val	Ile	Thr	Ser	Cys	Gln	Ser	Ile	Asp	Ser	885	890	895
Val	Pro	Glu	Tyr	Asn	Thr	Glu	Asp	Ile	Trp	Phe	Gln	Phe	Ala	Leu	Leu	900	905	910
Ile	Leu	Glu	Lys	Lys	Thr	Gly	His	Val	Phe	Asn	Lys	Thr	Ser	Thr	Leu	915	920	925
Thr	Tyr	Met	Pro	Trp	Glu	Arg	Lys	Leu	Gln	Trp	Thr	Asn	Glu	Gln	Ile	930	935	940
Gln	Ser	Ala	Lys	Lys	Gly	Glu	Asn	Ile	Pro	Val	Asn	Lys	Phe	Ile	Ile	945	950	955
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Pro Gln His Glu Lys Glu Asn Glu Leu Asn Thr Phe Gln Asn Lys Ile	
20 25 30	
gat agt cta aaa aca act tta aac aaa gac att att tct caa caa act	144
Asp Ser Leu Lys Thr Thr Leu Asn Lys Asp Ile Ile Ser Gln Gln Thr	
35 40 45	
cta ttg gca aaa cag gac agt aaa cat ccg cta tcc gca tcc ctt gaa	192
Leu Leu Ala Lys Gln Asp Ser Lys His Pro Leu Ser Ala Ser Leu Glu	
50 55 60	
aac gaa aat aaa ctt tta tta aaa caa ctc caa ttg gtt ctg caa gaa	240
Asn Glu Asn Lys Leu Leu Leu Lys Gln Leu Gln Leu Val Leu Gln Glu	
65 70 75 80	
ttt aaa aaa ata tat acc tat aat caa gca tta gaa gca aag cta gaa	288
Phe Lys Lys Ile Tyr Thr Tyr Asn Gln Ala Leu Glu Ala Lys Leu Glu	
85 90 95	
aaa gat aag caa aca aca tca ata aca gat tta tat aat gaa gtc gct	336
Lys Asp Lys Gln Thr Thr Ser Ile Thr Asp Leu Tyr Asn Glu Val Ala	
100 105 110	
aaa agt gat tta ggg tta gtc aaa gaa acc aac agc gca aat cca tta	384
Lys Ser Asp Leu Gly Leu Val Lys Glu Thr Asn Ser Ala Asn Pro Leu	
115 120 125	
gtc agt att atc atg aca tct cac aat aca gcg caa ttt atc gaa gct	432
Val Ser Ile Ile Met Thr Ser His Asn Thr Ala Gln Phe Ile Glu Ala	
130 135 140	
tct att aat tca tta ttg tta caa aca tat aaa aac ata gaa att att	480
Ser Ile Asn Ser Leu Leu Leu Gln Thr Tyr Lys Asn Ile Glu Ile Ile	
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att gta gat gat gat agc tcg gat aat aca ttt gaa att gcc tcg aga	528
Ile Val Asp Asp Asp Ser Ser Asp Asn Thr Phe Glu Ile Ala Ser Arg	
165 170 175	
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Ile Ala Asn Thr Thr Ser Lys Val Arg Val Phe Arg Leu Asn Ser Asn	
180 185 190	
cta gga act tac ttt gcg aaa aat aca ggc ata tta aaa tct aaa ggt	624
Leu Gly Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser Lys Gly	
195 200 205	

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caa tgt gat cat ttt cat att tat ctt gat gcc tat gta gaa atc cct Gln Cys Asp His Phe His Ile Tyr Leu Asp Gly Tyr Val Glu Ile Pro 420 425 430	1296
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Glu Leu Ile Glu Lys Asn Gln Asp Gly Tyr Tyr Ile Thr Cys Asp Asp	
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Pro Ser Arg Met Thr Lys Tyr Phe Ser Ala Asp Arg Leu Val Tyr Ser	
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Phe Tyr Lys Pro Leu Glu Lys Asp Lys Ala Val Asn Val Leu Gly Thr	
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Gly Thr Val Ser Phe Arg Val Ser Leu Phe Asn Gln Phe Ser Leu Ser	
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      35      40      45
Leu Leu Ala Lys Gln Asp Ser Lys His Pro Leu Ser Ala Ser Leu Glu
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Asn Glu Asn Lys Leu Leu Leu Lys Gln Leu Gln Leu Val Leu Gln Glu
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Phe Lys Lys Ile Tyr Thr Tyr Asn Gln Ala Leu Glu Ala Lys Leu Glu
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290     295     300
Glu Lys Ile Lys Asn Leu Leu Leu Pro Leu Tyr Tyr Asn Thr Met Arg
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370     375     380
Lys Leu Ser Asn Pro Lys Ile Pro Val Tyr Ile Asn Ile Cys Ser Ile
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Pro Ser Arg Ile Ala Gln Leu Gln Arg Ile Ile Gly Ile Leu Lys Asn
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Gln Cys Asp His Phe His Ile Tyr Leu Asp Gly Tyr Val Glu Ile Pro
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Asp Phe Ile Lys Asn Leu Gly Asn Lys Ala Thr Val Val His Cys Lys
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Asp Lys Asp Asn Ser Ile Arg Asp Asn Gly Lys Phe Ile Leu Leu Glu
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95

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 485 490 495
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 565 570 575
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 Ile Thr Pro Glu Ser Phe Ser Lys Leu Ile Lys Tyr Trp Asn Glu Ala
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 Thr Val Trp Asn Asp Asn Glu Asp Lys Lys Ile Met Gln Tyr Asn Pro
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atc atc atc gaa aga aag atc att cat gaa ctg ggg cta tgt ggc cac	288
Ile Ile Ile Glu Arg Lys Ile Ile His Glu Leu Gly Leu Cys Gly His	
85 90 95	
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Ile Glu Asp Ile Ala Val Asn Ser Lys Tyr Gln Gly Gln Gly Leu Gly	
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Lys Leu Leu Ile Asp Gln Leu Val Thr Ile Gly Phe Asp Tyr Gly Cys	
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Tyr Lys Ile Ile Leu Asp Cys Asp Glu Lys Asn Val Lys Phe Tyr Glu	
130 135 140	
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Thr Val Trp Asn Asp Asn Glu Asp Lys Lys Ile Met Gln Tyr Asn Pro
50 55 60
Met Val Ile Val Asp Lys Arg Thr Glu Thr Val Ala Ala Thr Gly Asn
65 70 75 80
Ile Ile Ile Glu Arg Lys Ile Ile His Glu Leu Gly Leu Cys Gly His
85 90 95
Ile Glu Asp Ile Ala Val Asn Ser Lys Tyr Gln Gly Gln Gly Leu Gly
100 105 110
Lys Leu Leu Ile Asp Gln Leu Val Thr Ile Gly Phe Asp Tyr Gly Cys
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 Ser Asn Leu Glu Gln Ile Ser Ser Lys Arg Ser Pro Ala Lys Leu Leu
 35 40 45

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 85 90 95

 gaa gct att ggc aag ggt gaa gtc gca gtg att tta atg gct ggc gga 336
 Glu Ala Ile Gly Lys Gly Glu Val Ala Val Ile Leu Met Ala Gly Gly
 100 105 110

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 Gln Gly Thr Arg Leu Gly Ser Ser Gln Pro Lys Gly Cys Tyr Asp Ile
 115 120 125

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 Gly Leu Pro Ser Lys Lys Ser Leu Phe Gln Ile Gln Ala Glu Lys Leu
 130 135 140

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 Tyr Ile Met Thr Ser Gly Pro Thr Arg Ala Ala Thr Glu Ala Tyr Phe
 165 170 175

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 180 185 190

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Asn Gln Gly Thr Leu Pro Ala Phe Asp Leu Thr Gly Lys His Phe Leu	
195 200 205	
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Arg Gly Ile Lys His Val Tyr Met Tyr Cys Val Asp Asn Val Leu Ser	
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Lys Ile Ala Asp Pro Val Phe Ile Gly Phe Ala Ile Lys His Gly Phe	
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275 280 285	
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Gly Leu Ile Ala Thr Lys Asn Glu Lys Pro Cys Val Ile Glu Tyr Ser	
290 295 300	
gaa att tcc aat gaa ttg gct gaa gca aag gat aaa gat ggc tta tta	960
Glu Ile Ser Asn Glu Leu Ala Glu Ala Lys Asp Lys Asp Gly Leu Leu	
305 310 315 320	
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Lys Leu Arg Ala Gly Asn Ile Val Asn His Tyr Tyr Leu Val Asp Leu	
325 330 335	
cta aaa cgt gat ttg gat cag tgg tgt gag aat atg cca tat cac att	1056
Leu Lys Arg Asp Leu Asp Gln Trp Cys Glu Asn Met Pro Tyr His Ile	
340 345 350	
gcg aag aag aaa att cca gct tat gat agt gtt acc ggc aag tac act	1104
Ala Lys Lys Lys Ile Pro Ala Tyr Asp Ser Val Thr Gly Lys Tyr Thr	
355 360 365	
aag cct acc gaa cca aac ggt ata aaa tta gag caa ttc ata ttt gat	1152
Lys Pro Thr Glu Pro Asn Gly Ile Lys Leu Glu Gln Phe Ile Phe Asp	
370 375 380	
gtc ttt gac act gta cca ctg aac aag ttt ggg tgc tta gaa gta gat	1200
Val Phe Asp Thr Val Pro Leu Asn Lys Phe Gly Cys Leu Glu Val Asp	
385 390 395 400	
aga tgc aaa gaa ttt tca cct tta aaa aac ggt cct ggt tct aag aac	1248
Arg Cys Lys Glu Phe Ser Pro Leu Lys Asn Gly Pro Gly Ser Lys Asn	
405 410 415	
gat aat cct gag acc agc aga cta gca tat ttg aaa cta gga acc tcg	1296
Asp Asn Pro Glu Thr Ser Arg Leu Ala Tyr Leu Lys Leu Gly Thr Ser	
420 425 430	

tgg	ttg	gaa	gat	gca	ggc	gct	att	gta	aaa	gat	ggg	gta	cta	gtc	gaa	1344
Trp	Leu	Glu	Asp	Ala	Gly	Ala	Ile	Val	Lys	Asp	Gly	Val	Leu	Val	Glu	
		435					440					445				

gtt	tcc	agc	aaa	ttg	agt	tat	gca	ggg	gaa	aat	cta	tcc	cag	ttc	aaa	1392
Val	Ser	Ser	Lys	Leu	Ser	Tyr	Ala	Gly	Glu	Asn	Leu	Ser	Gln	Phe	Lys	
		450				455					460					

ggg	aaa	gtc	ttt	gac	aga	agt	ggg	ata	gta	tta	gaa	aaa	taa			1434
Gly	Lys	Val	Phe	Asp	Arg	Ser	Gly	Ile	Val	Leu	Glu	Lys				
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<211> 477

<212> PRT

<213> *Saccharomyces cerevisiae*

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			20					25					30			
Ser	Asn	Leu	Glu	Gln	Ile	Ser	Ser	Lys	Arg	Ser	Pro	Ala	Lys	Leu	Leu	
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Glu	Asp	Cys	Gln	Asn	Ala	Ile	Lys	Phe	Ser	Leu	Ala	Asn	Ser	Ser	Lys	
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Asp	Thr	Gly	Val	Glu	Ile	Ser	Pro	Leu	Pro	Pro	Thr	Ser	Tyr	Glu	Ser	
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Leu	Ile	Gly	Asn	Ser	Lys	Lys	Glu	Asn	Glu	Tyr	Trp	Arg	Leu	Gly	Leu	
			85					90					95			
Glu	Ala	Ile	Gly	Lys	Gly	Glu	Val	Ala	Val	Ile	Leu	Met	Ala	Gly	Gly	
			100					105					110			
Gln	Gly	Thr	Arg	Leu	Gly	Ser	Ser	Gln	Pro	Lys	Gly	Cys	Tyr	Asp	Ile	
		115					120					125				
Gly	Leu	Pro	Ser	Lys	Lys	Ser	Leu	Phe	Gln	Ile	Gln	Ala	Glu	Lys	Leu	
	130					135					140					
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Tyr	Ile	Met	Thr	Ser	Gly	Pro	Thr	Arg	Ala	Ala	Thr	Glu	Ala	Tyr	Phe	
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Gln	Glu	His	Asn	Tyr	Phe	Gly	Leu	Asn	Lys	Glu	Gln	Ile	Thr	Phe	Phe	
			180					185					190			
Asn	Gln	Gly	Thr	Leu	Pro	Ala	Phe	Asp	Leu	Thr	Gly	Lys	His	Phe	Leu	
		195				200						205				
Met	Lys	Asp	Pro	Val	Asn	Leu	Ser	Gln	Ser	Pro	Asp	Gly	Asn	Gly	Gly	
	210					215					220					
Leu	Tyr	Arg	Ala	Ile	Lys	Glu	Asn	Lys	Leu	Asn	Glu	Asp	Phe	Asp	Arg	
225					230					235					240	
Arg	Gly	Ile	Lys	His	Val	Tyr	Met	Tyr	Cys	Val	Asp	Asn	Val	Leu	Ser	
			245					250						255		
Lys	Ile	Ala	Asp	Pro	Val	Phe	Ile	Gly	Phe	Ala	Ile	Lys	His	Gly	Phe	
		260						265					270			
Glu	Leu	Ala	Thr	Lys	Ala	Val	Arg	Lys	Arg	Asp	Ala	His	Glu	Ser	Val	
	275						280					285				
Gly	Leu	Ile	Ala	Thr	Lys	Asn	Glu	Lys	Pro	Cys	Val	Ile	Glu	Tyr	Ser	
	290					295					300					
Glu	Ile	Ser	Asn	Glu	Leu	Ala	Glu	Ala	Lys	Asp	Lys	Asp	Gly	Leu	Leu	
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100

Lys Leu Arg Ala Gly Asn Ile Val Asn His Tyr Tyr Leu Val Asp Leu
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 340 345 350
 Ala Lys Lys Lys Ile Pro Ala Tyr Asp Ser Val Thr Gly Lys Tyr Thr
 355 360 365
 Lys Pro Thr Glu Pro Asn Gly Ile Lys Leu Glu Gln Phe Ile Phe Asp
 370 375 380
 Val Phe Asp Thr Val Pro Leu Asn Lys Phe Gly Cys Leu Glu Val Asp
 385 390 395 400
 Arg Cys Lys Glu Phe Ser Pro Leu Lys Asn Gly Pro Gly Ser Lys Asn
 405 410 415
 Asp Asn Pro Glu Thr Ser Arg Leu Ala Tyr Leu Lys Leu Gly Thr Ser
 420 425 430
 Trp Leu Glu Asp Ala Gly Ala Ile Val Lys Asp Gly Val Leu Val Glu
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 Val Ser Ser Lys Leu Ser Tyr Ala Gly Glu Asn Leu Ser Gln Phe Lys
 450 455 460
 Gly Lys Val Phe Asp Arg Ser Gly Ile Val Leu Glu Lys
 465 470 475

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 <222> (8)..(352)
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 <222> (664)..(872)
 <223> polyA-signal of the nos gene from Agrobacterium tumefaciens

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 caaagtggtc cctaccacga tggaaaaact gtgcagtcgg tttggctttt tctgacgaac 180
 aaataagatt cgtggccgac aggtgggggt ccaccatgtg aaggcatctt cagactccaa 240
 taatggagca atgacgtaag ggcttacgaa ataagtaagg gtagtttggg aaatgtccac 300
 tcacccgtca gtctataaat acttagcccc tccctcattg ttaagggagc aagagctcgc 360
 ccgggatctc gaatcacgcg ttctaggatc cgaagcagat cgttcaaaca tttggcaata 420
 aagtttctta agattgaatc ctgttgccgg tcttgcatg attatcatat aatttctgtt 480
 gaattacggt aagcatgtaa taattaacat gtaatgcatg acgttattta tgagatgggt 540
 ttttatgatt agagtcccgc aattatacat ttaatacgcg atagaaaaca aaatatagcg 600
 cgcaaaactag gataaattat cgcgcgcggt gtcattctatg ttactagatc gggaagatct 660
 cgagacgtcg ggacaatcag taaattgaac ggagaatatt attcataaaa atacgatagt 720

101

aacgggtgat atattcatta gaatgaaccg aaaccggcgg taaggatctg agctacacat	780
gctcagggtt tttacaacgt gcacaacaga attgaaagca aatatcatgc gatcataggc	840
gtctcgcata tctcattaaa gcagggcatg cctgttttaa cattaattaa acctagggtga	900
cgtctaaaag ggcgaattc	919

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<210> 18
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aacaccagat cgaactgcaa

<210> 19
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103

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ggg gat gcg ccg atc aca cct gat ttt gtg ctt aag ctg ggt tgg gcc      96
Gly Asp Ala Pro Ile Thr Pro Asp Phe Val Leu Lys Leu Gly Trp Ala
          20          25          30

gcg ggt aaa gtg ctg gcg cgc cac ggc tcc cgt aag att att att ggt      144
Ala Gly Lys Val Leu Ala Arg His Gly Ser Arg Lys Ile Ile Ile Gly
          35          40          45

aaa gac acg cgt att tct ggc tat atg ctg gag tca gca ctg gaa gcg      192
Lys Asp Thr Arg Ile Ser Gly Tyr Met Leu Glu Ser Ala Leu Glu Ala
          50          55          60

ggg ctg gcg gca gcg ggc ctt tcc gca ctc ttc act ggc ccg atg cca      240
Gly Leu Ala Ala Ala Gly Leu Ser Ala Leu Phe Thr Gly Pro Met Pro
65          70          75          80

aca ccg gcc gtg gct tat ctg acg cgt acc ttc cgc gca gag gcc gga      288
Thr Pro Ala Val Ala Tyr Leu Thr Arg Thr Phe Arg Ala Glu Ala Gly
          85          90          95

att gtg ata tct gca tcg cat aac ccg ttc tac gat aat ggc att aaa      336
Ile Val Ile Ser Ala Ser His Asn Pro Phe Tyr Asp Asn Gly Ile Lys
          100          105          110

ttc ttc tct atc gac ggc acc aaa ctg ccg gat gcg gta gaa gag gcc      384
Phe Phe Ser Ile Asp Gly Thr Lys Leu Pro Asp Ala Val Glu Glu Ala
          115          120          125

atc gaa gcg gaa atg gaa aag gag atc agc tgc gtt gat tcg gca gaa      432
Ile Glu Ala Glu Met Glu Lys Glu Ile Ser Cys Val Asp Ser Ala Glu
          130          135          140

ctg ggt aaa gcc agc cgt atc gtt gat gcc gcg ggt cgc tat atc gag      480
Leu Gly Lys Ala Ser Arg Ile Val Asp Ala Ala Gly Arg Tyr Ile Glu
145          150          155          160

ttt tgc aaa gcc acg ttc ccg aac gaa ctt agc ctc agt gaa ctg aag      528
Phe Cys Lys Ala Thr Phe Pro Asn Glu Leu Ser Leu Ser Glu Leu Lys
          165          170          175

att gtg gtg gat tgt gca aac ggt gcg act tat cac atc gcg ccg aac      576
Ile Val Val Asp Cys Ala Asn Gly Ala Thr Tyr His Ile Ala Pro Asn
          180          185          190

gtg ctg cgc gaa ctg ggg gcg aac gtt atc gct atc ggt tgt gag cca      624
Val Leu Arg Glu Leu Gly Ala Asn Val Ile Ala Ile Gly Cys Glu Pro
          195          200          205

aac ggt gta aac atc aat gcc gaa gtg ggg gct acc gac gtt cgc gcg      672
Asn Gly Val Asn Ile Asn Ala Glu Val Gly Ala Thr Asp Val Arg Ala
          210          215          220

ctc cag gct cgt gtg ctg gct gaa aaa gcg gat ctc ggt att gcc ttc      720
Leu Gln Ala Arg Val Leu Ala Glu Lys Ala Asp Leu Gly Ile Ala Phe
225          230          235          240

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104

gac ggc gat ggc gat cgc gtg att atg gtt gac cat gaa ggc aat aaa	768
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245 250 255	
gtc gat ggc gat cag atc atg tat atc atc gcg cgt gaa ggt ctt cgt	816
Val Asp Gly Asp Gln Ile Met Tyr Ile Ile Ala Arg Glu Gly Leu Arg	
260 265 270	
cag ggc cag ctg cgt ggt ggc gct gtg ggt aca ttg atg agc aac atg	864
Gln Gly Gln Leu Arg Gly Gly Ala Val Gly Thr Leu Met Ser Asn Met	
275 280 285	
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Gly Leu Glu Leu Ala Leu Lys Gln Leu Gly Ile Pro Phe Ala Arg Ala	
290 295 300	
aaa gtg ggt gac cgc tac gta ctg gaa aaa atg cag gag aaa ggc tgg	960
Lys Val Gly Asp Arg Tyr Val Leu Glu Lys Met Gln Glu Lys Gly Trp	
305 310 315 320	
cgt atc ggt gca gag aat tcc ggt cat gtg atc ctg ctg gat aaa act	1008
Arg Ile Gly Ala Glu Asn Ser Gly His Val Ile Leu Leu Asp Lys Thr	
325 330 335	
act acc ggt gac ggc atc gtt gct ggc ttg cag gtg ctg gcg gcg atg	1056
Thr Thr Gly Asp Gly Ile Val Ala Gly Leu Gln Val Leu Ala Ala Met	
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gca cgt aac cat atg agc ctg cac gac ctt tgc agc ggc atg aaa atg	1104
Ala Arg Asn His Met Ser Leu His Asp Leu Cys Ser Gly Met Lys Met	
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ttc ccg cag att ctg gtt aac gta cgt tac acc gca ggt agc ggc gat	1152
Phe Pro Gln Ile Leu Val Asn Val Arg Tyr Thr Ala Gly Ser Gly Asp	
370 375 380	
cca ctt gag cat gag tca gtt aaa gcc gtg acc gca gag gtt gaa gct	1200
Pro Leu Glu His Glu Ser Val Lys Ala Val Thr Ala Glu Val Glu Ala	
385 390 395 400	
gcg ctg ggc aac cgt gga cgc gtg ttg ctg cgt aaa tcc ggc acc gaa	1248
Ala Leu Gly Asn Arg Gly Arg Val Leu Leu Arg Lys Ser Gly Thr Glu	
405 410 415	
ccg tta att cgc gtg atg gtg gaa ggc gaa gac gaa gcg cag gtg act	1296
Pro Leu Ile Arg Val Met Val Glu Gly Glu Asp Glu Ala Gln Val Thr	
420 425 430	
gaa ttt gca cac cgc atc gcc gat gca gta aaa gcc gtt taa	1338
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<210> 22

<211> 445

<212> PRT

<213> Escherichia coli

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Ala Gly Lys Val Leu Ala Arg His Gly Ser Arg Lys Ile Ile Ile Gly
35      40      45
Lys Asp Thr Arg Ile Ser Gly Tyr Met Leu Glu Ser Ala Leu Glu Ala
50      55      60
Gly Leu Ala Ala Ala Gly Leu Ser Ala Leu Phe Thr Gly Pro Met Pro
65      70      75      80
Thr Pro Ala Val Ala Tyr Leu Thr Arg Thr Phe Arg Ala Glu Ala Gly
85      90      95
Ile Val Ile Ser Ala Ser His Asn Pro Phe Tyr Asp Asn Gly Ile Lys
100     105     110
Phe Phe Ser Ile Asp Gly Thr Lys Leu Pro Asp Ala Val Glu Glu Ala
115     120     125
Ile Glu Ala Glu Met Glu Lys Glu Ile Ser Cys Val Asp Ser Ala Glu
130     135     140
Leu Gly Lys Ala Ser Arg Ile Val Asp Ala Ala Gly Arg Tyr Ile Glu
145     150     155     160
Phe Cys Lys Ala Thr Phe Pro Asn Glu Leu Ser Leu Ser Glu Leu Lys
165     170     175
Ile Val Val Asp Cys Ala Asn Gly Ala Thr Tyr His Ile Ala Pro Asn
180     185     190
Val Leu Arg Glu Leu Gly Ala Asn Val Ile Ala Ile Gly Cys Glu Pro
195     200     205
Asn Gly Val Asn Ile Asn Ala Glu Val Gly Ala Thr Asp Val Arg Ala
210     215     220
Leu Gln Ala Arg Val Leu Ala Glu Lys Ala Asp Leu Gly Ile Ala Phe
225     230     235     240
Asp Gly Asp Gly Asp Arg Val Ile Met Val Asp His Glu Gly Asn Lys
245     250     255
Val Asp Gly Asp Gln Ile Met Tyr Ile Ile Ala Arg Glu Gly Leu Arg
260     265     270
Gln Gly Gln Leu Arg Gly Gly Ala Val Gly Thr Leu Met Ser Asn Met
275     280     285
Gly Leu Glu Leu Ala Leu Lys Gln Leu Gly Ile Pro Phe Ala Arg Ala
290     295     300
Lys Val Gly Asp Arg Tyr Val Leu Glu Lys Met Gln Glu Lys Gly Trp
305     310     315     320
Arg Ile Gly Ala Glu Asn Ser Gly His Val Ile Leu Leu Asp Lys Thr
325     330     335
Thr Thr Gly Asp Gly Ile Val Ala Gly Leu Gln Val Leu Ala Ala Met
340     345     350
Ala Arg Asn His Met Ser Leu His Asp Leu Cys Ser Gly Met Lys Met
355     360     365
Phe Pro Gln Ile Leu Val Asn Val Arg Tyr Thr Ala Gly Ser Gly Asp
370     375     380
Pro Leu Glu His Glu Ser Val Lys Ala Val Thr Ala Glu Val Glu Ala
385     390     395     400
Ala Leu Gly Asn Arg Gly Arg Val Leu Leu Arg Lys Ser Gly Thr Glu
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420     425     430
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106

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<210> 25
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<309> 2007-05-19

<313> (3721481)..(3722851)

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acg cgc atg tat tcc gat ctt ccg aaa gtg ctg cat acc ctt gcc ggc 96
Thr Arg Met Tyr Ser Asp Leu Pro Lys Val Leu His Thr Leu Ala Gly
20 25 30

aaa gcg atg gtt cag cat gtc att gat gct gcg aat gaa tta ggc gca 144
Lys Ala Met Val Gln His Val Ile Asp Ala Ala Asn Glu Leu Gly Ala
35 40 45

gcg cac gtt cac ctg gtg tac ggt cac ggc ggc gat ctg cta aaa cag 192
Ala His Val His Leu Val Tyr Gly His Gly Gly Asp Leu Leu Lys Gln
50 55 60

gcg ctg aaa gac gac aac ctt aac tgg gtg ctt cag gca gag cag ctg 240
Ala Leu Lys Asp Asp Asn Leu Asn Trp Val Leu Gln Ala Glu Gln Leu
65 70 75 80

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ggt acg ggt cat gca atg cag cag gcc gca cct ttc ttt gcc gat gat	288
Gly Thr Gly His Ala Met Gln Gln Ala Ala Pro Phe Phe Ala Asp Asp	
85 90 95	
gaa gac att tta atg ctc tac ggc gac gtg ccg ctg atc tct gtc gaa	336
Glu Asp Ile Leu Met Leu Tyr Gly Asp Val Pro Leu Ile Ser Val Glu	
100 105 110	
aca ctc cag cgt ctg cgt gat gct aaa ccg cag ggt ggc att ggt ctg	384
Thr Leu Gln Arg Leu Arg Asp Ala Lys Pro Gln Gly Gly Ile Gly Leu	
115 120 125	
ctg acg gtg aaa ctg gat gat ccg acc ggt tat gga cgt atc acc cgt	432
Leu Thr Val Lys Leu Asp Asp Pro Thr Gly Tyr Gly Arg Ile Thr Arg	
130 135 140	
gaa aac ggc aaa gtt acc ggc att gtt gag cac aaa gat gcc acc ggc	480
Glu Asn Gly Lys Val Thr Gly Ile Val Glu His Lys Asp Ala Thr Gly	
145 150 155 160	
gag cag cgt cag att cag gag atc aac acc ggc att ctg att gcc aac	528
Glu Gln Arg Gln Ile Gln Glu Ile Asn Thr Gly Ile Leu Ile Ala Asn	
165 170 175	
ggc gca gat atg aaa cgc tgg ctg gcg aag ctg acc aac aat aat gct	576
Gly Ala Asp Met Lys Arg Trp Leu Ala Lys Leu Thr Asn Asn Asn Ala	
180 185 190	
cag ggc gaa tac tac atc acc gac att att gcg ctg gcg tat cag gaa	624
Gln Gly Glu Tyr Tyr Ile Thr Asp Ile Ile Ala Leu Ala Tyr Gln Glu	
195 200 205	
ggg cgt gaa atc gtc gcc gtt cat ccg caa cgt tta agc gaa gta gaa	672
Gly Arg Glu Ile Val Ala Val His Pro Gln Arg Leu Ser Glu Val Glu	
210 215 220	
ggc gtg aat aac cgc ctg caa ctc tcc cgt ctg gag cgt gtt tat cag	720
Gly Val Asn Asn Arg Leu Gln Leu Ser Arg Leu Glu Arg Val Tyr Gln	
225 230 235 240	
tcc gaa cag gct gaa aaa ctg ctg tta gca ggc gtt atg ctg cgc gat	768
Ser Glu Gln Ala Glu Lys Leu Leu Leu Ala Gly Val Met Leu Arg Asp	
245 250 255	
cca gcg cgt ttt gat ctg cgt ggt acg cta act cac ggg cgc gat gtt	816
Pro Ala Arg Phe Asp Leu Arg Gly Thr Leu Thr His Gly Arg Asp Val	
260 265 270	
gaa att gat act aac gtt atc atc gag ggc aac gtg act ctc ggt cat	864
Glu Ile Asp Thr Asn Val Ile Ile Glu Gly Asn Val Thr Leu Gly His	
275 280 285	
cgc gtg aaa att ggc acc ggt tgc gtg att aaa aac agc gtg att ggc	912
Arg Val Lys Ile Gly Thr Gly Cys Val Ile Lys Asn Ser Val Ile Gly	
290 295 300	
gat gat tgc gaa atc agt ccg tat acc gtt gtg gaa gat gcg aat ctg	960
Asp Asp Cys Glu Ile Ser Pro Tyr Thr Val Val Glu Asp Ala Asn Leu	
305 310 315 320	

108

gca gcg gcc tgt acc att ggc ccg ttt gcc cgt ttg cgt cct ggt gct	1008
Ala Ala Ala Cys Thr Ile Gly Pro Phe Ala Arg Leu Arg Pro Gly Ala	
325 330 335	
gag ttg ctg gaa ggt gct cac gtc ggt aac ttc gtt gag atg aaa aaa	1056
Glu Leu Leu Glu Gly Ala His Val Gly Asn Phe Val Glu Met Lys Lys	
340 345 350	
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Ala Arg Leu Gly Lys Gly Ser Lys Ala Gly His Leu Thr Tyr Leu Gly	
355 360 365	
gat gcg gaa att ggc gat aac gtt aac atc ggc gcg gga acc att acc	1152
Asp Ala Glu Ile Gly Asp Asn Val Asn Ile Gly Ala Gly Thr Ile Thr	
370 375 380	
tgc aac tac gat ggt gcg aat aaa ttt aag acc att atc ggc gac gat	1200
Cys Asn Tyr Asp Gly Ala Asn Lys Phe Lys Thr Ile Ile Gly Asp Asp	
385 390 395 400	
gtg ttt gtt ggt tcc gac act cag ctg gtg gcc ccg gta aca gta ggc	1248
Val Phe Val Gly Ser Asp Thr Gln Leu Val Ala Pro Val Thr Val Gly	
405 410 415	
aaa ggc gcg acc att gct gcg ggt aca act gtg acg cgt aat gtc ggc	1296
Lys Gly Ala Thr Ile Ala Ala Gly Thr Thr Val Thr Arg Asn Val Gly	
420 425 430	
gaa aat gca tta gct atc agc cgt gtg ccg cag act cag aaa gaa ggc	1344
Glu Asn Ala Leu Ala Ile Ser Arg Val Pro Gln Thr Gln Lys Glu Gly	
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Trp Arg Arg Pro Val Lys Lys Lys	
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 Ala His Val His Leu Val Tyr Gly His Gly Gly Asp Leu Leu Lys Gln
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 Gly Thr Gly His Ala Met Gln Gln Ala Ala Pro Phe Phe Ala Asp Asp
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 Thr Leu Gln Arg Leu Arg Asp Ala Lys Pro Gln Gly Gly Ile Gly Leu
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Glu Asn Gly Lys Val Thr Gly Ile Val Glu His Lys Asp Ala Thr Gly
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Glu Gln Arg Gln Ile Gln Glu Ile Asn Thr Gly Ile Leu Ile Ala Asn
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Gly Ala Asp Met Lys Arg Trp Leu Ala Lys Leu Thr Asn Asn Asn Ala
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Gln Gly Glu Tyr Tyr Ile Thr Asp Ile Ile Ala Leu Ala Tyr Gln Glu
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Gly Arg Glu Ile Val Ala Val His Pro Gln Arg Leu Ser Glu Val Glu
210                      215                      220
Gly Val Asn Asn Arg Leu Gln Leu Ser Arg Leu Glu Arg Val Tyr Gln
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Ser Glu Gln Ala Glu Lys Leu Leu Leu Ala Gly Val Met Leu Arg Asp
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Pro Ala Arg Phe Asp Leu Arg Gly Thr Leu Thr His Gly Arg Asp Val
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Glu Ile Asp Thr Asn Val Ile Ile Glu Gly Asn Val Thr Leu Gly His
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Arg Val Lys Ile Gly Thr Gly Cys Val Ile Lys Asn Ser Val Ile Gly
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Asp Asp Cys Glu Ile Ser Pro Tyr Thr Val Val Glu Asp Ala Asn Leu
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Ala Ala Ala Cys Thr Ile Gly Pro Phe Ala Arg Leu Arg Pro Gly Ala
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Glu Leu Leu Glu Gly Ala His Val Gly Asn Phe Val Glu Met Lys Lys
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Ala Arg Leu Gly Lys Gly Ser Lys Ala Gly His Leu Thr Tyr Leu Gly
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Cys Asn Tyr Asp Gly Ala Asn Lys Phe Lys Thr Ile Ile Gly Asp Asp
385                      390                      395                      400
Val Phe Val Gly Ser Asp Thr Gln Leu Val Ala Pro Val Thr Val Gly
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Lys Gly Ala Thr Ile Ala Ala Gly Thr Thr Val Thr Arg Asn Val Gly
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Met	Lys	Val	Asp	Tyr	Glu	Gln	Leu	Cys	Lys	Leu	Tyr	Asp	Asp	Thr	Cys	
1				5				10						15		

cgc	aca	aag	aat	gtg	cag	ttc	agt	tac	ggg	acg	gcc	gga	ttc	aga	acg	96
Arg	Thr	Lys	Asn	Val	Gln	Phe	Ser	Tyr	Gly	Thr	Ala	Gly	Phe	Arg	Thr	
			20					25					30			

ctg	gcc	aag	aat	ttg	gat	acg	gtg	atg	ttc	agt	act	ggg	ata	ctg	gcg	144
Leu	Ala	Lys	Asn	Leu	Asp	Thr	Val	Met	Phe	Ser	Thr	Gly	Ile	Leu	Ala	
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gtt	ctc	agg	tcg	ctg	aag	ctt	cag	ggg	cag	tat	gtg	ggg	gtg	atg	atc	192
Val	Leu	Arg	Ser	Leu	Lys	Leu	Gln	Gly	Gln	Tyr	Val	Gly	Val	Met	Ile	
	50						55				60					

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Thr	Ala	Ser	His	Asn	Pro	Tyr	Gln	Asp	Asn	Gly	Val	Lys	Ile	Val	Glu	
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cca	gac	gga	tcg	atg	ctt	ttg	gcc	aca	tgg	gag	cca	tat	gcc	atg	cag	288
Pro	Asp	Gly	Ser	Met	Leu	Leu	Ala	Thr	Trp	Glu	Pro	Tyr	Ala	Met	Gln	
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Leu	Ala	Asn	Ala	Ala	Ser	Phe	Ala	Thr	Asn	Phe	Glu	Glu	Phe	Arg	Val	
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Glu	Leu	Ala	Lys	Leu	Ile	Glu	His	Glu	Lys	Ile	Asp	Leu	Asn	Thr	Thr	
		115					120					125				

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Val	Val	Pro	His	Ile	Val	Val	Gly	Arg	Asp	Ser	Arg	Glu	Ser	Ser	Pro	
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Tyr	Leu	Leu	Arg	Cys	Leu	Thr	Ser	Ser	Met	Ala	Ser	Val	Phe	His	Ala	
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Gln	Val	Leu	Asp	Leu	Gly	Cys	Val	Thr	Thr	Pro	Gln	Leu	His	Tyr	Ile	
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Ala Thr Glu Gln Asp Tyr Tyr Ser Phe Phe Ile Gly Ala Phe Asn Glu	
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ctc ttc gcc acg tat cag ctg gag aag agg ctg tct gtc cca aaa ttg	672
Leu Phe Ala Thr Tyr Gln Leu Glu Lys Arg Leu Ser Val Pro Lys Leu	
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Phe Ile Asp Thr Ala Asn Gly Ile Gly Gly Pro Gln Leu Lys Lys Leu	
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Asn Asp Arg Ser Asp Val Pro Glu Leu Leu Asn Phe Glu Cys Gly Ala	
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Asp Tyr Val Lys Thr Asn Gln Arg Leu Pro Lys Gly Leu Ser Pro Ser	
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Val Phe Tyr Tyr Val Asp Ser Gly Ser Lys Phe His Leu Leu Asp Gly	
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Asp Lys Ile Ser Thr Leu Phe Ala Lys Phe Leu Ser Lys Gln Leu Glu	
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Tyr Ala Asn Gly Ser Ser Thr Ala Tyr Ile Lys Asn Thr Leu His Cys	
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ccc gtg tct tgc act aag aca ggt gtt aaa cac ttg cat cat gaa gct	1152
Pro Val Ser Cys Thr Lys Thr Gly Val Lys His Leu His His Glu Ala	
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Thr Ile Ile Phe Ser Glu Lys Phe His Arg Thr Ile Lys Ser Glu Leu	
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Asp Trp Asp Glu Glu Tyr Thr Asp Leu Pro Asn Lys Leu Val Lys Cys	
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Ile Val Pro Asp Arg Ser Ile Phe Gln Thr Thr Asp Gln Glu Arg Lys	
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 115 120 125

Val	Val	Pro	His	Ile	Val	Val	Gly	Arg	Asp	Ser	Arg	Glu	Ser	Ser	Pro
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Gln	Val	Leu	Asp	Leu	Gly	Cys	Val	Thr	Thr	Pro	Gln	Leu	His	Tyr	Ile
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Thr	Asp	Leu	Ser	Asn	Arg	Arg	Lys	Leu	Glu	Gly	Asp	Thr	Ala	Pro	Val
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Asn	Asp	Arg	Ser	Asp	Val	Pro	Glu	Leu	Leu	Asn	Phe	Glu	Cys	Gly	Ala
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Ser	Phe	Asp	Ser	Leu	Tyr	Cys	Ser	Phe	Asp	Gly	Asp	Ala	Asp	Arg	Val
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Val	Phe	Tyr	Tyr	Val	Asp	Ser	Gly	Ser	Lys	Phe	His	Leu	Leu	Asp	Gly
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Asp	Lys	Ile	Ser	Thr	Leu	Phe	Ala	Lys	Phe	Leu	Ser	Lys	Gln	Leu	Glu
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Pro	Val	Ser	Cys	Thr	Lys	Thr	Gly	Val	Lys	His	Leu	His	His	Glu	Ala
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Ala	Thr	Gln	Tyr	Asp	Ile	Gly	Ile	Tyr	Phe	Glu	Ala	Asn	Gly	His	Gly
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Thr	Ile	Ile	Phe	Ser	Glu	Lys	Phe	His	Arg	Thr	Ile	Lys	Ser	Glu	Leu
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Ser	Lys	Ser	Lys	Leu	Asn	Gly	Asp	Thr	Leu	Ala	Leu	Arg	Thr	Leu	Lys
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Cys	Phe	Ser	Glu	Leu	Ile	Asn	Gln	Thr	Val	Gly	Asp	Ala	Ile	Ser	Asp
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Met	Leu	Ala	Val	Leu	Ala	Thr	Leu	Ala	Ile	Leu	Lys	Met	Ser	Pro	Met
	450					455					460				
Asp	Trp	Asp	Glu	Glu	Tyr	Thr	Asp	Leu	Pro	Asn	Lys	Leu	Val	Lys	Cys
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Ile	Val	Pro	Asp	Arg	Ser	Ile	Phe	Gln	Thr	Thr	Asp	Gln	Glu	Arg	Lys
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Ala	Val	Arg	Val	Tyr	Ala	Glu	Cys	Lys	Asp	Ser	Ser	Lys	Leu	Gly	Gln
	530					535					540				
Phe	Cys	Asp	Glu	Val	Val	Glu	His	Val	Lys	Ala	Ser	Ala			
545					550							555			

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CLAIMS:

1. A genetically modified plant cell comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.
2. The cell of claim 1 that is a cell of a crop plant.
3. The cell of claim 1 or 2 that is a seed cell.
4. The cell of claim 1 or 2 that is a leaf cell.
5. A process for producing a plant, which comprises
 - a) genetically modifying a plant cell, wherein the genetic modification comprises steps i), ii) and iii) below:
 - i) introduction of a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase into the plant cell,
 - ii) introduction of a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase into the plant cell, and
 - iii) introduction of a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase into the plant cell,wherein individual steps i), ii) and iii) are carried out in any order; or any combination of steps i), ii) and iii) are carried out simultaneously;
 - b) regenerating a plant from the plant cell produced in step a); and
 - c) optionally, generating further plants using the plant obtained in step b).

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6. The process of claim 5, further comprising the step of isolating cells from the plant produced in step b) or c); and repeating steps a) to c) until a plant is generated which has a foreign nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.

7. Use of the cell of any one of claims 1 to 4, for producing a plant comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.

8. Use of a plant comprising a foreign nucleic acid molecule coding for a glucosaminoglycan synthase, a foreign nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a foreign nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for producing progeny plants or seeds.

9. A process for producing glucosaminoglycans, which comprises the step of extracting glucosaminoglycans from a genetically modified plant comprising the cell of any one of claims 1 to 4, or from a plant that is obtained by the process of claim 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.

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10. Use of a genetically modified plant comprising the cell of any one of claims 1 to 4, or a plant that is obtained by the process of claim 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for producing glucosaminoglycans.
11. A composition comprising the genetically modified plant cell of any one of claims 1 to 4, a nucleic acid molecule coding for a protein having the activity of a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.
12. A process for preparing a composition comprising glucosaminoglycans, which comprises comminuting a genetically modified plant comprising the cell of any one of claims 1 to 4 or a plant that is obtained by the process of claim 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase.
13. Use of a genetically modified plant comprising the cell of any one of claims 1 to 4, or a plant that is obtained by the process of claim 5 or 6 and which comprises a nucleic acid molecule coding for a glucosaminoglycan synthase, a nucleic acid molecule coding for a protein having the activity of a glucosamine 6-phosphate acetyltransferase, and a nucleic acid molecule coding for a protein having the activity of a monofunctional UDP-N-acetyl-glucosamine pyrophosphorylase, for preparing the composition of claim 11.