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(54) **Title:** CEREAL COMPRISING STARCH WITH LOW GELATINISATION TEMPERATURE

(57) **Abstract:** The present invention relates to the field of providing cereal plants with low starch gelatinisation temperature. Such cereal plants are for example useful for production of beverages, such as alcoholic beverages.

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Cereal comprising starch with low gelatinisation temperature

Technical field

The present invention relates to the field of providing cereal plants with low starch gelatinisation temperature. Such cereal plants are for example useful for production of beverages, such as alcoholic beverages.

Background

Starch is the principal source of sugars in production of cereal based beverages. In particular, production of alcoholic, cereal based beverages relies on aqueous cereals extracts containing high levels of fermentable sugars. In cereal grains, for example in barley or malted grains, starch is stored as insoluble inclusions known as granules. Enzymatic digestion of starch is greatly enhanced by heating the starch granules in the presence of water in a process known as gelatinisation. Once gelatinized, starch can be more easily degraded to fermentable sugars through the action of appropriate enzymes. Usually, the gelatinisation temperature of starch granules from barley is in the range of 59 to 64 °C, but may sometimes increase with up to 5 °C depending on the environmental conditions during cereal cultivation. In order to obtain fermentable sugars, the starch needs to be degraded through the action of enzymes like α-amylase, β-amylase and limit dextrinase after gelatinisation. When using barley malt as a starch source, this enzymatic activity is partly or fully provided by endogenous enzymes present in the malt. However, some of these endogenous barley enzymes are totally or partially inactivated following extensive heat treatment. As a consequence, there is a narrow temperature window, which allows gelatinisation of starch without negative effects on endogenous enzyme activity.

Summary

There is, thus, a need for cereal plants containing starch with low gelatinisation temperature. The present invention provides barley plants carrying a mutation in the *mPGES2* gene. Such barley plants contain starch with reduced gelatinisation temperature compared to barley plants not carrying said mutation, but otherwise identical.

It is thus an aspect of the invention to provide a barley plant, progeny thereof or parts thereof carrying a mutation in the *mPGES2* gene, wherein said mutation is a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *mPGES2* gene (SEQ ID NO: 2). Said mutant *HvmPGES2* gene encodes a mutant HvmPGES2 protein comprising a mutation of amino acid 265 of SEQ ID NO:4.

Another aspect of the invention is to provide a barley plant, progeny thereof or parts thereof carrying a mutation in the *mPGES2* gene, wherein the mutant *mPGES2* gene comprises the sequence SEQ ID NO:8 or a sequence at least 98% identical thereto, wherein said sequence comprises at least nucleotide 4657 of SEQ ID NO:8.

Another aspect of the invention is to provide a barley plant, progeny thereof or parts thereof carrying a mutation, wherein said mutation is a C to G exchange of the nucleotide corresponding to the nucleotide 501 of SEQ ID NO:11.

Another aspect of the invention is to provide a barley plant or a part thereof, or a progeny thereof, wherein the genome of said plant, comprises a genetic element consisting of a genomic region of barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, wherein said genomic region is positioned on chromosome 2H between the genomic position corresponding to 10,497,647 and the genomic position corresponding to 31,153,440 of the barley genome assembly by IBSC of 14-07-2016, and said genomic region comprises a mutant *mPGES2* gene carrying a G->A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *mPGES2* gene (SEQ ID NO:2). The barley genome assembly by IBSC of 14-07-2016 is available as IBSC (2016-07-14): Pseudomolecules of the map-based reference genome assembly of barley cv. Morex, DOI: 10.5447/IPK/2016/34 from Masher et al. 2017. Position 10,497,647 at chr 2H corresponds to position 501 in SEQ ID NO:9 and position 31,153,440 at chr 2H correspond to position 501 in SEQ ID NO:10. As will be appreciated by the person skilled in the art, genomic sequences may differ slightly between different barley plants, and positional information may vary between different releases of the reference genome, however the skilled person will be able to identify the nucleotide corresponding to 10,497,647 and 31,153,440 of aforementioned barley genome assembly in any given barley genome by aligning the relevant parts of the genomic sequence of said given barley genome with SEQ ID NO:9 and SEQ ID NO:10.

A further aspect of the invention is to provide a plant product comprising the barley plant as disclosed herein or a part thereof.

An even further aspect of the invention is to provide a method of preparing malt, said method comprising the steps of

(i) providing kernels of a barley plant as disclosed herein;
(ii) steeping said kernels;
(iii) germinating the steeped kernels under predetermined conditions;
(iv) drying said germinated kernels.

An even further aspect of the invention is to provide a method of producing a beverage, said method comprising the steps of:

(i) Providing kernels of a barley plant according to the present disclosure and/or malt according to the present disclosure
(ii) Preparing an aqueous extract of said kernels and/or said malt
(iii) processing said aqueous extract into a beverage.

Another aspect of the invention is to provide a method of preparing a barley plant containing starch with a low gelatinisation temperature, the method comprising the steps of

a) providing barley plants; and
b) introducing a mutation in the *mPGES2* gene or a deletion of the *mPGES2* of said barley plant; and
c) Selecting barley plants carrying a mutated *HvmPGES2* gene or a deletion of the *HvmPGES2* gene.

Description of Drawings

Figure 1 shows the gelatinisation temperature of starch from barley cv. Quench (black) and mutant line MQF08-1101 (white) propagated in the field 12 times. The figure shows the gelatinisation temperature of extracted starch from 2010 to 2014 and starch gelatinisation temperature determined directly on flour from 2015 to 2016 from a

representative sample from each pool. The bar graphs show the mean of several replicates (+standard deviation) for each line. Under each bar graph, the year of the field trial and the respective location, the Danish Island of Fyn or New Zealand, is noted.

Figure 2 shows the gelatinisation temperature of malt from barley grown on Fyn in 2014 and 2015 from three micromalted biological replicates of cv. Quench (black) and MQF08-1101 (white). The bar graphs show the mean of the three biological replicates (+standard deviation).

Figure 3 shows the starch gelatinisation temperature of mature grains of Quench (black) and MQF08-1101 (white) grown in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 16 to 21 days after flowering. The bar graph shows the mean of five individuals (+standard deviation).

Figure 4 shows the content of amylose as percentage of the total starch weight during development. A, the content of amylose as percentage of starch weight in mature grains of Quench (black) and MQF08-1101 (white) grown in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 16 to 21 days after flowering. The bar graph shows the mean of five biological replicates (+standard deviation). One biological replicate depicts one spike that were taken from different plants. B, the content of amylose from day 15 after flowering and until day 39 for Quench (solid line) and MQF08-1101 (dotted line) grown in controlled conditions using the same temperature program throughout the growth. Each point represents the mean of three biological samples (±standard deviation). One biological replicate depicts one half spike, which were taken from different plants. C, the content of amylose from day 15 after flowering and until day 39 for Quench (solid line) and MQF08-1101 (dotted line) grown in controlled conditions with appliance of a heat treatment from 16 to 21 days after flowering. Each point represents the mean of three biological samples (±standard deviation). One biological replicate depicts one half spike, which were taken from different plants.

Figure 5 shows the molar fraction of selected chain length ranges in debranched amylopectin isolated from starch. DP stands for degree of polymerization, which relates

to the number of α-1-4-linked glucose unit in amylopectin chains. A, B, illustrate molar fractions of specific DP ranges in starch of mature Quench (black) and MQF08-1101 (white) grown in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 16 to 21 days after flowering. The bar graph shows the mean of five biological replicates (+standard deviation). One biological replicate depicts one spike that were taken from different plants.

C, D, E, F, illustrate molar fractions of specific DP ranges during development from 15 days after flowering to 39 days after flowering for Quench (solid line) and MQF08-1101 (dotted line) grown in controlled conditions using the same temperature program throughout the growth (C, E), or with appliance of a heat treatment from 16 to 21 days after flowering (D, F). Each point represents the mean of three biological samples (±standard deviation). One biological replicate depicts one half spike, which were taken from different plants.

Figure 6 shows the average starch gelatinisation temperature of the individuals in a DH population of a cross between MQF08-1101 and the barley cultivar Melius. The individuals were split according to the genotype of the QTL linked to low starch gelatinisation temperature. The bar graph shows the average (+standard deviation) of starch gelatinisation temperature for 21 individuals with the MQF08-1101 allele (white) and 23 individuals with the Melius WT allele (black) in four different environments.

Figure 7 shows starch gelatinisation temperature of F2 plants from a cv. Quench x MQF08-1101 cross. The bar graphs show the average (+standard deviation) of the starch gelatinisation temperature of grains from the first tiller from the 26 plants homozygous for the MQF08-1101 A allele (white) and for the 28 plants homozygous for the Quench G allele (black).

Figure 8 shows starch gelatinisation temperature of plants carrying either the MQF08-1101 A allele or Quench G allele in a mixed genetic background. Plants previously crossed with MQF08-1101 were genotyped for MQF08-1101 A allele (white) or Quench G allele (black). Subsequently, the starch gelatinisation temperature was measured on a representative sample of grains from a plot in three replicates. The bar graph shows the mean (+standard deviation) of the technical replicates.

Figure 9 shows a model of HvmPGES2 based on the structure 1z9h.pdb of the Microsomal Prostaglandin E Synthase Type 2 (mPGES2) from crab-eating macaque. The protein main chain is shown in ribbon format and backbone and sidechains are shown for the active site cysteine (in grey) and glycine G265 (in black).

Figure 10 shows a multiple sequence alignment of Cys-type GSTs. The sequence of HvmPGES2 from barley was aligned with the sequences of various Cys-type GSTs. Sequences representing the six subfamilies of Cys-GSTs present in plants (GSTL, DHAR, GHR, GSTH, GSTI and mPGES2) were taken from Lallement et al.,(2014) and supplemented with additional mPGES2 sequences from tobacco (NtmPGES2), tomato (SlmPGES2) and tree cotton (GamPGES2) as well as the sequence of the macaque protein used for structure threading (PGES2_MACFA). The variable N-terminal regions were removed up to the active site cysteine and the truncated sequences were aligned in Clustal omega. Black shading indicates residues conserved in ≥90% of the aligned sequences. Residues in bold are absolutely conserved in the aligned mPGES2 sequences. The position of the SNP mutation (D) and other residues(*) where mutations based on protein structure might affect the protein function are indicated above the alignment. The boxed regions were used to generate a phylogram in CLC Main Workbench.

Figure 11 shows a phylogram of Cys-type GSTs. The boxed regions shown in figure 10 were used to generate a phylogram in CLC Main Workbench.

Detailed description

Definitions

As used herein, "a" can mean one or more, depending on the context in which it is used.

The term “amino acid corresponding to X” is used herein to describe amino acids of a given polypeptide (e.g. a mutant mPGES2 polypeptide) in relation to amino acids of a reference polypeptide (e.g. HvmPGES2 of SEQ ID NO:4). Following alignment between said polypeptide and the reference polypeptide, an amino acid is corresponding to X if it is in the same position as X in said alignment. The term “nucleotide corresponding to X” is used in a similar manner. Thus, following alignment

between a DNA sequence and a reference DNA sequence, a nucleotide is corresponding to X if it is in the same position as X in said alignment.

The term “amylose” refers to homopolymers of α-D-glucose. Amylose has a linear molecular structure, as its glucose units are almost exclusively linked by α-1-4-glycosidic bonds.

The term “amylopectin” refers to homopolymers of α-D-glucose. Amylopectin molecules contains frequent α-1-6-glycosidic linkages. These introduce branch points into the otherwise α-1-4-linked glucose chains resulting in clusters of parallel chains appearing in regular intervals along the molecule’s axis.

The term "barley" in reference to the process of making barley based beverages, such as beer, particularly when used to describe the malting process, means barley kernels. In all other cases, unless otherwise specified, "barley" means the barley plant (*Hordeum vulgare*, L.), including any breeding line or cultivar or variety, whereas part of a barley plant may be any part of a barley plant, for example any tissue or cells.

The term “barley flour” as used herein refers to milled barley kernels.

A "cereal" plant, as defined herein, is a member of the Poaceae plant family, cultivated primarily for their starch-containing seeds or kernels. Cereal plants include, but are not limited to barley (Hordeum), wheat (Triticum), rice (Oryza), maize (Zea), rye (Secale), oat (Avena), sorghum (Sorghum), and Triticale, a rye–wheat hybrid.

The term “DP” as used herein refers to the degree of polymerization, and indicates the number of α-1,4-linked glucose units in amylopectin side chains. Thus, by way of example DP3 refers to amylopectin side chains consisting of a sequence of 3 α-1,4-linked glucose units.

By "encoding" or "encoded", in the context of a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid or polynucleotide encoding a protein may comprise non-translated sequences, e.g. introns, within translated regions of the nucleic acid, or may lack such intervening non-

translated sequences, e.g. in cDNA. The information by which a protein is encoded is specified by the use of codons.

As used herein, "expression" in the context of nucleic acids is to be understood as the transcription and accumulation of mRNA. "Expression" used in the context of proteins refers to translation of mRNA into a polypeptide.

The term “gelatinisation temperature” as used herein refers to the peak temperature of a temperature range during which starch loses its semi-crystalline structure in water under the impact of heat and forms a gel. Preferably, gelatinisation temperature is determined as described in Example 2 below. Reference to a cereal having a particular gelatinisation temperature refers to a cereal comprising starch with said gelatinisation temperature.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (promoter and terminator). Furthermore, plant genes generally consist of exons interrupted by introns.

The term "kernel" is defined to comprise the cereal caryopsis, also denoted internal seed. In addition, the kernel may comprise the lemma and palea. In most barley varieties, the lemma and palea adhere to the caryopsis and are a part of the kernel following threshing. However, naked barley varieties also occur. In these, the caryopsis is free of the lemma and palea and threshes out free as in wheat. The terms "kernel" and "grain" are used interchangeably herein.

The term "malting" as used herein refers to a controlled germination of cereal kernels (in particular barley kernels) taking place under controlled environmental conditions in for example steep tanks and/or germination boxes. The malting process may comprise steeping followed by germination. The malting process may be stopped by drying of the germinated kernels, for example, in a kiln drying process at elevated temperatures.

The term “malt” as used herein refers to cereal kernels, which have been malted. Malt may be milled malt.

"Mashing" is the incubation of milled malt and/or cereal kernels in water. Mashing is preferably performed at a specific temperature, and in a specific volume of water.

The term “milled” refers to material (e.g. barley kernels or malt), which has been finely divided, e.g. by cutting, milling, grinding or crushing. The barley kernels can be milled while moist using e.g. a grinder or a wet mill. Milled barley kernels or milled malt is sufficiently finely divided to render the material useful for aqueous extracts. Milled barley kernels or milled malt cannot be regenerated into an intact plant by essentially biological methods.

"Mutations" include deletions, insertions, substitutions, transversions, and point mutations in the coding and noncoding regions of a gene. Deletions may be of the entire gene, or of only a portion of the gene. Point mutations may concern changes of one base pair, and may result in premature stop codons, frameshift mutations, mutation of a splice site or amino acid substitutions.

By the term "plant product" is meant a product resulting from the processing of a plant or plant material. Said plant product may thus, for example, be malt, wort, a fermented or non-fermented beverage, a food, or a feed product.

By the term “progeny” as used herein is meant a plant, which directly or indirectly is off-spring of a given plant. Thus, progeny is not confined to direct off-spring but also includes off-spring after numerous generations. In general, progeny of a barley plant carrying a specific mutation also carries that specific mutation. Thus, progeny of a barley plant carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene, also carry that mutation.

The term “sequence identity” as used herein refers to the % of identical amino acids or nucleotides between a candidate sequence and a reference sequence following alignment. Thus, a candidate sequence sharing 80% amino acid identity with a reference sequence requires that, following alignment, 80% of the amino acids or nucleotides in the candidate sequence are identical to the corresponding amino acids or nucleotides in the reference sequence. Identity according to the present invention is determined by aid of computer analysis, such as, without limitations, the Clustal Omega computer alignment program for alignment of polypeptide sequences (Sievers

et al. *(2011 October 11) Molecular Systems Biology 7 :539,* PMID: 21988835; Li et al. *(2015 April 06) Nucleic Acids Research 43 (W1) :W580-4* PMID: 25845596; McWilliam et al., *(2013 May 13) Nucleic Acids Research 41 (Web Server issue) :W597-600* PMID: 23671338), and the default parameters suggested therein. The Clustal Omega software is available from EMBL-EBI at https://www.ebi.ac.uk/Tools/msa/clustalo/. Using this program with its default settings, the mature (bioactive) part of a query and a reference polypeptide are aligned. The number of fully conserved residues are counted and divided by the length of the reference polypeptide. The MUSCLE or MAFFT algorithms may be used for alignment of nucleotide sequences. Sequence identities may be calculated in a similar way as indicated for amino acid sequences. Sequence identity as provided herein is thus calculated over the entire length of the reference sequence.

The term “starch” as used herein refers to a composition of one or both of the discrete macromolecules: amylose and amylopectin.

The term “stop codon” as used herein refers to a nucleotide triplet in the genetic code, which within mRNA results in termination of translation. The term “stop codon” as used herein also refers to a nucleotide triplet within a gene encoding the stop codon in mRNA. The stop codon in DNA typically has one of the following sequences: TAG, TAA or TGA.

The term "wild-type" barley refers to a conventionally generated barley plant.

By the term "wort" is meant a liquid extract of malt and/or cereal kernels, such as milled malt and/or milled cereal kernels and optionally additional adjuncts. Wort is in general obtained by mashing, optionally followed by "sparging", in a process of extracting residual sugars and other compounds from spent grains after mashing with hot water. Sparging is typically conducted in a lauter tun, a mash filter, or another apparatus to allow separation of the extracted water from spent grains. The wort obtained after mashing is generally referred to as "first wort", while the wort obtained after sparging is generally referred to as the "second wort". If not specified, the term wort may be first wort, second wort, or a combination of both. During conventional beer production, wort is boiled together with hops. Wort without hops, may also be referred to as "sweet wort", whereas wort boiled with hops may be referred to as "boiled wort" or simply as wort.

mPGES2

The present invention provides a barley plant carrying a mutation in *mPGES2* gene. More specifically, the invention provides a barley plant carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene.

The sequence of a wild type *mPGES2* gene from barley cultivar Morex supplemented with sequence information from barley cultivar Haruma Nijo is provided herein as SEQ ID NO:1. The skilled person will understand that other wild type cereal plants comprises an *mPGES2* gene with a sequence differing from SEQ ID NO:1 to some extent. For example, a cereal *mPGES2* gene is a gene encoding an mPGES2 polypeptide and sharing at least 70%, more preferably at least 80%, even more preferably at least 85%, yet more preferably at least 90%, even more preferably at least 95% sequence identity to SEQ ID NO:2 or SEQ ID NO:3. For example, a cereal *mPGES2* gene is a gene encoding an mPGES2 polypeptide sharing at least 70%, more preferably at least 80%, even more preferably at least 85%, yet more preferably at least 90%, even more preferably at least 95% sequence identity to SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6. In addition to sharing aforementioned sequence identity, the polypeptide encoded by a cereal *mPGES2* gene preferably also comprises at least the amino acids corresponding to amino acids 99-100, 138, 266, 273 of SEQ ID NO:4, more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99, 209, 223, 263, 265, 266 and 269 of SEQ ID NO:4, more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99-101, 104,138, 150, 153-154, 157, 258, 263, 266, 273-274, 280, 298, 301, 305 of SEQ ID NO:4, yet more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99-102, 104-105, 107-110, 116, 120-123, 128, 130, 132, 134, 136-138, 150, 153-154,157, 171-172, 174-176, 178, 180, 183-185, 189-190, 192-193, 197-199, 201-202, 204-205, 209, 211, 215, 220-221, 223-224, 227-228, 232, 234-235, 242, 244, 247-248, 250-251, 254, 257, 261, 265-266, 268-271, 273-275, 278-281, 286, 291, 296, 298, 301-302, 304, 306 of SEQ ID NO:4, even more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99-102, 104-111, 114, 116-124, 126, 128-132, 134-140, 143, 145, 147, 149-154,157, 161, 171-172, 174-176, 178, 180-181, 183-193, 196-199, 201-205, 209-211, 215, 220-221, 223-

235, 237-238, 240, 242, 247-252, 254, 257-258, 261, 263-266, 268-271, 273-275, 277-281, 283, 286, 288, 291-298, 301-302, 304-306, 309, 313 of SEQ ID NO:4, yet more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99-105, 107-111, 116-117, 120-123, 125-128, 130-132, 134, 136-138, 142-145, 150, 153-154, 157, 171-172, 174-176, 178-180, 182-185, 187, 189-193, 195-202, 204-205, 208-211, 214-216, 220-221, 223-224, 227-228, 230, 232, 234-237, 239-242, 244-245, 247-248, 250-251, 253-254, 257-258, 261, 264-266, 268-271, 273-275, 278-286, 289, 291-293, 296, 298-299, 301-302, 304-306, 310, 314-315 of SEQ ID NO:4, even more preferably the polypeptide encoded by a cereal *mPGES2* gene also comprises at least the amino acids corresponding to amino acids 99-111, 114, 116-140, 142-145, 147-151, 153-155, 157, 160-161, 171-172, 174-176, 178-193, 195-206, 208-211, 213-217, 220-221, 223-242, 244-254, 256-258, 261, 263-266, 268-271, 273-275, 277-286, 288-302, 304-306, 309-310, 313-315 of SEQ ID NO:4.

In a preferred embodiment of the invention the cereal plant is a barley plant, in which case the wild type *HvmPGES2* gene preferably encodes the polypeptide of SEQ ID NO:4 or SEQ ID NO:6.

Cereal plant carrying a mutation in the *mPGES2* gene

The present invention provides a cereal plant, in particular a barley plant carrying a mutation in *mPGES2* gene. More specifically, the invention provides a barley plant or progeny thereof carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene. Whereas the cereal plant of the invention preferably is a barley plant carrying said mutation, cereal plants carrying other mutations are also disclosed herein.

A mutation including one or more of the following is disclosed:

- A deletion of the *mPGES2* gene;
- A deletion of part of the *mPGES2* gene, e.g. a deletion of the coding region resulting in a mutant *mPGES2* gene encoding a mutant mPGES2 protein lacking one or more amino acids of SEQ ID NO:4 or SEQ ID NO:6
- A mutation resulting in a shift in reading frame, e.g. a deletion or an insertion

- A substitution of one or more base pairs, e.g. a substitution of one or more base pairs resulting in a mutant *mPGES2* gene encoding a mutant mPGES2 protein with a substitution of one or more amino acids.
- An insertion e.g. an insertion resulting in a shift in reading frame

The mutation may be a mutation resulting in a mutant *mPGES2* gene encoding a mutant mPGES2 protein lacking at least the amino acid corresponding to amino acid 265 of SEQ ID NO:4.

Said mutant mPGES2 protein can be lacking a given amino acid for several reasons including (but not limited to) the following:

- The mutant mPGES2 polypeptide may contain a deletion of said amino acids
- The mutant mPGES2 polypeptide may contain a deletion of several amino acids including the amino acid lacking
    - The mPGES2 polypeptide may contain a substitution of the amino acid to another amino acid.

The mutant mPGES2 may be a truncated polypeptide comprising only the N-terminal part of mPGES2. This may for example be the case when the mutation in the *mPGES2* gene is a mutation introducing a premature stop codon, a mutation in a splice site or a frame shift mutation.

Said truncated mPGES2 may be a mutant mPGES2 protein lacking at least the 8 most C-terminal amino acids, such as at least the 18 most C-terminal amino acids, for example at least the 30 most C-terminal amino acids 49, for example at least the 69 most C-terminal amino acids, preferably at least the 77 most C-terminal amino acids for example at least the 145 most C-terminal amino acids, such as at least the 148 most C-terminal amino acids, preferably at least the 192 most C-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6.

Said truncated mPGES2 may be an N-terminal fragment of mPGES2 comprising at the most the 314 N-terminal amino acids, such as at the most the 312 N-terminal amino acids, for example at the most the 305 N-terminal amino acids, such as at the most the 272 N-terminal amino acids, preferably at the most the 264 N-terminal amino acids of mPGES2, such as at the most the 253 N-terminal amino acids of SEQ ID NO:4 or SEQ

ID NO:6, for example at the most the 177 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6, such as at the most the 174 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6, preferably at the most the 130 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6.

Cereal plants carrying a mutation in *mPGES2* gene are disclosed, wherein the mutation in the *mPGES2* gene results in an expression level of *mPGES2* mRNA, which is at the most 50% of the expression level of *HvmPGES2* in a cereal plant not carrying said mutation, but otherwise identical. Such mutations could for example be a mutation in the non-coding regions of the mPGES2 gene for example in the promoter region or in an intron.

Cereal plants carrying a mutation in *mPGES2* gene are disclosed, wherein the mutation in the *mPGES2* gene results in an expression level of mPGES2 protein, which is at the most 50% of the expression level mPGES2 protein in a cereal plant not carrying said mutation, but otherwise identical.

In one embodiment, the mutation in *mPGES2* gene as disclosed herein is a marker indicating that the starch of a cereal plant carrying said mutation has a lower gelatinisation temperature.

Polymorphisms of *mPGES2* gene exist, some resulting in polymorphic mPGES2 protein. Polymorphic mPGES2 proteins may not result in or be associated with low starch gelatinisation temperature. For example, polymorphic mPGES2 proteins comprising any one of the following polymorphism:

- Valine (V) to Leucine (L) in position 142; and/or
- Glutamine (Q) to Arginine (R) in position 158; and/or
- Leucine (L) to isoleucine (I) in position 199, which is in a region of mPGES2 characterized by absolute conservation ≥90 % (Table 5); and/or
- Serine (S) to Proline (P) in position 267; and/or
- Glutamine (Q) to Arginine (R) in position 297,

may not result in or be associated with low starch gelatinisation temperature.

The mutant *mPGES2* gene as disclosed herein may encode a mutant mPGES2 protein, wherein the mutant mPGES2 protein is not a polymorphic mPGES2 protein comprising any one of the following polymorphisms:

- Valine (V) to Leucine (L) in position 142; and/or
- Glutamine (Q) to Arginine (R) in position 158; and/or
- Leucine (L) to isoleucine (I) in position 199; and/or
- Serine (S) to Proline (P) in position 267; and/or
- Glutamine (Q) to Arginine (R) in position 297.

The mutant *mPGES2* gene as disclosed herein may encode a mutant mPGES2 protein, wherein the mutant mPGES2 protein comprises one or more of the mutations described herein above and in addition comprises one or more of the following polymorphisms:

- Valine (V) to Leucine (L) in position 142; and/or
- Glutamine (Q) to Arginine (R) in position 158; and/or
- Leucine (L) to isoleucine (I) in position 199; and/or
- Serine (S) to Proline (P) in position 267; and/or
- Glutamine (Q) to Arginine (R) in position 297.

Barley plant carrying a mutation in the *HvmPGES2* gene

The present invention provides a cereal plant carrying a mutation in the *mPGES2* gene More specifically, the invention provides a barley plants or progeny thereof

- carrying a G-> A mutation of nucleotide 794 of the coding sequence of the barley *mPGES2* gene
- carrying a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the barley *mPGES2* gene of SEQ ID NO:2
- carrying a mutation in the *mPGES2* gene, wherein said mutant *HvmPGES2* gene encodes a mutant HvmPGES2 protein comprising a mutation of amino acid 265 of SEQ ID NO:4;
- carrying a mutation in the *mPGES2* gene, wherein said mutant *HvmPGES2* gene encodes a mutant HvmPGES2 protein of SEQ ID NO:5;

- carrying a mutation in the *mPGES2* gene, wherein said mutant *mPGES2* gene comprises a coding sequence of SEQ ID NO:3.

- carrying a mutation in the *mPGES2* gene, wherein the mutant *mPGES2* gene comprises the sequence SEQ ID NO:8
- carrying a mutation in the *mPGES2* gene, wherein the mutant *mPGES2* gene comprises a sequence at least 98% identical to SEQ ID NO:8, wherein said sequence comprises at least nucleotide 4657 of SEQ ID NO:8; and/or
- carrying a mutation, wherein said mutation is a C to G exchange the nucleotide corresponding to the nucleotide at position 501 of SEQ ID NO:11; and/or
- wherein the genome of said plant, comprises a genetic element consisting of a genomic region of barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, wherein said genomic region is positioned on chromosome 2H between the genomic position corresponding to 10,497,647 and the genomic position corresponding to 31,153,440 of the barley genome assembly (DOI: 10.5447/IPK/2016/34 from Masher et al. 2017).

Thus, in one embodiment the barley plant of the invention carries a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene. Accordingly, the barley plant may carry a G-> A mutation of the nucleotide corresponding to nucleotide 794 of SEQ ID NO:2. In particular, the barley plant of the invention may carry a G-> A mutation of nucleotide 794 of SEQ ID NO:2. As noted, there may be slight variations in the sequence of the *mPGES2* gene. The coding sequence consists of the parts of the sequence of the *mPGES2* gene coding for the mPGES2 protein, and thus the coding sequence excludes e.g. promoter regions and introns.

In one embodiment, the barley plant according to invention comprises a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein comprising a mutation of amino acid 265 of SEQ ID NO:4 from Gly to Asp.

For the purposes of this patent application seeds of barley plant (*Hordeum vulgare*) designated “MQF08-1101” has been deposited with NCIMB Ltd. Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA Scotland under the provisions of the Budapest Treaty. The MQF08-1101 barley plant was deposited on 12 November 2018 and has received the accession number NCIMB 43272.

In one embodiment, the barley plant of the invention is the barley plant deposited on

12 November2018 with NCIMB Ltd. under accession number NCIMB 43272 and referred to as " MQF08-1101" or progeny thereof. Thus, the barley plant of the invention may be barley plant MQF08-1101 deposited with NCIMB Ltd. on 12 November 2018 and having accession number NCIMB 43272, or any progeny barley plant thereof, wherein the mPGES2 gene of the barley plant comprises the coding sequence as set out in SEQ ID NO:3 and/or the genomic sequence of SEQ ID NO:8 or a sequence at least 98% identical thereto, wherein said sequence comprises at least nucleotide 4657 of SEQ ID NO:8.

In one embodiment, the barley plant is the barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, or progeny thereof.

Whereas the barley plant of the invention preferably comprises aforementioned mutations, other mutations are also disclosed herein.

The mutant HvmPGES2 may be a truncated polypeptide comprising only the N-terminal part of HvmPGES2 of SEQ ID NO:4 or 6. This may for example be the case when the mutation in the *mPGES2* gene is a mutation introducing a premature stop codon, a mutation in a splice site or a frame shift mutation.

Thus, the barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking at least the 8 most C-terminal amino acids, such as at least the 18 most C-terminal amino acids, for example at least the 30 most C-terminal amino acids 49, for example at least the 69 most C-terminal amino acids, preferably at least the 77 most C-terminal amino acids for example at least the 145 most C-terminal amino acids, such as at least the 148 most C-terminal amino acids, preferably at least the 192 most C-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a truncated HvmPGES2 protein comprising an N-terminal fragment of HvmPGES2 comprising at the most the 314 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6, such as at the most the 312 N-terminal amino acids of SEQ ID NO: 4 or SEQ ID NO:6, for example at the most the 305 N-terminal amino acids of SEQ ID NO: 4 or SEQ ID NO:6, such as at the most the 272 N-terminal amino acids of SEQ ID NO: 4 or SEQ ID NO:6, preferably at the most the 264 N-terminal amino acids of SEQ ID NO: 4 or SEQ ID NO:6, such as at the most the 253 N-terminal amino acids of SEQ ID NO:4 or SEQ ID

NO:6, for example at the most the 177 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6, such as at the most the 174 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6, preferably at the most the 130 N-terminal amino acids of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to any of the amino acids of SEQ ID NO:4 mentioned in Table 5.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-111, 114, 116-140, 142-145, 147-151, 153-155, 157, 160-161, 171-172, 174-176, 178-193, 195-206, 208-211, 213-217, 220-221, 223-242, 244-254, 256-258, 261, 263-266, 268-271, 273-275, 277-286, 288-302, 304-306, 309-310 or 313-315 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-105, 107-111, 116-117, 120-123, 125-128, 130-132, 134, 136-138, 142-145, 150, 153-154, 157, 171-172, 174-176, 178-180, 182-185, 187, 189-193, 195-202, 204-205, 208-211, 214-216, 220-221, 223-224, 227-228, 230, 232, 234-237, 239-242, 244-245, 247-248, 250-251, 253-254, 257-258, 261, 264-266, 268-271, 273-275, 278-286, 289, 291-293, 296, 298-299, 301-302, 304-306, 310, or 314-315 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-102, 104-111, 114, 116-124, 126, 128-132, 134-140, 143, 145, 147, 149-151, 153-154,157, 161, 171-172, 174-176, 178, 180-181, 183-193, 196-199, 201-205, 209-211, 215, 220-221, 223-235, 237-238, 240, 242, 244, 247-252, 254, 257-258, 261, 263-266, 268-271, 273-275, 277-281, 283, 286, 288, 291-298, 301-302, 304-306, 309, or 313 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-102, 104-105, 107-110, 116, 120-123, 128, 130, 132, 134, 136-138, 150, 153-

154,157, 171-172, 174-176, 178, 180, 183-185, 189-190, 192-193, 197-199, 201-202, 204-205, 209, 211, 215, 220-221, 223-224, 227-228, 232, 234-235, 242, 244, 247-248, 250-251, 254, 257, 261, 265-266, 268-271, 273-275, 278-281, 286, 291, 296, 298, 301-302, 304 or 306 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-101, 104,138, 150, 153-154, 157, 258, 263, 265, 266, 273-274, 280, 298, 301 or 305 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99-100, 138, 265, 266 or 273 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking one or more of the amino acids corresponding to amino acids 99, 209, 223, 263, 265, 266 or 269 of SEQ ID NO:4 or SEQ ID NO:6.

In one embodiment, the barley plant of the invention comprises a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein lacking at least the amino acid corresponding to amino acid 265 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *mPGES2* gene encoding a mutant mPGES2 protein containing a mutation of one or more of the amino acids corresponding to amino acids 99-266 or 268 to 315 of SEQ ID NO:4.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids of SEQ ID NO:4 or SEQ ID NO:6 mentioned in Table 5.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-111, 114, 116-140, 142-145, 147-151, 153-155, 157, 160-161, 171-172, 174-176, 178-193, 195-206, 208-211, 213-217, 220-221, 223-242, 244-254, 256-258, 261, 263-266, 268-271, 273-275, 277-286, 288-302, 304-306, 309-310 or 313-315 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-105, 107-111, 116-117, 120-123, 125-128, 130-132, 134, 136-138, 142-145, 150, 153-154, 157, 171-172, 174-176, 178-180, 182-185, 187, 189-193, 195-202, 204-205, 208-211, 214-216, 220-221, 223-224, 227-228, 230, 232, 234-237, 239-242, 244-245, 247-248, 250-251, 253-254, 257-258, 261, 264-266, 268-271, 273-275, 278-286, 289, 291-293, 296, 298-299, 301-302, 304-306, 310, or 314-315 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-102, 104-111, 114, 116-124, 126, 128-132, 134-140, 143, 145, 147, 149-151, 153-154,157, 161, 171-172, 174-176, 178, 180-181, 183-193, 196-199, 201-205, 209-211, 215, 220-221, 223-235, 237-238, 240, 242, 244, 247-252, 254, 257-258, 261, 263-266, 268-271, 273-275, 277-281, 283, 286, 288, 291-298, 301-302, 304-306, 309, or 313 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-102, 104-105, 107-110, 116, 120-123, 128, 130, 132, 134, 136-138, 150, 153-154,157, 171-172, 174-176, 178, 180, 183-185, 189-190, 192-193, 197-199, 201-202, 204-205, 209, 211, 215, 220-221, 223-224, 227-228, 232, 234-235, 242, 244, 247-248, 250-251, 254, 257, 261, 265-266, 268-271, 273-275, 278-281, 286, 291, 296, 298, 301-302, 304 or 306 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-101, 104,138, 150, 153-154, 157, 258, 263, 265, 266, 273-274, 280, 298, 301 or 305 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 99-100, 138, 265, 266 or 273 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein containing a mutation of one or more of the amino acids 69, 99, 145, 148, 192, 209, 223, 263, 265, 266 or 269 of SEQ ID NO:4 or SEQ ID NO:6.

In one embodiment, the barley plant according to invention may comprise a mutant *HvmPGES2*gene encoding a mutant HvmPGES2 protein comprising a mutation of amino acid 265 of SEQ ID NO:4 or SEQ ID NO:6.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein of SEQ ID NO:4 or SEQ ID NO:6, wherein said mutant HvmPGES2 protein comprises one or more of the following mutations: C99Y, G209D, G223D, F263S, G265D, G266D or P269L.

The barley plant may comprise a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein carrying a mutation leading to a stop codon at amino acid position 131, 175, 178 or 254 of SEQ ID NO:4 or SEQ ID NO:6.

In one embodiment, the mutation in *HvmPGES2* gene as disclosed herein is a marker for barley plants comprising starch having a lower gelatinisation temperature. Accordingly, in one embodiment the invention relates to a barley plant carrying a mutation in the *mPGES2* gene at position 794 of the coding sequence (SEQ ID NO:2), wherein said mutation may be a marker for a low-gelatinisation temperature phenotype. In one embodiment the invention relates to a barley plant comprising an *mPGES2* gene containing the nucleotide A at position 794 of the coding sequence (SEQ ID NO:2), wherein said mutation may be a marker for a low-gelatinisation temperature phenotype. In one embodiment the invention relates to a barley plant comprising an *mPGES2* gene containing the coding sequence provided as SEQ ID NO:3 herein.

In one embodiment the invention relates to a method for identifying a barley plant having a low-gelatinisation temperature phenotype, wherein said method comprises determining whether said barley plant carries a mutation at position 794 of the coding sequence of the *mPGES2* gene (SEQ ID NO:2), for example determining whether said barley plant carries a *mPGES2* gene containing the nucleotide A at position 794 of the coding sequence (SEQ ID NO:2), for example determining whether said barley plant comprises an *mPGES2* gene containing the coding sequence provided as SEQ ID NO:3 herein, wherein the presence of aforementioned mutations is a marker for said barley plant having a low-gelatinisation phenotype.

Said low-gelatinisation phenotype preferably is a phenotype wherein starch of said barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a barley plant of cv. Quench grown under similar conditions.

Cereal plant

The cereal plant according to the invention may be any cereal plant. In preferred embodiments of the invention the cereal plant is a barley plant.

The barley plant may be any plant of the species *Hordeum vulgare*, including any breeding line or cultivar or variety.

"Wild barley", *Hordeum vulgare* ssp. spontaneum, is considered the progenitor of today's cultivated forms of barley. Domesticated, but heterogenous mixtures of barley are referred to as barley landraces. Today, most of the landraces have been displaced in advanced agricultures by pure line cultivars. Compared with landraces, modern barley cultivars have numerous improved properties (Nevo, 1992; Pelger et al., 1992).

Within the present invention, the term "barley plant" comprises any barley plant, such as barley landraces or modern barley cultivars. Thus, the invention relates to any barley plant comprising a mutation in the *HvmPGES2* gene.

However, preferred barley plants for use with the present invention are modern barley cultivars or pure lines. The barley cultivar to be used with the present invention may, for example, be selected from the group consisting of Sebastian, Quench, Celeste, Lux, Prestige, Saloon, Neruda, Harrington, Klages, Manley, Schooner, Stirling, Clipper, Franklin, Alexis, Blenheim, Ariel, Lenka, Maresi, Steffi, Gimpel, Cheri, Krona, Camargue, Chariot, Derkado, Prisma, Union, Beka, Kym, Asahi 5, KOU A, Swan Hals, Kanto Nakate Gold, Hakata No. 2, Kirin – choku No. 1, Kanto late Variety Gold, Fuji Nijo, New Golden, Satukio Nijo, Seijo No. 17, Akagi Nijo, Azuma Golden, Amagi Nijpo, Nishino Gold, Misato golden, Haruna Nijo, Scarlett, Rosalina and Jersey preferably from the group consisting of Haruna Nijo, Sebastian, Quench, Celeste, Lux, Prestige, Saloon, Neruda and Power, preferably from the group consisting of Harrington, Klages, Manley, Schooner, Stirling, Clipper, Franklin, Alexis, Blenheim, Ariel, Lenka, Maresi, Steffi, Gimpel, Cheri, Krona, Camargue, Chariot, Derkado, Prisma, Union, Beka, Kym,

Asahi 5, KOU A, Swan Hals, Kanto Nakate Gold, Hakata No. 2, Kirin – choku No. 1, Kanto late Variety Gold, Fuji Nijo, New Golden, Satukio Nijo, Seijo No. 17, Akagi Nijo, Azuma Golden, Amagi Nijpo, Nishino Gold, Misato golden, Haruna Nijo, Scarlett and Jersey preferably from the group consisting of Haruna Nijo, Sebastian, Tangent, Lux, Prestige, Saloon, Neruda, Power, Quench, NFC Tipple, Barke, Class, Vintage, Applaus, Bowie, Broadway, Champ, Chanson, Charles, Chimbon, Cosmopolitan, Crossway, Dragoon, Ellinor, Embrace, Etoile, Evergreen, Flair, Highway, KWS Beckie, KWS Cantton, KWS Coralie, KWS Fantex, KWS Irina, KWS Josie, KWS Kellie, LG Diablo, LG Figaro, LG Nabuco, LG Tomahawk, Laureate, Laurikka, Lauxana, Luther, Odyssey, Ovation, Prospect, RGT Elysium, RGT Observer, RGT Planet, Rotator, Sarbi, Scholar, Subway and Golden Promise.

The barley plant may be in any suitable form. For example, the barley plant according to the invention may be a viable barley plant, a dried plant, a homogenized plant, or a milled barley kernel. The plant may be a mature plant, an embryo, a kernel, a germinated kernel, a malted kernel, a milled malted kernel, a milled kernel or the like.

Parts of barley plants may be any suitable part of the plant, such as kernels, embryos, leaves, stems, roots, flowers, or fractions thereof. A fraction may, for example, be a section of a kernel, embryo, leaf, stem, root, or flower. Parts of barley plants may also be a fraction of a homogenate or a fraction of a milled barley plant or kernel.

In one embodiment of the invention, parts of barley plants may be cells of said barley plant, such as viable cells that may be propagated in vitro in tissue cultures. In other embodiments, however, the parts of barley plants may be viable cells that are not capable of maturing into an entire barley plant, i.e. cells that are not a reproductive material.

Characteristics of cereal plants carrying a mutation in *mPGES2*

The invention provides plants, wherein the starch of said cereal plants has a lower gelatinisation temperature. Gelatinisation temperature is dependent on many factors including the cultivation conditions. However, the cereal plants of the invention consistently have a lower gelatinisation temperature compared to similar cereal plants grown under the same conditions.

Preferably, the starch of the cereal plant of the invention has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a cereal plant not carrying said mutation, but otherwise identical; and grown under similar or the same conditions.

Thus, in one embodiment the invention provides barley plants, wherein the starch of the barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a barley plant not carrying said mutation, but otherwise identical; and grown under similar conditions.

For example, the barley plant may be a barley plant, wherein starch of said barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a barley plant of cv. Quench grown under similar or the same conditions.

Interestingly, the structure of the starch of the cereal plants of the invention differs from the starch structure in wild type plants.

Thus, the cereal plants, and in particular the barley plants of the invention in general contain starch with a high amylose content at the time of maturity. For example, in one embodiment the barley plants of the invention may contain starch comprising at least 2 percentage points, such as at least 3 percentage points, for example at least 4 percentage points more amylose than starch of a barley plant not carrying said mutation, but otherwise identical; and grown under similar or the same conditions. This may apply whether or not the barley plants have undergone a heat treatment during growth.

In one embodiment the barley plant may contain starch having an average amylose content of at least 22% at maturity.

The cereal plants, and in particular the barley plants of the invention may in one embodiment contain starch with amylopectin having a relatively high abundance of chain length DP7-10 at the time of maturity. For example, the barley plants of the invention may in one embodiment contain starch comprising amylopectin, wherein the molar fraction of chain length DP7-10 is at least at least 0.5 percentage points higher compared to amylopectin of a barley plant not carrying said mutation, but otherwise

identical; and grown under similar or the same conditions. This may apply whether or not the barley plants have undergone a heat treatment during growth.

In one embodiment the barley plant contains starch comprising amylopectin, wherein the amylopectin has a molar fraction with chain length DP7-10 of at least 18.4 %. This may apply whether or not the barley plants have undergone a heat treatment during growth.

The cereal plants, and in particular the barley plants of the invention may in one embodiment contain starch with amylopectin having a relatively low abundance of chain length DP11-21 at the time of maturity. For example, the mature barley plants of the invention may in one embodiment contain starch comprising amylopectin, wherein the molar fraction of chain length DP11-21 is at least 0.7 percentage points lower compared to amylopectin of a barley plant not carrying said mutation, but otherwise identical; and grown under similar or the same conditions. This may apply whether or not the barley plants have undergone a heat treatment during growth.

In one embodiment the barley plant contains starch comprising amylopectin, wherein the amylopectin has a molar fraction with chain length DP11-21 of at the most 62.8%.

Plant products

The invention also provides plant products prepared from a cereal plant carrying a mutation in the *mPGES2* gene, ,more specifically a barley plant or progeny thereof carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene.

The plant product may be any product prepared from a cereal plant, for example a food, a feed or a beverage. Thus the plant product may be any of the beverages described herein below in the section “Beverage and method of production thereof”. The plant product may also be an aqueous extract of the cereal plant and/or of malt of said cereal plant, for example the plant product may be wort. Said aqueous extract may for example be prepared as described herein below in the section “Aqueous extract and methods of production thereof”.

In one embodiment the plant product may be malt, e.g. any of the malts described herein below in the section “Malt and methods of production thereof” or a malt based

product, such as malt based beverages. Although the primary use of malt is for beverage production, it can also be utilized in other industrial processes, for example as an enzyme source in the baking industry, or in the food industry as a flavouring and colouring agent, e.g. in the form of malt or malt flour or indirectly as a malt syrup, etc. Thus, the plant product according to the invention may be any of the aforementioned products.

In another aspect, the plant products according to the invention comprise, or even consist of syrup, such as a barley syrup, or a barley malt syrup. The plant product may also be an extract of barley or malt. Thus, the plant product may be wort.

Malt and methods of production thereof

The invention also provides malt prepared from a cereal plant carrying a mutation in the mPGES2 gene, more specifically a barley plant or progeny thereof carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene as described herein.

Malt may be prepared by malting, i.e. by germination of steeped barley kernels in a process taking place under controlled environmental conditions followed by a drying step. Said drying step may preferably be kiln drying of the germinated kernels at elevated temperatures.

Thus, a method of malting according to the present invention preferably comprises the steps of:

(a) providing kernels of a cereal plant, notably a barley plant, carrying a mutation in the *mPGES2* gene;
(b) steeping said cereal kernels;
(b) germinating said cereal kernel; and
(c) drying said germinated cereal kernels, preferably by kiln drying.

Steeping may be performed by any conventional method known to the skilled person. One non-limiting example involves steeping at a temperature in the range of 10 to 25°C with alternating dry and wet conditions. During steeping, for example, the cereal kernels may be incubated wet for in the range of 30 min to 3 h followed by incubation

dry for in the range of 30 min to 3 h and optionally repeating said incubation scheme in the range of 2 to 5 times. The final water content after steeping may, for example, be in the range of 40 to 50%.

Germination of grains may be performed by any conventional method known to the skilled person. One non-limiting example involves germination at a temperature in the range of 10 to 25°C, optionally with changing temperature in the range of 1 to 4 days.

The kiln drying may be performed at conventional temperatures, such as at least 75°C, for example in the range of 80 to 90°C, such as in the range of 80 to 85°C. Thus, the malt may, for example be produced by any of the methods described by Hough et al. (1982). However, any other suitable method for producing malt may also be used with the present invention, such as methods for production of specialty malts, including, but not limited to, methods of roasting the malt.

Malt may be further processed, for example by milling. Thus, the plant product according to the invention may be any kind of malt, such as unprocessed malt or milled malt, such as flour. Milled malt and flour thereof comprise chemical components of the malt and dead cells that lack the capacity to re-germinate.

Preferably milling is performed in a dry state, i.e. the malt is milled while dry. Thus, it is preferred that malt is not milled under water.

One advantage of malt prepared from cereal plants carrying a mutation in the mPGES2 gene is that the starch contained in said malt has a low gelatinisation temperature. Thus, starch of said malt may have an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of malt of a cereal plant not carrying said mutation, but otherwise identical; and grown under similar or the same conditions, wherein said malts have been prepared by similar or identical methods.

In one embodiment, the cereal plant is a barley plant, and starch of malt prepared from said barley plant may have an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of malt of a barley

plant of cv. Quench grown under similar or the same conditions and malted by the same or similar methods.

Aqueous extract and methods of production thereof

The invention provides cereal based beverages as well as methods of preparing the same, wherein the cereal is a cereal plant carrying a mutation in the *mPGES2* gene, more specifically the cereal may be a barley plant or progeny thereof carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene.

Frequently, methods for preparing a beverage comprise a step of preparing an aqueous extract of kernels of the cereal plants of the invention and/or of malts prepared from cereal plants of the invention.

The aqueous extract may, in general, be prepared by incubating cereal flour and/or malt flour in water or in an aqueous solution. In particular, the aqueous extract may be prepared by mashing.

In general said aqueous solution may be water, such as tap water to which one or more additional agents may be added. The additional agents may be present in the aqueous solution from the onset or they may be added during the process of preparing an aqueous extract. Said additional agents may be enzymes. Thus, the aqueous solution may comprise one or more enzymes. Said enzymes may be added to the aqueous solution from the onset, or subsequently, during the process.

Said enzymes may, for example, be one or more hydrolytic enzymes. Suitable enzymes include lipases, starch degrading enzymes (e.g. amylases), glucanases [preferably (1-4)- and/or (1,3;1,4)-β-glucanases], and/or xylanases (such as arabinoxylanases), and/or proteases, or enzyme mixtures comprising one or more of the aforementioned enzymes, e.g. Cereflo, Ultraflo, or Ondea Pro (Novozymes). For example,the aqueous solution may comprise one or more hydrolytic enzymes selected from the group consisting of α-amylase, β-amylase, limit dextrinase, pullulanase, β--glucanase (e.g. endo-(1,3;1,4)-β-glucanase or endo-1,4-β-glucanase),xylanase (e.g. endo- or exo-1,4-xylanase, an arabinofuranosidase or a ferulic acid esterase), glucoamylase and protease.

Said additional agents, preferably of food grade quality, may also be a salt, for example $CaCl_2$, or an acid, for example H_3PO_4.

The aqueous extract is generally prepared by incubation of the cereal flour and/or malt flour in the aqueous solution at one or more predetermined temperature(s). Said predetermined temperature may also be referred to as "mashing temperature" herein. Said mashing temperatures may for example be conventional temperatures used for mashing. However, one advantage of the present invention is that the low gelatinisation temperature of the cereal plants of the invention may allow use of relatively low mashing temperature. In particular, the mashing in temperature may be relatively low. The mashing temperature is in general either kept constant (isothermal mashing), or gradually increased, for example increased in a sequential manner. In either case, soluble substances in the cereal grains and/or malt are liberated into said aqueous solution thereby forming an aqueous extract.

The mashing temperature(s) are typically temperature(s) in the range of 30 to 90°C, such as in the range of 40 to 85°C, for example in the range of 50 to 85°C. The mashing temperatures may be chosen according to the cereal type used. In particular, a relatively low mashing-in temperature may be used, e.g. a temperature in the range of 50-60°C. In a preferred embodiment the mashing in temperature is higher than the gelatinisation temperature of the starch of the cereal plant, for example in the range of 0.5 to 5°C higher than the gelatinisation temperature of the starch of the cereal plant. Frequently, incubation with the aqueous solution includes a final step of heating to a higher temperature, e.g. to a temperature in the range of 75 to 80°C.

Subsequent to incubation in the aqueous solution in e.g. a mashing vessel, the aqueous solution may be transferred to another container, e.g. a lauter tun and incubated for additional time at elevated temperature.

Non-limiting examples of useful mashing protocols can be found in the literature of brewing, e.g. in Hough et al. (supra).

Mashing (i.e. incubation of the cereal flour and/or malt flour in aqueous solution) can occur in the presence of adjuncts, which is understood to comprise any carbohydrate source other than malt, such as, but not limited to, barley, barley syrups, or maize, or

rice – either as whole kernels or processed products like grits, syrups or starch. All of the aforementioned adjuncts may be used principally as an additional source of extract (syrups are typically dosed during wort heating). The requirements for processing of the adjunct in the brewery depend on the state and type of adjunct used, and in particular on the starch gelatinisation or liquefaction temperatures. If the gelatinisation temperature is above that for the cereal plant starch of the invention, then starch of the adjunct may be gelatinized before addition to the mash.

After incubation in the aqueous solution, the aqueous extract may typically be separated, e.g. through filtration into the aqueous extract and residual non-dissolved solid particles, the latter also denoted "spent grain". Filtering may for example be performed in a lauter tun. Alternatively, the filtering may be filtering through a mash filter. The aqueous extract thus obtained may also be denoted "first wort". Additional liquid, such as water may be added to the spent grains during a process also denoted sparging. After sparging and filtration, a "second wort" may be obtained. Further worts may be prepared by repeating the procedure. Thus, the aqueous extract may be wort, e.g. a first wort, a second wort, a further wort or a combination thereof.

Beverage and method of production thereof

The present invention also provides cereal based beverages and methods of producing such beverages, wherein the cereal is a cereal plant carrying a mutation in the *mPGES2* gene, more specifically a barley plant or progeny thereof carrying a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene.

Said beverage may be an alcoholic cereal based beverages or non-alcoholic cereal based beverages. Alcoholic cereal based beverages may for example be beer or a distilled alcohol.

Said beer may be any kind of beer, for example lager or ale. Thus, the beer may for example be selected from the group consisting of altbier, Amber ale, Barley wine, Berliner Weisse, Bière de Garde, Bitter, Blonde Ale, Bock, Brown ale, California Common, Cream Ale, Dortmunder Export, Doppelbock, Dunkel, Dunkelweizen, Eisbock, Fruit lambic, Golden Ale, Gose, Gueuze, Hefeweizen, Helles, India pale ale, Kölsch, Lambic, Light ale, Maibock, Malt liquor, Mild, Märzenbier, Old ale, Oud bruin,

Pale ale, Pilsener, Porter, Red ale, Roggenbier, Saison, Scotch ale, Steam beer, Stout, Schwarzbier, lager, Witbier, Weissbier and Weizenbock.

Said distilled alcohol may be any kind of distilled alcohol. In particular the distilled alcohol may be based on a cereal, e.g. a malted cereal, e.g. a barley malt. Non-limiting examples of such distilled alcohol include whiskey and vodka.

The beverage may be a non-alcoholic beverage, such as a non-alcoholic cereal based beverage, e.g. non-alcoholic beer or non-alcoholic malt beverages, such as maltina.

The beverage may for example be prepared by a method comprising the steps of:

(i) Providing kernels of a cereal plant according to the invention and/or malt prepared from kernels of a cereal plant according to the invention
(ii) Preparing an aqueous extract of said kernels and/or said malt, e.g. as described herein below in the section preparing aqueous extract
(iii) processing said aqueous extract into a beverage.

The aqueous extract may be boiled with or without hops where after it may be referred to as boiled wort. First, second and further worts may be combined, and thereafter subjected to boiling. The aqueous extract may be boiled for any suitable amount of time, e.g. in the range of 60 min to 120 min.

Step (iii) may in particular comprises fermentation of said aqueous extract, e.g. by fermentation of wort. Thus, the beverage may be prepared by fermentation of the aqueous extract with yeast.

Once the aqueous extract has been prepared it may be processed into beer by any method including conventional brewing methods. Non-limited descriptions of examples of suitable methods for brewing can be found, for example, in publications by Hough et al. (1982). Numerous, regularly updated methods for analyses of barley and beer products are available, for example, but not limited to, American Association of Cereal Chemists (1995), American Society of Brewing Chemists (1992), European Brewery Convention (1998), and Institute of Brewing (1997). It is recognized that many specific procedures are employed for a given brewery, with the most significant variations

relating to local consumer preferences. Any such method of producing beer may be used with the present invention.

The first step of producing beer from the aqueous extract preferably involves boiling said aqueous extract as described herein above, followed by a subsequent phase of cooling and optionally whirlpool rest. One or more additional compounds may be added to the aqueous extract, e.g. one or more of the additional compounds described below in the section "Additional compounds". After being cooled, the aqueous extract may be transferred to fermentation tanks containing yeast, e.g. brewing yeast, such as *S. pastorianus* or *S. cerevisiae*. The aqueous extract may be fermented for any suitable time period, in general in the range of 1 to 20 days, such as 1 to 10 days. The fermentation is performed at any useful temperature e.g. at a temperature in the range of 10 to 20°C. The methods may also comprise addition of one or more enzymes, e.g. one or more enzymes may be added to the wort prior to or during fermentation. In particular, said enzyme may be a proline-specific endoprotease. A non-limiting examples of a proline-specific endoprotease is "Brewer's Clarex" available from DSM. In other embodiments, no exogenous enzymes are added during the methods.

During the several-day-long fermentation process, sugar is converted to alcohol and CO_2 concomitantly with the development of some flavour substances. The fermentation may be terminated at any desirable time, e.g. once no further drop in %P is observed.

Subsequently, the beer may be further processed, for example chilled. It may also be filtered and/or lagered – a process that develops a pleasant aroma and a less yeast-like flavour. Additives may also be added. Furthermore, CO_2 may be added. Finally, the beer may be pasteurized and/or filtered, before it is packaged (e.g. transferred to containers or kegs, bottled or canned). The beer may also be pasteurized by standard methods.

Additional compounds

The methods of the invention may comprise the step of adding one or more additional compounds. Said additional compounds may for example be a flavor compound, a preservative, a functional ingredient, a color, a sweetener, a pH regulating agent or a salt. The pH regulating agent may for example be a buffer or an acid, such as phosphoric acid.

Functional ingredients may be any ingredient added to obtain a given function. Preferably a functional ingredient renders the beverage healthier. Non-limiting examples of functional ingredients includes vitamins or minerals.

The preservative may be any food grade preservative, for example it may be benzoic acid, sorbic acid, sorbates (e.g. potassium sorbate), sulphites and/or salts thereof.

The additional compound may also be CO_2. In particular, CO_2 may be added to obtain a carbonated beverage.

The flavour compound to be used with the present invention may be any useful flavour compound. The flavour compound may for example be selected from the group consisting of aromas, plant extracts, plant concentrates, plant parts and herbal infusions. In particular the flavor compounds may be hops.

Method of preparing a cereal plant carrying a mutation in mPGES2

Cereal plants carrying a mutation in mPGES2 may be prepared in any useful manner.

For example, such cereal plants can be prepared by a method comprising the steps of:

- subjecting a plurality of cereal plants or cereal kernels to random mutagenesis, e.g. by irradiation or chemical treatment, e.g. treatment with sodium azide;
- identifying cereal plants or cereal kernels carrying a mutation in *mPGES2*.

Such methods may also include one or more steps of reproducing said cereal plants/cereal kernels in order to obtain multiple barley plants/kernels each carrying random mutations.

In particular, cereal plants (such as barley plants) carrying a particular mutation in *mPGES2* may be prepared and identified essentially as described in international patent application PCT/EP2017/065516 using primers and probes designed to identify a mutation in the *HvmPGES2* gene.

Cereal plants carrying a mutation in the *mPGES2* gene may also be prepared using various site directed mutatgenesis methods, which for example can be designed based on the sequence of the *mPGES2* gene provided herein. In one embodiment, the cereal plant is prepared using any one of CRISPR, a TALEN, a zinc finger, meganuclease,

and a DNA-cutting antibiotic as described in WO 2017/138986. In one embodiment, the cereal plant is prepared using CRISPR/cas9 technique, e.g. using RNA-guided Cas9 nuclease. This may be done as described in Lawrenson et al., Genome Biology (2015) 16:258; DOI 10.1186/s13059-015-0826-7 except that the single guide RNA sequence is designed based on the genes sequences provided herein. In one embodiment, the cereal plant is prepared using a combination of both TALEN and CRISPR/cas9 techniques, e.g. using RNA-guided Cas9 nuclease. This may be done as described in Holme et al., Plant Mol Biol (2017) 95:111-121; DOI: 10.1007/s11103-017-0640-6) except that the TALEN and single guide RNA sequence are designed based on the genes sequences provided herein.

In one embodiment, the cereal plant is prepared using homology directed repair, a combination of a DNA cutting nuclease and a donor DNA fragment. This may be done as described in Sun et al., Molecular Plant (2016) 9:628-631; DOI: https://doi.org/10.1016/j.molp.2016.01.001 except that the DNA cutting nuclease is designed based on the genes sequences provided herein and the donor DNA fragment is designed based on the coding sequence of the mutated cereal variant provided herein.

In one embodiment of the invention, the objective is to provide agronomical useful cereal plants carrying a mutation in the *mPGES2* gene, in particular agronomical useful barley plants carrying a G-> A mutation of nucleotide 794 of the coding sequence of the mPGES2 gene. In addition to the mutation in the *mPGES2* gene, there are additional factors which also may be considered in the art of generating a commercial cereal variety useful for malting and/or brewing and/or as base for beverages, for example kernel yield and size, growth properties and other parameters that relate to malting performance or brewing performance. Since many – if not all – relevant traits have been shown to be under genetic control, the present invention also provides modern, homozygous, high-yielding malting cultivars, which may be prepared from crosses with the cereal plants that are disclosed in the present publication. The skilled barley breeder will be able to select and develop cereal plants, which – following crossings with other cereal plants – will result in superior cultivars. Alternatively, the breeder may utilize plants of the present invention for further mutagenesis to generate new cultivars carrying additional mutations in addition to the mutation of the *mPGES2* gene.

The invention also comprise cereal plants carrying a mutation in the *mPGES2* gene prepared from plant breeding method, including methods of selfing, backcrossing, crossing to populations, and the like. Backcrossing methods can be used with the present invention to introduce into another cultivar the mutation of the *mPGES2* gene. Thus, the invention also relates to progeny of the above described barley plants, wherein said progeny also carries a G-> A mutation of nucleotide 794 of the coding sequence of the *mPGES2* gene.

In one embodiment, the invention relates to progeny of the barley plant deposited on 12 November 2018 with NCIMB Ltd. under the accession number NCIMB 43272 and referred to as MQF08-1101. Said progeny may be prepared by any useful method, including but not limited to selfing, backcrossing, or crossing to other populations. In particular, said progeny may also comprise a genetic element consisting of a genomic region of barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, wherein said genomic region is positioned on chromosome 2H between the genomic position corresponding to 10,497,647 and the genomic position corresponding to 31,153,440 of the barley genome assembly by IBSC of 14-07-2016, DOI:10.5447/IPK/2016/34. Said progeny may also carry a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *mPGES2* gene.

A way to accelerate the process of plant breeding comprises the initial multiplication of generated mutants by application of tissue culture and regeneration techniques. Thus, another aspect of the present invention is to provide cells, which upon growth and differentiation produce cereal plants carrying the mutation of the *mPGES2* gene. For example, breeding may involve traditional crossings, preparing fertile anther-derived plants or using microspore culture.

In one embodiment the barley plant of the invention has not exclusively been obtained by means of an essentially biological process. Progeny of a barley plant obtained by a technical process is herein considered as not being exclusively obtained by means of an essentially biological process, because the parent plant is obtained by a technical process.

In one embodiment the barley plant carries a mutation in the *mPGES2 gene*, wherein said mutation has been induced by chemical and/or physical agents.

In one embodiment the barley plant has been prepared by a method involving a step of induced mutagenesis or said plant is progeny of a plant prepared by a method involving a step of induced mutagenesis. Thus, the barley plant may be a plant prepared by a method comprising the following steps or progeny of a plant prepared by a method comprising the following steps:

- Mutagenizing barley plants or parts thereof, for example with a chemical mutagenizing agent such as NaN_3
- Selecting barley plants carrying a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *mPGES2* gene (SEQ ID NO:2).

Sequence Listing

SEQ ID NO:1

Sequence of *HvmPGES2* based on the gene model HORVU2Hr1G01103 from the genomic sequence of *Hordeum vulgare*, cv. Morex. Sequencing gaps in the current public genome assembly, represented by "N"s, in the gene model were filled using NCBI accession AK373333 (in italics) from cv. *Haruna Nijo*. Coding sequence is provided in capital letters. The nucleotide identified as mutated in MQF08-1101 is indicated in bold.

catgacttgatgatgtggcatgattgcatgagaagaaaaaatagatagtgggttacaactatttaagaat
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acggacgcgtttggctc

SEQ ID NO:2

Coding sequence of *HvmPGES2* in Quench sequenced by Sanger sequencing based on the sequence HORVU2Hr1G01103 from *Hordeum vulgare*, cv. Morex and NCBI accession AK373333 from cv. *Haruna Nijo.* The G that is mutated in MQF08-1101 into A is marked in bold. The underlined C is different from Morex, but encodes a silent mutation.

ATGAGGTCGCTCCGCGCGGCGCAGACCCTGCTCGCCTCCCGCTCGTTCCTCCTCTCCTCGCGCG
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GCCTACGCCGACGCCCTGCCCGGGGCCGTCGTCCCGGGCCGGGATCGCCGGCGCGGTCTCCTTC
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CCGAGCGGTTTGCTGTGAAGTATGCTGGTGCTGCAGCAATGTACTTCGTGGCTAAGAAGCTTAA
GAAGAAATACAACATTACTGATGAGCGTGCCTCATTGTATGATGCGGCCAACACGTGGACGGAA
GCCCTGAATGGAAGAAATTTTCTCG**G**TGGCTCCAAGCCTAACTTGGCAGATCTGGCAGCATTTG
GTGTTCTGAGACCTATCAGATACCTGCAGTCTGGAAAAGACATGGTTGAGCATACCCAAATCGG
CGAGTGGTACCAGCGGATGGAAGATGCGGTCGGAGAACCATCGAGAATCCCGGAGGGCCAGTAC
CAGGAGTAG

SEQ ID NO: 3

Coding sequence *HvmPGES2* in MQF08-1101 sequenced by Sanger sequencing based on the sequence HORVU2Hr1G01103 from *Hordeum vulgare*, cv. Morex and NCBI accession AK373333 from cv. *Haruna Nijo.*. The identified mutation A compared to Quench are marked in bold. The underlined C is different from Morex, but encodes a silent mutation.

ATGAGGTCGCTCCGCGCGGCGCAGACCCTGCTCGCCTCCCGCTCGTTCCTCCTCTCCTCGCGCGCGCTCC
ACGCCGCCGCGGCCTCCCCCGCCGCGGCCGGGCGCTGGGGCAACGCCCCGCCGCCGCCGCCTACGCCGAC
GCCCTGCCCGGGGCCGTCGTCCCGGGCCGGGATCGCCGGCGCGGTCTCCTTCTCCCTGACGTTCGCCACG
GTGGCGGTGGCCGAGGTGCAGGCCAAGGAGCGGCTGCCCTCGGATCTGCTGCCCCGGAACGTCGTGCTCT
ACCAGTACCAGGCCTGCCCCTTCTGCAACAAGGTCAGAGCTTTCCTAGACTATCATGATATACCCTACAA

AGTTGTGGAAGTTAATCCACTGAGCAAAAAGGAAATCAAGTGGTCTGAGTACAAGAAGGTCCCAATATTG
ACGGTAGATGGTGAACATCTGGTTGATTCTACAGACATAATCAATATATTACAGCACAGGATCAGCCCTG
ATGATGAAGTCACTAATGAAGAGGAAACAAAATGGCGCAAGTGGGTTGATGAGCACCTTGTGCATGTATT
GTCACCAAATATATACCGGACGACTTCAGAGGCTCTAGAATCTTTTGACTACATTGCAAAACATGGTAAC
TTCAGTTACACCGAGCGGTTTGCTGTGAAGTATGCTGGTGCTGCAGCAATGTACTTCGTGGCTAAGAAGC
TTAAGAAGAAATACAACATTACTGATGAGCGTGCCTCATTGTATGATGCGGCCAACACGTGGACGGAAGC
CCTGAATGGAAGAAATTTTCTCG**A**TGGCTCCAAGCCTAACTTGGCAGATCTGGCAGCATTTGGTGTTCTG
AGACCTATCAGATACCTGCAGTCTGGAAAAGACATGGTTGAGCATACCCAAATCGGCGAGTGGTACCAGC
GGATGGAAGATGCGGTCGGAGAACCATCGAGAATCCCGGAGGGCCAGTACCAGGAGTAG

SEQ ID NO:4

Amino acid sequence of polypeptide encoded by coding region of the sequence set forth as SEQ ID NO:2. This polypeptide is also referred to as *mPGES2* of *Hordeum vulgare*, cv. Morex and cv. Quench herein.

MRSLRAAQTLLASRSFLLSSRALHAAAASPAAAGRWGNAPPPPPTPTPCPGPSSRAGIAGAVSFSLTFAT
VAVAEVQAKERLPSDLLPRNVVLYQYQACPFCNKVRAFLDYHDIPYKVVEVNPLSKKEIKWSEYKKVPIL
TVDGEHLVDSTDIINILQHRISPDDEVTNEEETKWRKWVDEHLVHVLSPNIYRTTSEALESFDYIAKHGN
FSYTERFAVKYAGAAAMYFVAKKLKKKYNITDERASLYDAANTWTEALNGRNFL**G**GSKPNLADLAAFGVL
RPIRYLQSGKDMVEHTQIGEWYQRMEDAVGEPSRIPEGQYQE

SEQ ID NO:5

Amino acid sequence of the mutant polypeptide encoded by SEQ ID NO:3

MRSLRAAQTLLASRSFLLSSRALHAAAASPAAAGRWGNAPPPPPTPTPCPGPSSRAGIAGAVSFSLTFAT
VAVAEVQAKERLPSDLLPRNVVLYQYQACPFCNKVRAFLDYHDIPYKVVEVNPLSKKEIKWSEYKKVPIL
TVDGEHLVDSTDIINILQHRISPDDEVTNEEETKWRKWVDEHLVHVLSPNIYRTTSEALESFDYIAKHGN
FSYTERFAVKYAGAAAMYFVAKKLKKKYNITDERASLYDAANTWTEALNGRNFL**D**GSKPNLADLAAFGVL
RPIRYLQSGKDMVEHTQIGEWYQRMEDAVGEPSRIPEGQYQE

SEQ ID NO:6

Amino acid sequence of HvmPGES2 NCBI accession AK373333 from cv. *Haruna Nijo*. The difference to SEQ ID NO:4 is underlined.

MRSLRAAQTLLASRSFLLSSRALHAAAASPAAAGRWGNAPPPPPTPTPCPGPSSRAGIAGAVSFSLTFAT
VAVAEVQAKERLPSDLLPRNVVLYQYQACPFCNKVRAFLDYHDIPYKVVEVNPLSKKEIKWSEYKKVPIL
TVDGEHLVDSTDIINILQHRISPDDEVTNEEETKWRKWVDEHLVHVLSPNIYRTTSEALESFDYIAKHGN
FSYTERFAVKYAGAAAMYFVAKKLKKKYNITDERASLYDAANTWTEALNGRNFL**G**GPKPNLADLAAFGVL
RPIRYLQSGKDMVEHTQIGEWYQRMEDAVGEPSRIPEGQYQE

SEQ ID NO:7

Genomic sequence of *HvmPGES2* in Quench ranging from the start to the stop codon of the contained coding sequence based on mapping of genomic short reads from NGS sequencing to SEQ ID NO:1.

ATGAGGTCGCTCCGCGCGGCGCAGACCCTGCTCGCCTCCCGCTCGTTCCTCCTCTCCTCGCGCGCGCTCC
ACGCCGCCGCGGCCTCCCCCGCCGCGGCCGGGCGCTGGGGCAACGCCCCGCCGCCGCCGCCTACGCCGAC
GCCCTGCCCGGGGCCGTCGTCCCGGGCCGGGATCGCCGGCGCGGTCTCCTTCTCCCTGACGTTCGCCACG
GTGGCGGTGGCCGAGGTGCAGGCCAAGGAGCGGCTGCCCTCGGATCTGCTGCCCCGGAACGTCGTGCTCT
ACCAGTACCAGGCCTGCCCCTTCTGCAACAAGGTCAGAGgtgaggttgctccccaattgctccctcggat

ccatattgattgacgctgccttagtgctagcatttttcgattcgcgcctcggctctttagaaaatggcg
agatcccgttgcgctgacagattcgatcagagttggcatggctttaaaatctagagaacgattttgtagt
gtattagggtagatttgtatgttggttagaagaatttgttctcatcggatcgaattggactgtaatcgga
gcttgtcaacatattatttctgctcctgctagagactaaagggctgttggggaaatctatggctaagcac
ctatagagttattcatctctgcttgtatggctttataattggcaaatgtagaatgctttctgacctggtg
gtcccatctggtgccatttctgcgtgtattggcatggttcaagatcaataagtattgtgttattcttgtt
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taactgttttctattggctttgtttagCTTTCCTAGACTATCATGATATACCCTACAAAGTTGTGGAAGT
TAATCCACTGAGCAAAAAGGAAATCAAGTGGTCTGAGTACAAGAAGGTCCCAATATTGACGGTAGATGGT
GAACATCTGGTTGATTCTACAGgttacgcttgttacctacaagtttcttttgcaattcatgtccttcact
ttgttgcagcttcaagtactcttgtgttaacatttatttatttcttgttggcagACATAATCAATATATT
ACAGCACAGGATCAGCCCTGATGATGAAGTCACTAATGAAGAGGAAACAAAATGGCGCAAgtatgtgatt
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GGGTTGATGAGCACCTTGTGCATGTATTGTCACCAAATATATACCGGACGACTTCAGAGGCTCTAGAATC
TTTTGACTACATTGCAAAACATGgtaaaattctggaacttttatcttccttttgctgccaataaatttac
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tccatttgatgggttttattattgttcaattacaataagtggattttgttgtttgcactgcaatgcttgt
aaaatgtgattttgtgaaccagactggaaggtaattgcacaatacatgagacaaatattattattctgtg
aagcccacaaaacataactaaacaggctagtcagtttccatacaccgatttctcccgtgatgaatcctaa
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gttcctttctcgacccaaactgtgatgcagGTAACTTCAGTTACACCGAGCGGTTTGCTGTGAAGTATGC
TGGTGCTGCAGCAATGTACTTCGTGGCTAAGAAGCTTAAGAAGAAATACAACATTACTGATGAGCGTGCC

TCATTGTATGATGCGGCCAACACGTGGACGGAAGCCCTGAATGGAAGAAATTTTCTCGgtatcttattta
tccttaggccaaaagaaacatattgggagctacaggaaaattagaatacagcgagtttattatattcctt
agcattaatttggttttcactttctttgtcatccagGTGGCTCCAAGCCTAACTTGGCAGATCTGGCAGC
ATTTGGTGTTCTGAGACCTATCAGATACCTGCAGTCTGGAAAAGACATGGTTGAGCATACCCAAATCGGC
GAGTGGTACCAGCGGATGGAAGATGCGGTCGGAGAACCATCGAGAATCCCGGAGGGCCAGTACCAGGAGT
AG

SEQ ID NO:8

Genomic sequence of *HvmPGES2* in MQF08-1101 based on mapping of genomic short reads from NGS sequencing to SEQ ID NO:1. Coding sequence is provided in capital letters.

ATGAGGTCGCTCCGCGCGGCGCAGACCCTGCTCGCCTCCCGCTCGTTCCTCCTCTCCTCGCGCGCGCTCC
ACGCCGCCGCGGCCTCCCCCGCCGCGGCCGGGCGCTGGGGCAACGCCCCGCCGCCGCCGCCTACGCCGAC
GCCCTGCCCGGGGCCGTCGTCCCGGGCCGGGATCGCCGGCGCGGTCTCCTTCTCCCTGACGTTCGCCACG
GTGGCGGTGGCCGAGGTGCAGGCCAAGGAGCGGCTGCCCTCGGATCTGCTGCCCCGGAACGTCGTGCTCT
ACCAGTACCAGGCCTGCCCCTTCTGCAACAAGGTCAGAGgtgaggttgctccccaattgctccctcggat
ccatattgattgacgctgccttagtgctagcatttttcgattcgcgcctcggctctttagaaaatggcg
agatcccgttgcgctgacagattcgatcagagttggcatggctttaaaatctagagaacgattttgtagt
gtattagggtagatttgtatgttggttagaagaatttgttctcatcggatcgaattggactgtaatcgga
gcttgtcaacatattatttctgctcctgctagagactaaagggctgttggggaaatctatggctaagcac
ctatagagttattcatctctgcttgtatggctttataattggcaaatgtagaatgctttctgacctggtg
gtcccatctggtgccatttctgcgtgtattggcatggttcaagatcaataagtattgtgttattcttgtt
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taactgttttctattggctttgtttagCTTTCCTAGACTATCATGATATACCCTACAAAGTTGTGGAAGT
TAATCCACTGAGCAAAAAGGAAATCAAGTGGTCTGAGTACAAGAAGGTCCCAATATTGACGGTAGATGGT
GAACATCTGGTTGATTCTACAGgttacgcttgttacctacaagtttcttttgcaattcatgtccttcact
ttgttgcagcttcaagtactcttgtgttaacatttatttatttcttgttggcagACATAATCAATATATT
ACAGCACAGGATCAGCCCTGATGATGAAGTCACTAATGAAGAGGAAACAAAATGGCGCAAgtatgtgatt
ttttaattgattggtgtgcaactatgcgttagatgttaatgttgtctttggtgctccctttctttagcat
tatatgttttagaggttcagttgcatatttctttccattacaaatacttagtacacacccattttactta
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cgtacaccttcttagctctggcacatctatgccccagcatagttgcgtccattcatttccttggccctgg
tgctcctccacatataggcctttgtgtttgtgctgctgatatactccctgagctgatgttctgctactgt
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gaaaactacacaagataaactttagtgatcactacttttaggcttttagcagatgtagctttggccaact
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aaatatatgcaagtaacttcttctgaacactgctcacagggtcacacccatgttccaaagccagttagga
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ggattgacatacttacaacatactcttatctactagagcatgacaagtttcctgcatctttgatctagGT
GGGTTGATGAGCACCTTGTGCATGTATTGTCACCAAATATATACCGGACGACTTCAGAGGCTCTAGAATC
TTTTGACTACATTGCAAAACATGgtaaaattctggaacttttatcttccttttgctgccaataaatttac
tatccttcagaattgtaatagcaaataccctttgtgaacttgtgctcattgtttattgaagtttccatag
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cacccaggatgttctaaaaatgtttatgtgattgtataaattggaggttatctgcttctaatcctattac
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aaaatgtgattttgtgaaccagactggaaggtaattgcacaatacatgagacaaatattattattctgtg
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gttcctttctcgacccaaactgtgatgcagGTAACTTCAGTTACACCGAGCGGTTTGCTGTGAAGTATGC
TGGTGCTGCAGCAATGTACTTCGTGGCTAAGAAGCTTAAGAAGAAATACAACATTACTGATGAGCGTGCC
TCATTGTATGATGCGGCCAACACGTGGACGGAAGCCCTGAATGGAAGAAATTTTCTCGgtatcttattta
tccttaggccaaaagaaacatattgggagctacaggaaaattagaatacagcgagtttattatattcctt
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ATTTGGTGTTCTGAGACCTATCAGATACCTGCAGTCTGGAAAAGACATGGTTGAGCATACCCAAATCGGC
GAGTGGTACCAGCGGATGGAAGATGCGGTCGGAGAACCATCGAGAATCCCGGAGGGCCAGTACCAGGAGT
AG

SEQ ID NO:9

Nucleotide sequence from the genomic sequence of *Hordeum vulgare*, cv. Morex wherein position 501 corresponds to position 10,497,647 on chromosome 2H of the barley genome assembly by IBSC of 14-07-2016

AATTCAGGAAGATTTTGATGAATTTAGTGATGTCGAGGATCTCTATAGCACATTGCCTATGGAGAAGGTT
GAAGCACTCGAAGACATGGTTTCACTTGCTCCTTCCAGTCTTATCAAGGTGGGCTGTGAGCTGTTAATGC
TTTGTTTTACTTTGGTTATTGTGATTTTGCTAATAGAAACAACTGGCAAACTTCCTTATGTTTTTTCTCC
ACAAATACTGCTTGTCTGCTATGCTCGTGGAGATCATTTCTCTACCATGTTTTTATGTTGTGCAGCTCTT
CATCTTTTAGTTATATGGGTAATTATACATTTTTCAACATTACTTCTTGTTTGGTTGGCAGGGTGTTGCT
GCTGTTTCCACTACTGCAGTTTTGAGTACGAAGAATCCGAAAGCAACTTCACCTACTCAGGTACATGCTA
TTCATTCCTAGTTATTTATTGAATATCTGTTGGTTTGTGATAGACGGAGACATAGTATTAGTATATCGTA
TACTCTAAACACAATGGTTTGTTATGTGCCCAGTGCCGGCTTAGTTGGCATCCTGGCTGCTGCTTTCTTG
TCGACCTTTACATGCTACTCCCTCCGTCCCATAATATAAGAGTGTTTTTAACACTAGTGTAGTGTCAAGA
ACGTGCTTATATTATGGGATAGAGGGAGTAGTATTGATTTATTTGGTTATCTTCTTACAAGTTAATCCTT
GAATATGAATACACGTGATTGTTGCTATTTGGGCATTTAATAAAAACACATGCTAACAAGATTGAGGAAT
TGGTTATTTCTAATGTACAATCCTGATTACTAATTGTCGATATGCTGATTTACCGACTCTGTCTCCCTTT
GCTTCAGGCTACTGTATCAACAATCTCACAAGGTACATCACAAGATCAAGCAGAGGAAACAACTTCAATA
GAAAGCAATCCTGAACCGGTGCCAAATATTACTTACTAATGCCCGCAAAGTATCGTTGTATATTATATTT
GTTATGGAGCTATGTATGTAT

SEQ ID NO:10

Nucleotide sequence from the genomic sequence of *Hordeum vulgare*, cv. Morex wherein position 501 corresponds to position 31,153,440 on chromosome 2H of the barley genome assembly by IBSC of 14-07-2016

CAAAATAAAAGTAACAGCAGAAAATCTACTTCGGATGTGCAGAGATAGGCTTATAAACAAATCTAAAGTC
ATAATTTACTCCATAGAGAGGTGATCCATAACGAAACTAATATGACAGTGGATCAGCTACTGTTTCTTTT
TCTTTGTTCTTTTTTGAGTGAGGATCAGCTACTACTGTTCTTTCCTGTTGGACACCATCAGCCACAATTC
CATAGTTTAAGTTAGGACTTGACAAGGACATTCGACATTAAAGCAATACAATTATTCTAACACCACCATA
CTAACAATAAGAAGCGTAGGCAGAGCCAGATGGCCTCGGTCACAGTATGACGTTCTAACGCACATTGTCA
TTGCTGTCATACAATACACTCGAGGAATCATAATTAACCAATGGTACTGAACAGAGGGTGACTAGGAATC
CGGGCGAGTCCACCAACAGTGGTTGATGCTATTCTCAACAAATTCACTCCATAGAGAGGTGATCTCGAAT
AAAATTGATACTGTAGTGGATCAGCTATTGTTCCTTCCTGCAGGAAACCCTCAGCTATTTTGTTCCATGG
TATTATTATTATGCTAGGACTGTCAATGACACTAGGAAAACTGCATCGAACATTAACATTAACGCAATAC
AATCATTCTAACACCACCGTGCTGACAGTAACAAACTTAGGCAGAGCCTAAATCGCAGCATGATAATATT
CTACCGCACATTGTCACTACTGTCAGCAAAAGGAAGTTGGAAAAAACACTCGATGCTTCATAATGAACCA
ACGGAGCTGAACAGAGAGTCTCACTAGGAATCCCGGCGAGTCCGCCAGTAGCGGTTGATGCTGTTCTTAA
AATAAACCGTGGCGAGCAGGCGCACGTCCTCGCGGCAGGACGAGCCCCTGGCGGAGATGATCTCCTGCAG
AAGCACCAGATCAGCGAGTCAGCACGCTCGAACCCCATGGCACCGCCCCCCAATCGCCCGTAGATCGGGG
TAGGCCCGGGAGGAGGAAAAG

SEQ ID NO: 11
The nucleotide sequence covering the substitution (marked in bold) at position 20,084,177 on chromosome 2H of the barley genome assembly by IBSC of 14-07-2016 found in MQF08-1101 compared to Quench

ACCCCCCCCCCCCCCGGGGAAGGAGGATCACCTCATGTGCGCCATCATGAAGCGTTCGATGAGGGAGGCGG
CGAAGGACGCCACACACAACCGCCGCGAGCTCGAGATCGAGTAGATCTTCCTCGAGCAGGGGGTCACAGC
TTAGCAGGCGAGCGCATCCAAGGAGGCGGACCTGTGCGTCCTGAAGGCCGAGCAGGACAAGATCTGGATC
GACCTTGACTCCGACGAGGACTGAGCTCCCCGGGCTCCTCCGCCGCATCATCGCCGCTGCACTAGTCATT
TTTGGTAGCCTTGGCTCGGGTACGCTGCACGGCCCGCAGATCCCCCTACCTAGTAGTAGTATGAAGAACT
GTTGTAGTTTGATGAAGAACTGGTGTAGTTTGACGAAGAACCATGATGTAGTTTGATGAAAACCATGATG
TAGTTTCACCCGTGAACCCTTTTTTTCCAAATTTACAATTTCATATACGGGGTTTGATCTTCGGTGCGCGA
AATGGCCCCG**C**AAAATTGCTCCGCAACGACGTGTAAACCCACTTGTGGGTCGCTCTTCTACGCGGTCTGC
TAGAGATGCTCTTAGAGCATCTATAGCAGATCATTTAAATCTTAAACTATAAAACTGGGATAAAAAGCTC
CTTCCTCTAGTCCGGCCGCCGCCGCCCCATCGCCCTTCGACCGCACCCCGTCCCTGCCGGTCGCGCCCCA
TCACCACGCTGACCGCGCCCCGTTGTCCGCCGACCACGCCCCGTCGACTCCGCTGTCCGAGCCTTGCTCC
ATTGCTAGCCGCACCTGCCGAGCCGTGCCCCTTTGCCTGCCCCGTCGGGAGGATTCCGGTGGCCAAGCTA
AGGTCATGGGAGGCCATAGCGAACCGACGGCGATCGATTGCGGCGTCTAGCGGACTTTTTCGGTGTACGG
TGATGAATTTCGACGAGATTAGGGCGAATTTCGACAAACTCCGCAGCGACGTGTTTGCTCCAACGAGGTT
TGACCGGTATACGGGGCCCCC

Examples

Example 1

Screening and mutant selection

Disintegration of starch observed after incubation in alkaline solution like KOH can be used to estimate differences in gelatinisation temperature of cereal starches (Little et al. 1958). A high-throughput procedure to screen for altered starch disintegration in alkaline solution was developed for single barley grains. The procedure involves extraction of starch from barley grains and subsequent disintegration of said starch in alkaline solution to identify mutants with lowered starch gelatinisation temperature. The screening was done on a chemically induced mutant population prepared by treating

barley kernels of the cultivar (cv.) Quench with sodium azide essentially as described by Kleinhofs, 1978. In total more than 11,000 grains were screened. Lines with potentially decreased starch gelatinisation temperature were propagated and starch was extracted from their offspring to measure gelatinisation temperature using differential scanning calorimetry (DSC), a method routinely used to measure gelatinisation temperature from starches (Gill et al. 2010; Sopade et al. 2004). Several mutant lines were picked up by the screening. Of these, one mutant line, depicted as MQF08-1101, was selected for further analysis as the gelatinisation temperature measured for this line was consistently lower than that of cv. Quench.

Example 2

Gelatinisation temperature of starch produced by mutant line MQF08-1101

Mutant line MQF08-1101 was propagated along with cv. Quench in twelve independent field trials and harvested in one pool per field plot. For the first nine propagations (harvest years 2010 to 2014), starch was extracted from grains to determine gelatinisation temperature using differential scanning calorimetry (DSC) (Mettler Toledo DSC1 instrument). For the following three propagations (harvest years 2015 to 2016), the grains were milled and the DSC analysis was done directly on the flour. The barley flour sample was mixed with three times the amount of water (w/w) and added to an aluminium crucible, which was then sealed. The crucible was heated in the DSC from 40 °C to 90 °C at a 10 °C/min rate and the endothermic peak temperature for the phase transition was recorded as the gelatinisation temperature. The gelatinisation temperature is dependent on the particular growth conditions, however, the analysis revealed that MQF08-1101 consistently has a 0.8-2.6 °C lower gelatinisation temperature than cv. Quench grown under similar conditions (Figure 1).

The gelatinisation temperature of malt prepared from grains of MQF08-1101 and cv. Quench plants grown on Fyn 2014 and 2015 was also determined. Three biological replicates that were grown in distinct trial plots in the same field were chosen for each year resulting in 12 samples. Prior to malting, all barley samples were evaluated for the parameters germination index, germination energy and water sensitivity. Data was based on two times sample sizes of 100 grains for a 4 mL germination test and a sample size of 100 barley grains for an 8 mL germination test according to Analytical-EBC Method 3.6.2 Germinative Energy of Barley: BRF Method (Table 1). Furthermore, the protein, water and starch content of the barley were determined using a Foss 1241 NIT instrument.

The 12 barley samples were then malted in duplicates using 100 g of barley per sample cup. The barley samples were steeped as a forced submission into water of all grains in a closed sample grid chamber. The steeping procedure was performed at day 1, 2 and 3. For the following 3 days the water content was adjusted by spraying with water. After these six days rootlets had appeared and standard kiln program was applied to dry the malt in a Thermax incubator for 21 hours. The temperature profile of the kiln process was a 2 step ramping profile and an isothermal terminal step monitored by a Eurotherm control unit. First ramping step started at set point 30 °C and a linear ramping at 2 °C/hour to the breakpoint at 55 °C. Second linear ramping was at 4 °C/hour and reaching a maximum at 85 °C. This temperature was kept constant for 1.5 hours. The process was based on a recirculating air flow using 80 % fresh air. The kiln samples were cured using a manual root removal system. Subsequently, a subset of each of the 12 samples was milled and gelatinisation temperature was measured on the flour by DSC. This showed a lower gelatinisation temperature of the starch from MQF08-1101 malt compared to that of cv. Quench in both years, even though the gelatinisation temperature varies between years (Figure 2).

Example 3

Differences in starch structure during development under controlled conditions

The gelatinisation temperature of starch produced by mutant line MQF08-1101 is consistently lower that than of starch produced by cv. Quench. A detailed analysis of the molecular composition of the starch produced by MQF08-1101 was undertaken and revealed structural changes in the starch that are believed to contribute to the lower starch gelatinisation temperature. To this end, both cv. Quench and MQF08-1101 were grown under 16 hour day and 8 hour night conditions with temperature and relative humidity set to 18 °C/70 % (day) and 12 °C/55 % (night). A heat treatment was applied to one cohort of the plants by moving them to a chamber with 33 °C/70 % (day) and 26 °C/55 % (night) from day 16 to 21 after flowering, while another cohort remained at control conditions. Samples were collected during development until maturity. The starch produced by MQF08-1101 could be compared to the structure of starch produced by the reference cv. Quench. The analysis concerning cv. Quench has been reported on in detail elsewhere (Valente, 2016).

First, the gelatinisation temperature of starch from mature grains was determined. At maturity, the first appearing spike was harvested from each individual and flour obtained from half a spike was used for the starch gelatinisation measurement. To this

end, dried grains from MQF08-1101 grown under control and heat conditions were crushed into flour in a 2010 Geno/Grinder (SPEX SamplePrep) and the gelatinisation temperature was measured by DSC. It has been described that gelatinisation temperature of starch increases when plants are exposed to elevated temperatures during grain filling (Tester et al. 1991; Myllärinen et al. 1998). The values obtained from MQF08-1101 were compared to respective samples from cv. Quench. In both cases an increase in starch gelatinisation temperature was observed for the plants subjected to heat. However, starch from MQF08-1101 plants showed a lower starch gelatinisation temperature than cv. Quench both under control condition and after heat stress (Figure 3).

Further, plants were analysed for their starch composition after growth in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 16 to 21 days after flowering.

To understand if the molecular composition of starch was different between cv. Quench and MQF08-1101 grown under control conditions, the amylose to amylopectin ratio was determined in grains sampled from day 15 after anthesis to maturity. A commercial amylose/amylopectin kit (K-AMYL, Megazyme, Ireland) was used with a protocol adapted to samples sizes of only 5 mg of flour. The analysis revealed differences in the amylose/amylopectin ratio expressed as the amylose content [%] of total starch during development grown in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 10 to 21 days after flowering. In mature grains, which are used for the malting, MQF08-1101 contained more amylose than cv. Quench, the same was observed during development except at day 29 after anthesis (Figure 4).

The chain-length distribution in the amylopectin was determined by resolving all α-1-6-glycosidic linkages in the starch produced by cv. Quench and MQF08-1101 during development grown in controlled conditions using the same temperature program throughout the growth, or with appliance of a heat treatment from 16 to 21 days after flowering. To prepare samples, the flour was washed extensively and beta glucans were solubilized and removed to isolate starch before debranching the amylopectin with isoamylase (Megazyme, E-ISAMY, 1000 U/mL from *Pseudomonas sphaeroides*) and pullulanase (Megazyme, E-PULBL, 900 U/mL from *Bacillus licheniformis*). The chain-lengths were analyzed using an ICS-3000 chromatography system (Dionex) with a 155 min long run with different ratios of 50 mM NaOH and 50 mM NaOH plus 1 M NaOAc. The resulting peaks, corresponding to the different chain-lengths, were

converted to molar fractions by comparison with a debranched and fluorescently labeled glycogen standard. Analysis of chain length distribution was done at the individual chain length level. The analysis showed changes at all developmental stages between cv. Quench and MQF08-1101, as MQF08-1101 contained a higher proportion of branches with chain lengths of 7-10 than cv. Quench as well as a lower proportion of branches with chain lengths of 11-21 (Figure 5). In particular, starch from mature grains of MQF08-1101 had higher amounts of chains of length 7-10 and smaller amounts of chains of length 11-21 than Quench. This, analysis reveals differences in the structure of amylopectin between cv. Quench and MQF08-1101, which are believed to be important for the gelatinisation temperature.

Example 4

Identification of genomic region and a SNP (single nucleotide polymorphism) associated with lowered starch gelatinisation temperature

The mutation associated with the lowered starch gelatinisation temperature in MQF08-1101 was determined employing a quantitative trait locus (QTL) mapping approach. To this end, a double haploid (DH) population was generated from a cross between MQF08-1101 and cv. Melius.

The resulting 43 DH plant lines were propagated in four different environments: three times in the field and once in the greenhouse with three individuals per line in each their pot. For the field grown samples several individuals from each line were grown in a plot and all grains per field plot were harvested in a pool. The starch gelatinisation temperature was measured by DSC on a representative sample of grains milled into flour essentially as described in Example 2. In the greenhouse, the spike of the first developing tiller for each individual plant was marked and harvested separately. The grains of these spikes were squeezed in a hydraulic press with 2 tons/cm^2 and lyophilised overnight followed by crushing them into flour in a 2010 Geno/Grinder (SPEX SamplePrep) for 6-8 min at 1.500 rpm. Subsequently, the starch gelatinisation temperature was measured on the flour using DSC.

Furthermore, each line was genotyped by a NGS approach and remapped to the barley genome pseduomolecules (Masher et al. 2017) to obtain a number of markers on each chromosome polymorphic between Melius and MQF08-1101. Based on the segregation pattern of the markers a genetic map with 104 markers were constructed using QTL IciMapping version 4.1 (Meng et al. 2015) with the kosambi function (Kosambi 1943).

The starch gelatinisation temperature for each barley line grown in the four different environments was then used as individual traits for QTL mapping. Windows QTL Cartographer Version 2.5 was utilized for both linear interval mapping (IM) and composite interval mapping (CIM) using an estimated significant threshold at a significance level of 0.05 on 1000 permutations for each environment (Wang et al., 2012). The QTL mapping identified a genomic region on chromosome 2H with the marker chr2H:11,398,210 showing the best association with the starch gelatinisation temperature in all environments. Splitting the population on whether the marker in the region was like MQF08-1101 or Melius, showed that plants having the Melius allele in the QTL had a higher starch gelatinisation temperature than plants containing the MQF08-1101 allele in the QTL (Figure 6).

The QTL is a genetic region without any cross-over events in the DH population. The two markers flanking the maximum size of the region without recombination are at position 10,497,647 and 31,153,440 on chromosome 2H. Within the current barley genome assembly annotated in May 2016 (Mascher et al. 2017) 959 high and low confidence genes are in the region. From these 959 genes six candidate genes, which potentially could be linked to starch biosynthesis and gelatinisation temperature (Table 2) were further investigated. In cases where no full-length sequence was present in the genome assembly, BLAST searches against other public data sets containing whole genome shotgun assemblies of the barley cultivars Morex, Barke and Bowman were performed (International Barley Genome Sequencing Consortium, 2012). However, for one gene, *Pho2,* no full-length sequence could be found. As a next step, the six genes were amplified from genomic DNA of cvs. Melius and Quench and mutant MQF08-1101 by PCR. The obtained PCR products were sequenced by Sanger sequencing (Eurofins Genomics or StarSeq GmbH, Mainz, Germany) and the results were analysed using CLC main workbench (Qiagen). The experiment revealed sequence deviations between cv. Melius and cv. Quench, however, no differences between cv. Quench and MQF08-1101 were identified. Thus, it is unlikely that any of the six candidate genes investigated is contributing to the lower gelatinisation temperature of starch produced by MQF08-1101.

A whole genome sequencing approach was next initiated to identify homozygous sequence deviations within the QTL region, which are associated with the phenotype. To this end, genomic DNA was extracted from leaf material of cv. Quench and MQF08-1101. A commercial provider (Eurofins Genomics) generated barcoded PCR-free

sequencing libraries for both lines with insert size of approximately 550 bp and sequenced those on an Illumina HiSeq 2500 with 2×125 bp paired-end sequencing. Reads were trimmed to remove leftover adapter sequences and poorly sequenced bases in the beginning of the reads with AdapterRemoval v. 2.2.0 with options "--trimns --trimqualities --minquality 20 --maxns 3 --minlength 70" (Schubert et al. 2016) and the first 5 bp were discarded with seqtk (https://github.com/lh3/seqtk). Trimmed reads were then mapped against the Morex barley genome pseudomolecules (Mascher et al. 2017) with "bwa mem" (Li 2013). Sorting, removing poorly mapped reads (below mapping quality 30) and cleaning read pair information were done with samtools v. 1.3.1 (Li 2011). Variants were called using "samtools mpileup" and "bcftools call --multiallelic-caller" v. 1.3.1 (Li 2011) excluding base calls with a quality less than 20. After filtering on genotype qualities of at least 20, depths of at least 5 and requiring that cv. Quench and MQF08-1101 were homozygous and different, only two variations were left in the QTL region, of which just one, a SNP at position 21,822,292 on chr2H, was in an annotated exon. At this position cv. Quench had a guanine (G), same as the reference Morex barley genome, while MQF08-1101 had an adenine (A). The other SNP was found at position 20,084,177 on the same chromosome and is a C to a G exchange marked in bold in SEQ ID NO: 11.

To further investigate the identified candidate SNP, the corresponding genomic region was amplified by PCR and Sanger sequenced in cv. Quench, MQF08-1101 and cv. Melius (Table 3). This showed that cvs. Melius and Quench both have a G in the identified position, whereas MQF08-1101 has an A. Thus, it is believed that this SNP is associated with the starch gelatinisation temperature differences observed in the cv. Melius x MQF08-1101 population. Furthermore, it is believed that this SNP may be a marker indicating that the starch of a barley plant carrying said mutation has a lower gelatinisation temperature compared to the corresponding wild type barley plant, such as a barley plant not carrying said mutation, but otherwise identical.

Example 5

SNP and gelatinisation temperature association in different barley backgrounds

The association between the identified SNP and the low gelatinisation temperature phenotype was determined in MQF08-1101 backcrossed to cv. Quench. The resulting F1 plants were grown to maturity and 119 F1 grains were sown in pots in the green house. From each germinated F2 plant one leaf was sampled and used to genotype for

the SNP using PCR (Table 3) and Sanger sequencing (Eurofins Genomics). 28 plants were identified as being homozygous for the Quench G allele, 26 plants homozygous for the MQF08-1101 A allele and 57 plants heterozygous. For the F2 plants homozygous at the allele, the starch gelatinisation temperature was measured on a pool of seeds from each plant. The seeds were milled into flour and the gelatinisation temperature was measured by DSC and reported as the peak temperature. The analysis showed a significant difference between the gelatinisation temperatures depending on whether the plants contained the MQF08-1101 A allele or Quench G allele (Figure 7). On average the plants with the MQF08-1101 has a 0.85 °C lower starch gelatinisation temperature then the plants with the Quench G allele.

In addition to crossing MQF08-1101 to cv. Quench, MQF08-1101 was integrated into the breeding pipeline by generating DH lines from crosses between MQF08-1101 and different cultivars. 13 DH lines from these crosses were chosen for further analysis based on their agronomic performance under field conditions in New Zealand 2017. From the 13 DH lines grown in field plots the grains from each plot were harvested in a pool. Following, DNA from a grain from each DH line was extracted and genotyped by PCR (Table 3) and Sanger sequencing (Eurofins Genomics). This resulted in the identification of seven plants carrying the MQF08-1101 allele with the A allele and six plants having the G version of the allele. Subsequently, a pool of grains per DH line was milled and the starch gelatinisation temperature was measured by DSC. This revealed that all plant lines carrying the G allele had higher gelatinisation temperature than the plants carrying the MQF08-1101 A allele (Figure 8). Thus, in all 13 DH breeding lines tested a correlation between the genotype of the identified allele and the starch gelatinisation temperature could be observed.

Example 6

mPGES2

The identified SNP is located in the coding sequence of the gene model HORVU2Hr1G011030, for which some sequence information for the first exon is missing in the barley reference genome assembly (Mascher et al. 2017). A Blastn search restricted to *Hordeum vulgare* accession within the sequence repository of the at National Center for Biotechnology Information (NCBI) nucleotide database identified AK373333, which represents a full-length mRNA sequence from barley cv. Haruna Nijo including complete sequence information of the first exon (Matsumoto et al. 2011). Thus, a full-length sequence for the gene was generated by completing gene model

HORVU2Hr1G011030 using sequence AK373333. The exons of the sequence was amplified and sequenced in Quench and MQF08-1101, confirming the presence of the sequence with only the identified mutation between Quench and MQF08-1101 differing. Several protein domains were found in the sequence: GST_C_mPGES2 cd03197, GST_N_mPGES2 cd03040, GST_N_3 pfam13417, GstA COG0625 and GrxC COG0695. These annotations together with an alignment and phylogram prepared with cysteine-containing gluthatione S-transferases (Cys-GSTs) (Lallement et al., 2014) (Figures 10 and 11) made it possible to classify the gene as a Cys-GSTs class mPGES2. Thus, the gene was designated *HvmPGES2*.

The identified SNP is located at the first nucleotide position in the 6th exon of *HvmPGES2*, thus, it could potentially affect mRNA splicing. To investigate this possibility, total RNA was isolated from single developing grains of cv. Quench and MQF08-1101 15 days after anthesis with the Aurum Total RNA Mini Kit (Biorad) and the following reverse transcription was done using iScript cDNA Synthesis Kit (BioRad). A PCR was performed using Phusion High-Fidelity PCR Master Mix with HF buffer (New England BioLabs) and the primers in Table 3. The amplicons were Sanger sequenced (Eurofins Genomics) and showed the same splicing pattern both in cv. Quench and MQF08-1101, thus, the SNP does not cause aberrant splicing. Consequently, the identified SNP encodes an amino acid change on protein level. The guanine (G) to adenosine (A) change found in *HVmPGES2* causes a glycine (G265) to aspartic acid (D) change in the protein when translated.

The genomic sequence of *HvmPGES2* from *Hordeum vulgare*, cv. Morex (i.e. the genomic sequence of *HvmPGES2* of the G-allele), wherein sequence gaps of the gene model HORVU2Hr1G011030 are filled by the sequence from AK373333 from *Hordeum vulgare* cv. Haruna Nijo is provided herein as SEQ ID NO:1.
The coding sequence of *HvmPGES2* from *Hordeum vulgare*, cv. Quench (i.e. the coding sequence of *HvmPGES2* of the G-allele) is provided as SEQ ID NO:2. The sequence of the polypeptide encoded by this gene in *Hordeum vulgare*, cv. Quench is provided as SEQ ID NO:4. The sequence of AK373333 from *Hordeum vulgare* cv. Haruna Nijo differs slightly from SEQ ID NO:4 in that amino acid 267 is a Pro rather than a Ser. Both the sequence of SEQ ID NO:4 and the sequence of SEQ ID NO:6, wherein amino acid 267 has been replaced by Pro are considered wild type sequences herein.

The coding sequence of *HvmPGES2* from mutant MQF08-1101 (i.e. the coding sequence of *HvmPGES2* of the A-allele) is provided as SEQ ID NO:3. SEQ ID NO:3 is identical to SEQ ID NO:2 except for the nucleotide at position 794, which is an A in SEQ ID NO:3. The sequence of the polypeptide encoded by this gene is provided herein as SEQ ID NO:5. SEQ ID NO:5 is identical to SEQ ID NO:4 expect that the Gly at position 265 has been replaced by Asp.

In order to further investigate HvmPGES2, it was aligned with sequences representing the six subfamilies of Cys-GSTs present in plants (GSTL, DHAR, GHR, GSTH, GSTI and mPGES-2) from Lallement et al.,(2014) supplemented with mPGES2 sequences from tobacco (NtmPGES2), tomato (SlmPGES2) and tree cotton (GamPGES2) and the sequence of the macaque protein (PGES2_MACFA) used for structure threading (see below) (figure 9). The variable N-terminal regions were removed up to the active site cysteine and the truncated sequences were aligned in Clustal omega (figure 10). This shows that HvmPGES2 shares many conserved residues with the other mPGES2 proteins as shown in bold. Among these conserved residues is the one containing the identified mutation marked with **D**, suggesting an important function for this amino acid.

In order to elucidate if this change has the potential to affect the functionality of the protein, fold recognition was done by submitting the HvmPGES2 protein sequence to SWISSMODEL (Biasini et al., https://swissmodel.expasy.org). The SWISS-MODEL template library (SMTL version 2017-10-23, PDB release 2017-10-13) was searched with Blast (Altschul et al., 1997) and HHBlits (Remmert, et al., 2012) for evolutionary related structures matching the target sequence. The structure 1z9h.pdb of the Microsomal Prostaglandin E Synthase Type 2 (mPGES2) from crab-eating macaque (Yamada et al., 2005) was found as the best template to generate a structural model of the barley sequence. The resulting model was analysed using Swiss-PdbViewer. The structure of HvmPGES2 predicted a loop distal to the active site, where the changed amino acid is located (Figure 9). The loop is believed to be involved in stabilising the helix packing in the protein, which may be impacted by the changed amino acid. The amino acid G266 which is neighbouring the changed amino acid is absolutely conserved among Cys-GSTs (marked in black) (Lallement et al. 2014). Therefore, it can be postulated that exchanging this amino acid would influence the activity of HvPGES2. Other residues where mutations are believed to affect the protein function

based on the structure and their conservation are indicated with * above the alignment (figure 10) and found in Table 4. Additionally, different regions with conserved amino acids were identified in the alignment. It is believe that mutations in these regions also change the enzymatic function. This being regions with an absolute conservation ≥90 % among Cys-GST's, with conservative substitutions and conservation ≥90 % among Cys-GST's, with absolute conservation ≥90 % among mPGES2's, with conservative substitutions and conservation ≥90 % among mPGES2's, with absolute conservation ≥90 % among plant mPGES's or with conservative substitutions and conservation ≥90 % among plant mPGES2's (Table 5) in all cases based on sequences included in the alignment.

Tables

Table 1

Results from germination test on barley from Fyn 2014 and Fyn 2015

	Germination index	Germination energy 4 mL	Germination energy 8 mL	Sensitivity
MQF08-1101A Fyn 2014	8.9	88.5	79.1	9.4
Quench1 Fyn 2014	7.9	95.0	80.0	15.0
MQF08-1101B Fyn 2014	8.4	91.5	92.0	-0.5
Quench2 Fyn 2014	7.1	95.5	72.0	23.5
MQF08-1101C Fyn 2014	8.5	93.5	94.0	-0.5
Quench3 Fyn 2014	8.5	93.5	83.0	10.5
MQF08-1101A Fyn 2015	7.9	98.0	86.0	12.0
Quench1 Fyn 2015	8.0	100.0	82.0	18.0
MQF08-1101B Fyn 2015	7.5	96.5	66.0	30.5
Quench2 Fyn 2015	7.2	97.0	70.0	27.0
MQF08-1101C Fyn 2015	7.7	97.0	76.0	21.0
Quench3 Fyn 2015	7.5	98.5	69.0	29.5

Table 2

Selected candidate genes in the QTL region

Chr	Gene	Annotation[1]	Comments
2H	HORVU2Hr1G006510	SWEET sugar transporter[2]	
2H	HORVU2Hr1G006520	SWEET sugar transporter[2]	
2H	HORVU2Hr1G006930	shrunken seed protein (SSE1)[3]	
2H	HORVU2Hr1G012130	MBOAT (membrane bound O-acyl transferase) family protein[4]	Not possible to amplify start of the gene
2H	HORVU2Hr1G012460	AGPPase[5] (Adenosine diphosphate glucose pyrophosphatase)[5]	
2H	HORVU2Hr1G012630	Pho2 (alpha-glucan phosphorylase 2)[6]	No full-length gene

[1](Mascher et al. 2017; Finn et al. 2016) [2](Ludewig & Flügge 2013) [3](Lin et al. 1999) [4](Ståhl et al. 2008) [5](Rodriguez-López et al. 2000) [6](Ma et al. 2013)

Table 3

Primers for amplification of sequence containing the identified SNP in the *HvmPGES2 gene*

Primer	Sequence
LMJ221_For	ATTACTGATGAGCGTGCC
LMJ222_Rev	GTTCTCCGACCGCATCTT

Table 4

AA change	Proposed effect
C99Y	Active site Cys
G209D	Destabilise turn/splice site
G223D	Change substrate-binding helix
F263S	Destabilise loop
G265D	The identified mutation
G266D	Destabilise loop
P269L	Destabilise loop
W131	Stop
W175	Stop
W178	Stop
W254	Stop

Table 5

Conservation	Region position in amino acid of *HvmPGES2* (SEQ ID NO:4)
Cys-GST's (absolute conservation ≥90 %)	99-100, 138, 266, 273
Cys-GST's (conservative substitutions allowed, conservation ≥90 %)	99-101, 104,138, 150, 153-154, 157, 258, 263, 266, 273-274, 280, 298, 301, 305
mPGES2 (absolute conservation ≥90 %)	99-102, 104-105, 107-110, 116, 120-123, 128, 130, 132, 134, 136-138, 150, 153-

	154,157, 171-172, 174-176, 178, 180, 183-185, 189-190, 192-193, 197-199, 201-202, 204-205, 209, 211, 215, 220-221, 223-224, 227-228, 232, 234-235, 242, 244, 247-248, 250-251, 254, 257, 261, 265-266, 268-271, 273-275, 278-281, 286, 291, 296, 298, 301-302, 304, 306
mPGES2 (conservative substitutions allowed, conservation ≥90 %)	99-102, 104-111, 114, 116-124, 126, 128-132, 134-140, 143, 145, 147, 149-151, 153-154,157, 161, 171-172, 174-176, 178, 180-181, 183-193, 196-199, 201-205, 209-211, 215, 220-221, 223-235, 237-238, 240, 242, 244, 247-252, 254, 257-258, 261, 263-266, 268-271, 273-275, 277-281, 283, 286, 288, 291-298, 301-302, 304-306, 309, 313
mPGES2 plants (absolute conservation ≥90 %)	99-105, 107-111, 116-117, 120-123, 125-128, 130-132, 134, 136-138, 142-145, 150, 153-154, 157, 171-172, 174-176, 178-180, 182-185, 187, 189-193, 195-202, 204-205, 208-211, 214-216, 220-221, 223-224, 227-228, 230, 232, 234-237, 239-242, 244-245, 247-248, 250-251, 253-254, 257-258, 261, 264-266, 268-271, 273-275, 278-286, 289, 291-293, 296, 298-299, 301-302, 304-306, 310, 314-315
mPGES2 plants (conservative substitutions allowed, conservation ≥90 %)	99-111, 114, 116-140, 142-145, 147-151, 153-155, 157, 160-161, 171-172, 174-176, 178-193, 195-206, 208-211, 213-217, 220-221, 223-242, 244-254, 256-258, 261, 263-266, 268-271, 273-275, 277-286, 288-302, 304-306, 309-310, 313-315

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Claims

1. A barley plant, progeny thereof or a part thereof wherein said barley plant:

- carries a mutation in the *HvmPGES2* gene, wherein said mutation is a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *HvmPGES2* gene (SEQ ID NO: 2); and/or
- comprises a mutant *HvmPGES2* gene encoding a mutant HvmPGES2 protein comprising a mutation of amino acid 265 of SEQ ID NO:4 from Gly to Asp; and/or
- carries a mutation in the *mPGES2* gene, wherein the mutant *mPGES2* gene comprises the sequence SEQ ID NO:8 or a sequence at least 98% identical to SEQ ID NO:8, wherein said sequence comprises at least nucleotide 4657 of SEQ ID NO:8.

2. A barley plant or progeny thereof or a part thereof, wherein the genome of said plant, comprises a genetic element consisting of a genomic region of barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, wherein said genomic region is positioned on chromosome 2H between the genomic position corresponding to 10,497,647 and the genomic position corresponding to 31,153,440 of the barley genome assembly by IBSC of 14-07-2016, wherein said genomic region comprises a mutant *mPGES2* gene carrying a G->A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *mPGES2* gene (SEQ ID NO:2).

3. The barley plant according to any one of the preceding claims, wherein the starch of said barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a barley plant not carrying said mutation, but otherwise identical; and grown under similar conditions.

4. The barley plant according to any one of the preceding claims, wherein starch of said barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of a barley plant of cv. Quench grown under similar conditions.

5. The barley plant according to any one of the preceding claims, wherein said part of the barley plant is kernels.

6. The barley plant according to any one of the preceding claims, with the proviso that the barley plant has not exclusively been obtained by means of an essentially biological process.

7. The barley plant according to any one of the preceding claims, wherein the barley plant has been prepared by a method comprising the following steps or the barley plant is progeny of a plant prepared by a method comprising the following steps:
   - Mutagenizing barley plants or parts thereof, for example with a chemical mutagenizing agent;
   - Selecting barley plants carrying a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *HvmPGES2* gene (SEQ ID NO:2).

8. The barley plant according to any one of the preceding claims, wherein the barley plant is the barley plant MQF08-1101 deposited with NCIMB Ltd. with accession no. NCIMB 43272, or progeny thereof.

9. A plant product comprising the cereal plant according to any one of the preceding claims or a part thereof.

10. The plant product according to claim 9, wherein the plant product is malt comprising processed kernel(s) of said cereal plant.

11. The malt according to claim 9, wherein the malt is milled malt.

12. The malt according to any one of claims 10 to 11, wherein the starch of said malt has an average gelatinisation temperature, which is at least 0.4°C lower than the average gelatinisation temperature of starch of malt of a barley plant not carrying said mutation, but otherwise identical; and grown under similar conditions.

13. The malt according to any one of claims 10 to 12, wherein the cereal plant is a barley plant, and wherein starch of said barley plant has an average gelatinisation temperature, which is at least 0.4°C lower than the average

gelatinisation temperature of starch of a barley plant of cv. Quench grown under similar conditions.

14. The plant product according to claim 8, wherein the plant product is barley flour.

15. The plant product according claim 9, wherein the plant product is wort prepared from kernels of said barley plant and/or from malt comprising processed kernel(s) of said barley plant.

16. The plant product according to claim 9, wherein the plant product is a beverage prepared from said barley plant of parts thereof.

17. The beverage according to claim 16, wherein said beverage is prepared from kernels of said barley plant and/or from malt comprising processed kernel(s) of said barley plant.

18. The beverage according to any one of claims 16 to 17, wherein the beverage is beer.

19. A method of preparing malt, said method comprising the steps of

(i) providing kernels of a barley plant according to any one of claims 1 to 8;
(ii) steeping said kernels;
(iii) germinating the steeped kernels under predetermined conditions;
(iv) drying said germinated kernels.

20. The method according to claim 18, wherein the malt is a malt according to any one of claims 10 to 13.

21. A method of producing a beverage, said method comprising the steps of:

(i) Providing kernels of a barley plant according to any one of claims 1 to 8 and/or malt according to any one of claims 10 to 13
(ii) Preparing an aqueous extract of said kernels and/or said malt
(iii) processing said aqueous extract into a beverage.

22. The method according to claim 21, wherein the method comprises a step of incubating said kernels and/or said malt in an aqueous suspension at a temperature, which is higher than the average gelatinisation temperature of said kernels or said malt.

23. The method according to claim 21, wherein step (iii) comprises fermentation of said aqueous extract.

24. A method of preparing a barley plant containing starch with a low gelatinisation temperature, the method comprising the steps of

a) providing barley plants; and

b) introducing a mutation in the *mPGES2* gene, for example by mutagenesis with a chemical mutagenizing agent; and

c) Selecting barley plants carrying a G-> A mutation of the nucleotide corresponding to nucleotide 794 of the coding sequence of the *HvmPGES2* gene (SEQ ID NO:2).

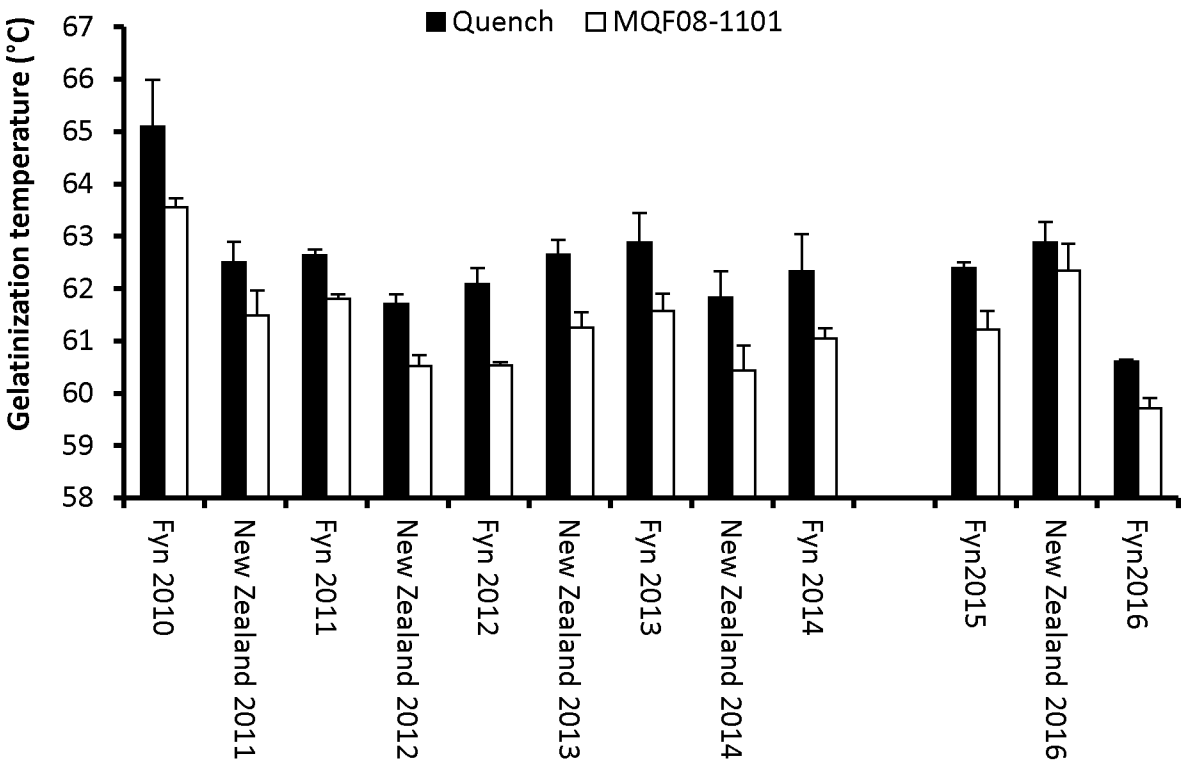


Fig. 1

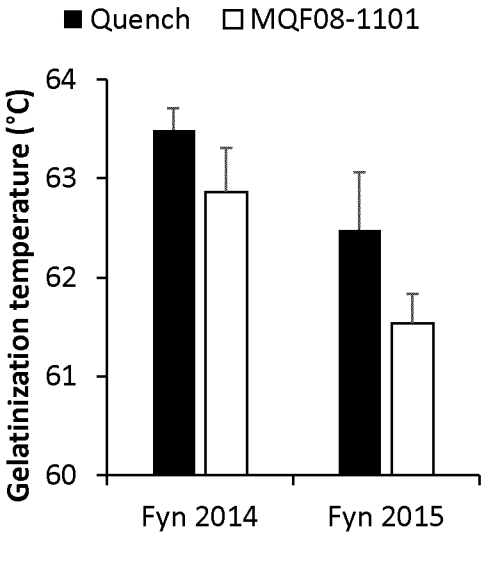


Fig. 2

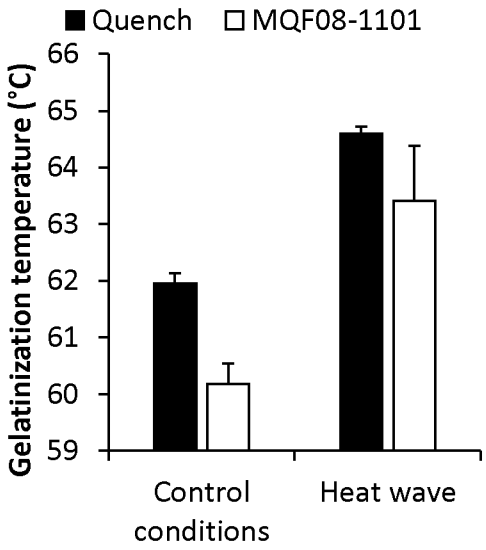


Fig. 3

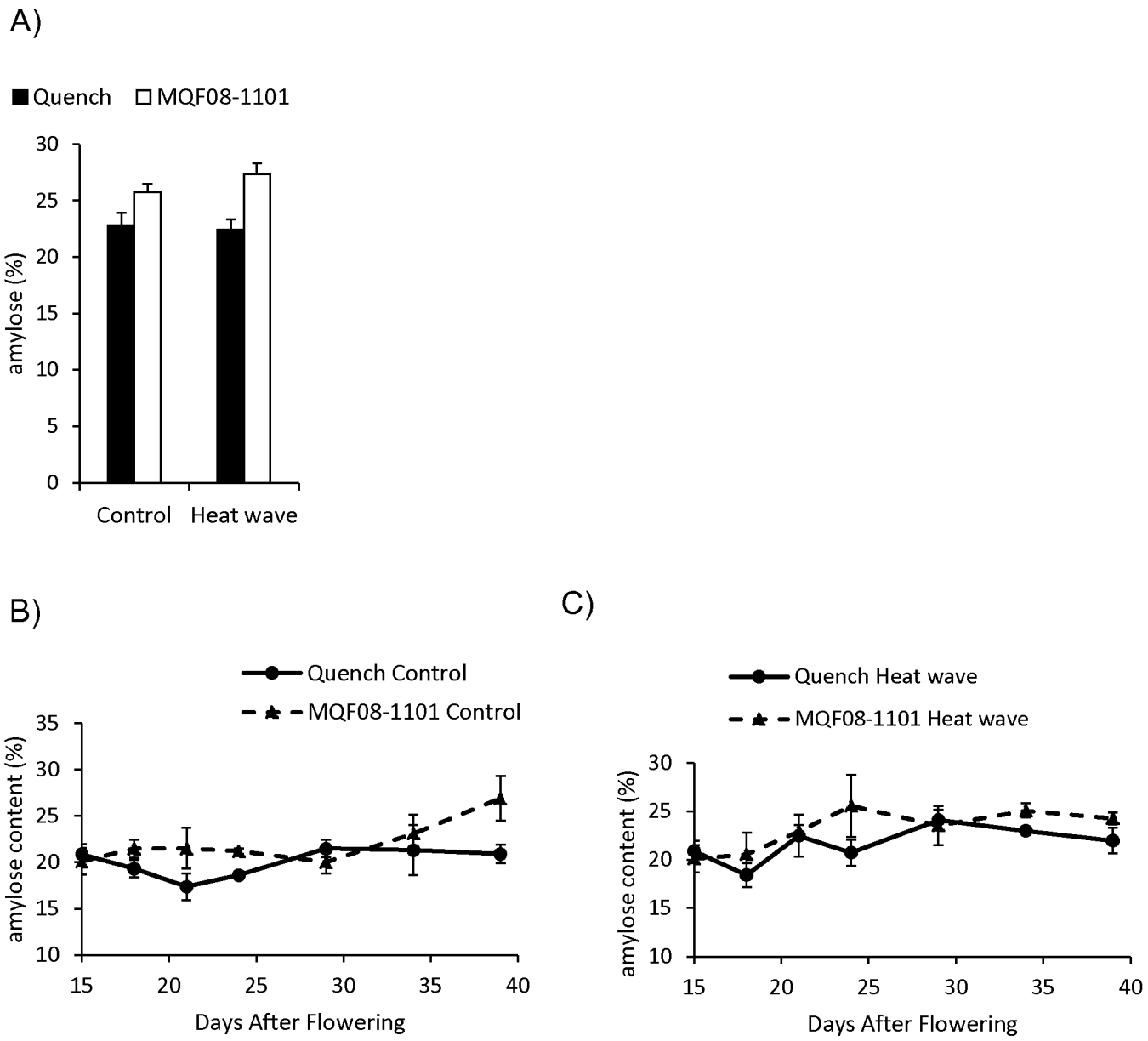

A)
Quench
MQF08-1101
amylose (%)
30
25
20
15
10
5
0
Control
Heat wave
B)
Quench Control
MQF08-1101 Control
amylose content (%)
35
30
25
20
15
10
15
20
25
30
35
40
Days After Flowering
C)
Quench Heat wave
MQF08-1101 Heat wave
amylose content (%)
30
25
20
15
10
15
20
25
30
35
40
Days After Flowering

Fig. 4

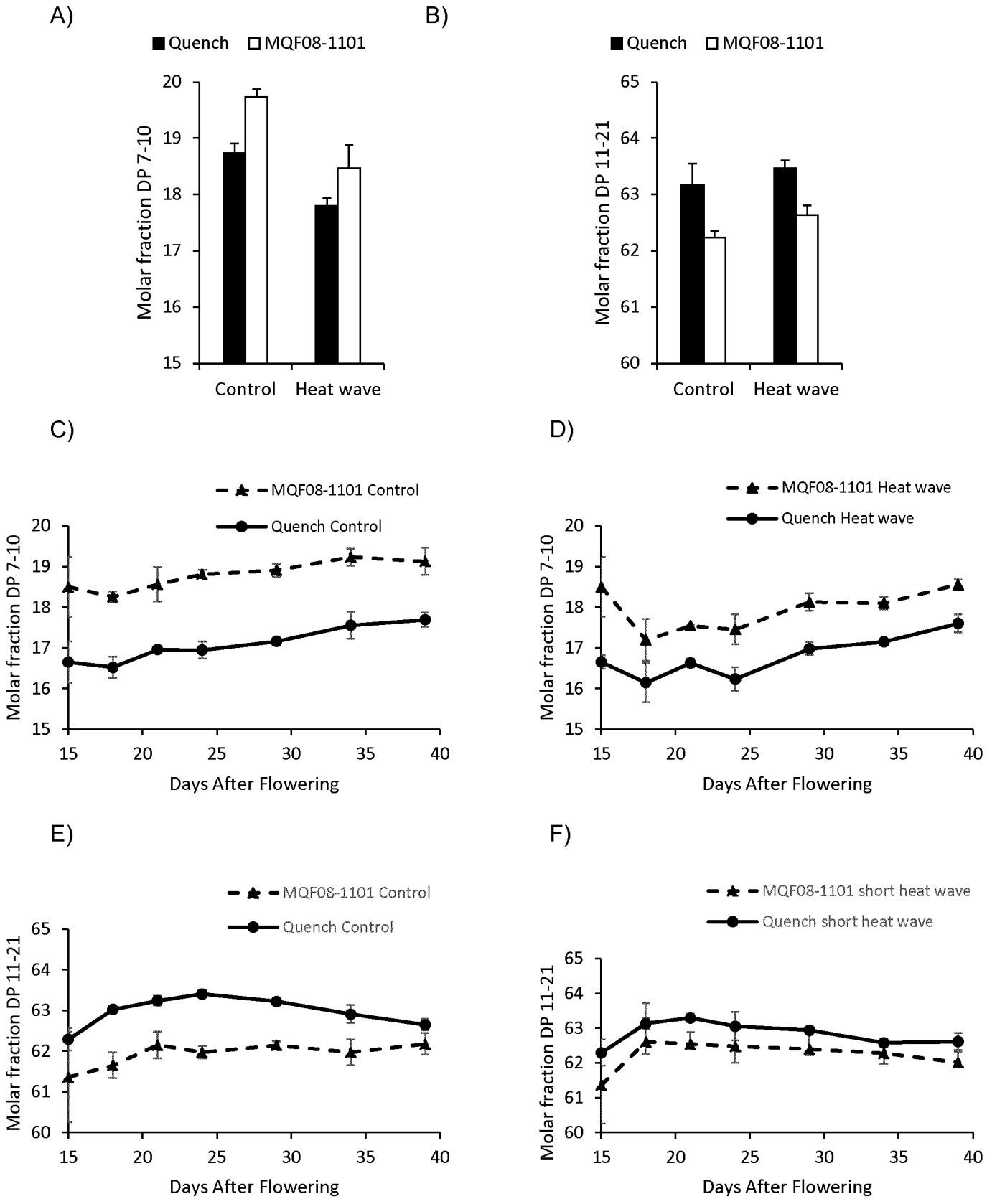

A)
Quench
MQF08-1101
Molar fraction DP 7-10
20
19
18
17
16
15
Control
Heat wave
B)
Quench
MQF08-1101
Molar fraction DP 11-21
65
64
63
62
61
60
Control
Heat wave
C)
MQF08-1101 Control
Quench Control
Molar fraction DP 7-10
Days After Flowering
D)
MQF08-1101 Heat wave
Quench Heat wave
Molar fraction DP 7-10
Days After Flowering
E)
MQF08-1101 Control
Quench Control
Molar fraction DP 11-21
Days After Flowering
F)
MQF08-1101 short heat wave
Quench short heat wave
Molar fraction DP 11-21
Days After Flowering

Fig. 5

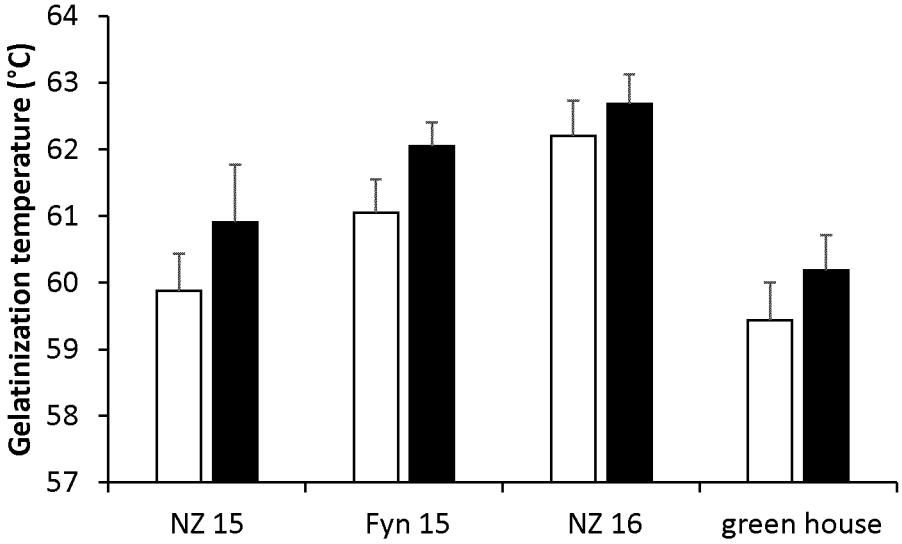

Gelatinization temperature (°C)
64
63
62
61
60
59
58
57
NZ 15
Fyn 15
NZ 16
green house

Fig. 6

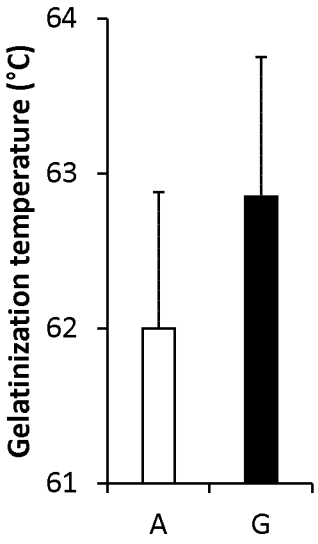

Gelatinization temperature (°C)
64
63
62
61
A
G

Fig. 7

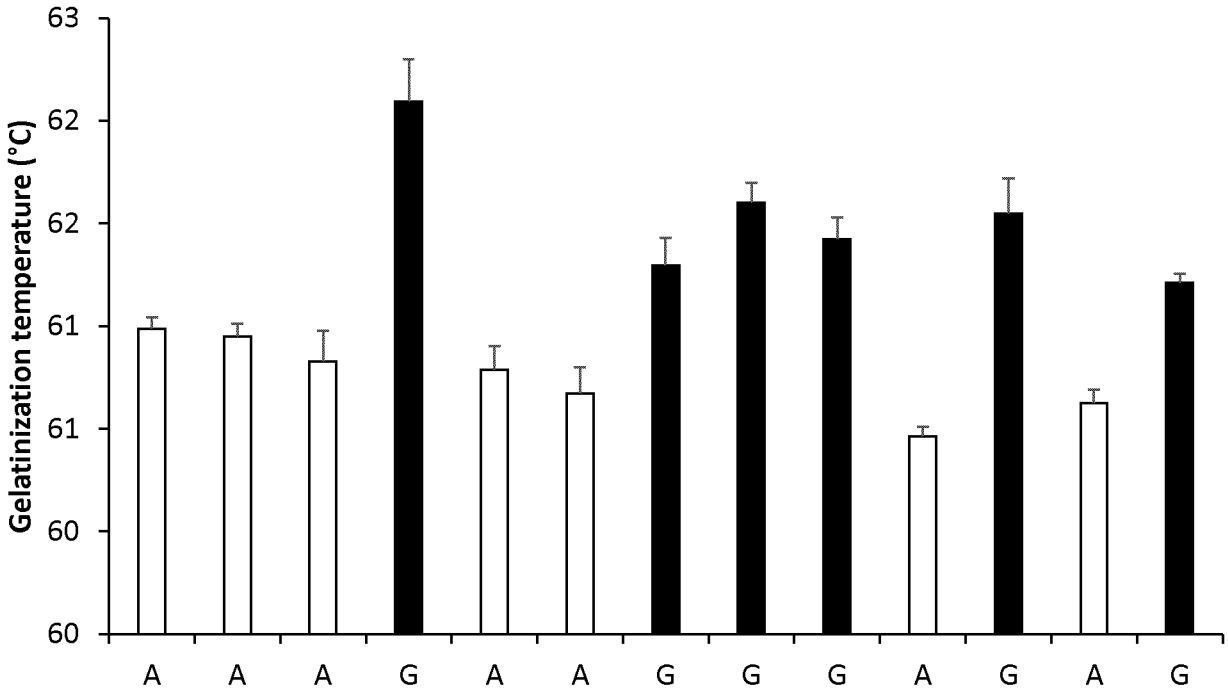

Gelatinization temperature (°C)
63
62
62
61
61
60
60
A A A G A A G G G A G A G

Fig. 8



Fig. 9

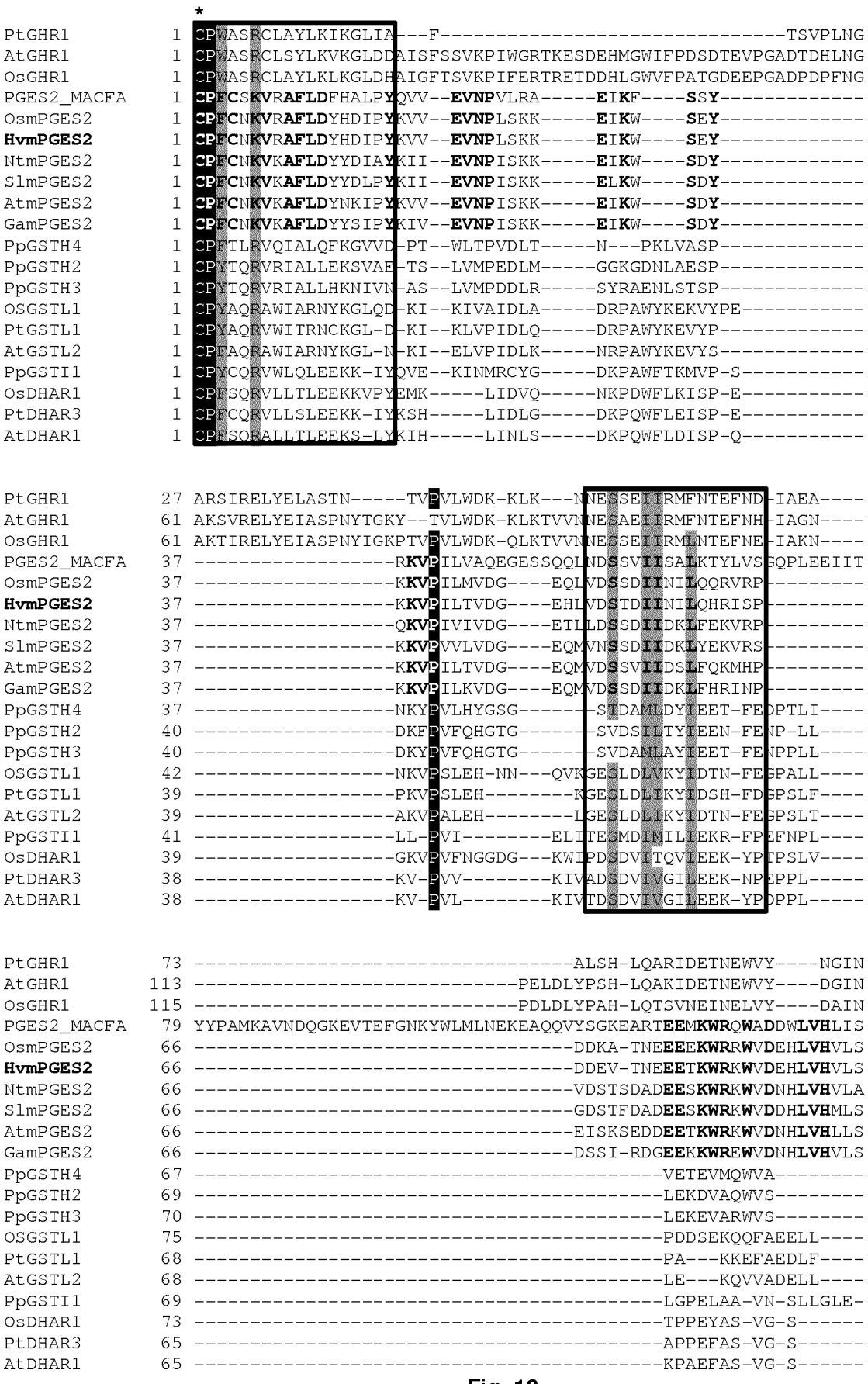


Fig. 10

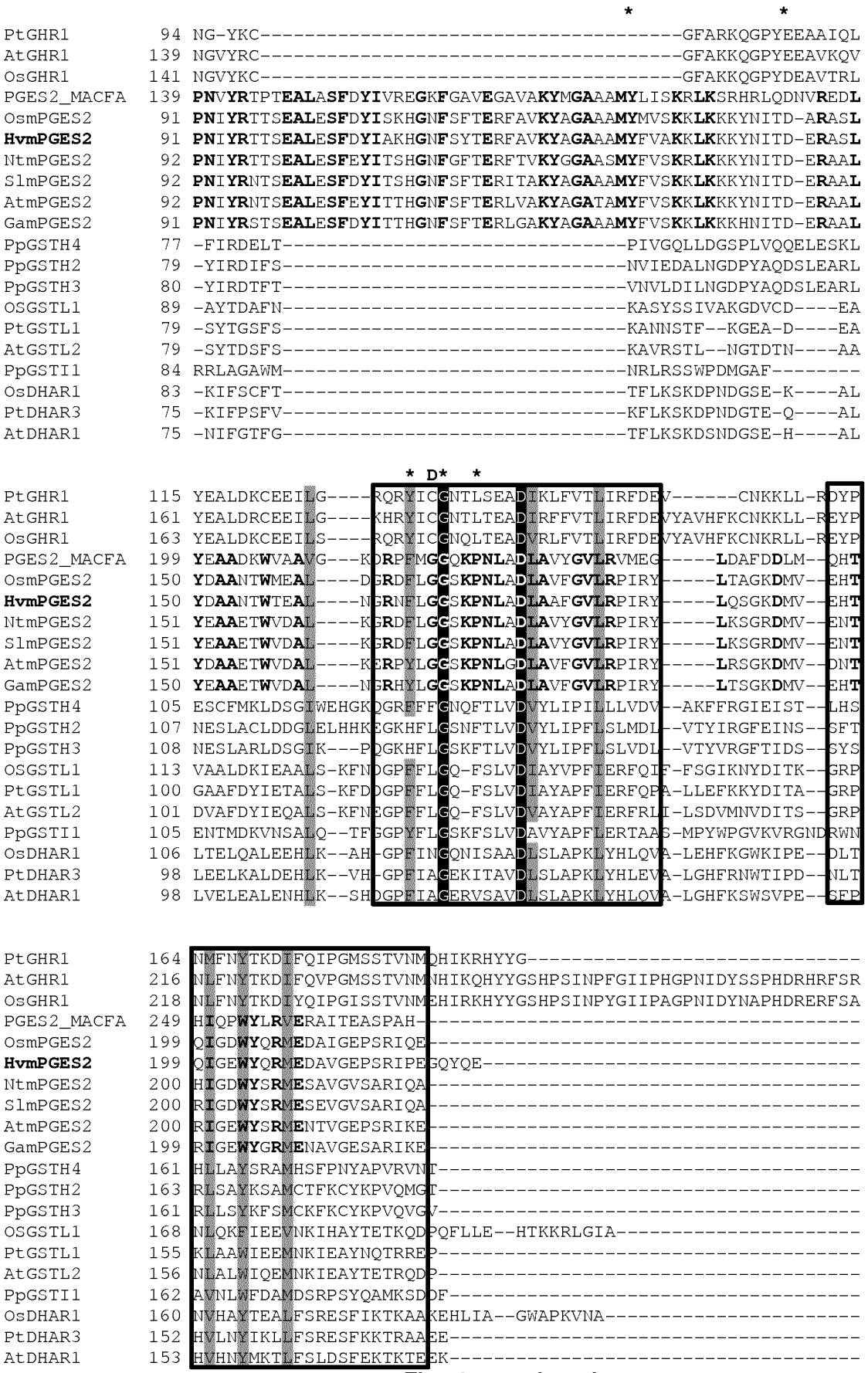


Fig 10 - continued

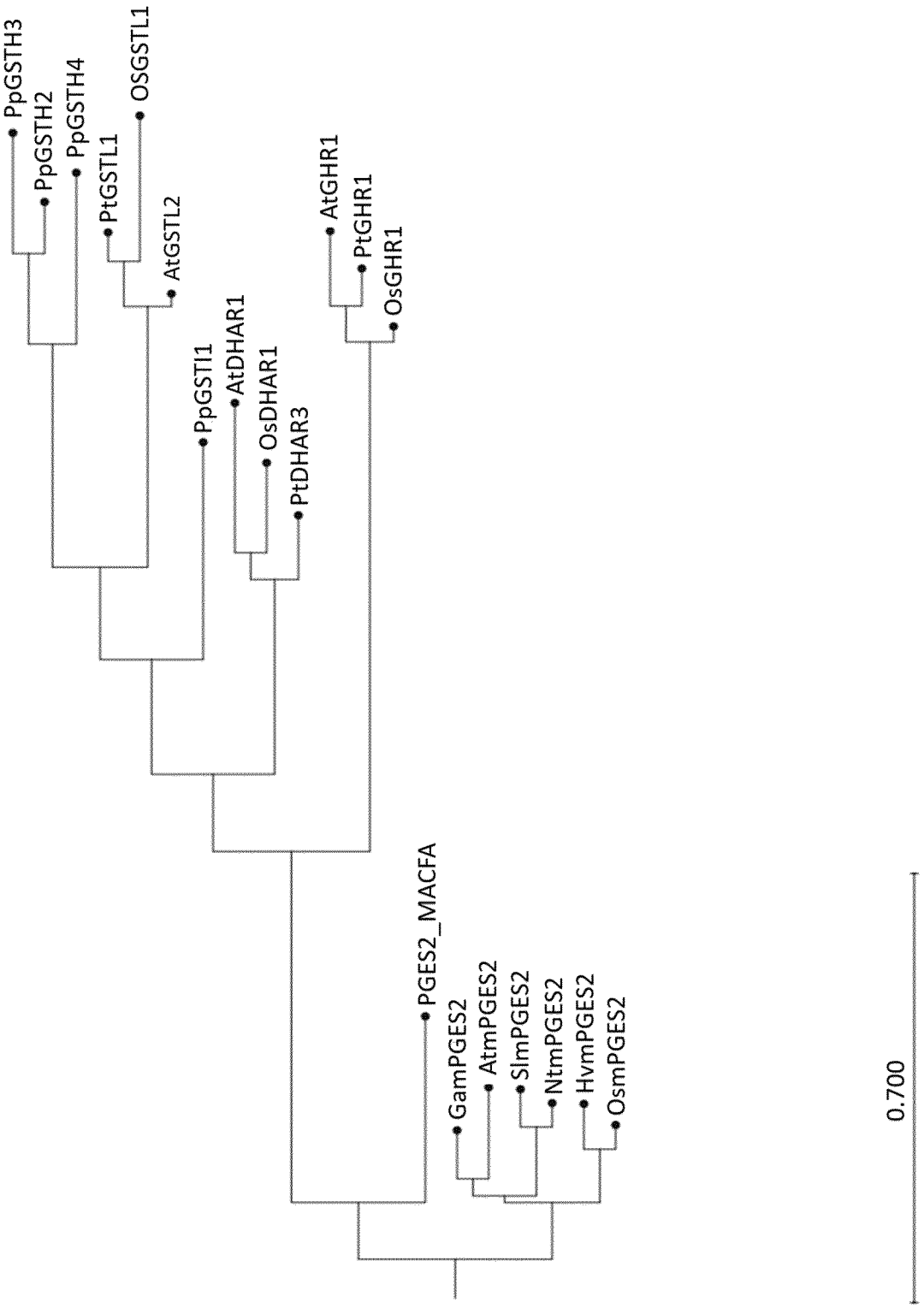

PpGSTH3
PpGSTH2
PpGSTH4
PtGSTL1
OSGSTL1
AtGSTL2
PpGSTl1
AtDHAR1
OsDHAR1
PtDHAR3
AtGHR1
PtGHR1
OsGHR1
PGES2_MACFA
GamPGES2
AtmPGES2
SlmPGES2
NtmPGES2
HvmPGES2
OsmPGES2
0.700

Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/050149

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H5/10 C07K14/415 C12C1/18 A01H6/46
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C07K C12C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MORELL M K ET AL: "Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties", THE PLANT JOURNAL, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 34, no. 2, 1 April 2003 (2003-04-01), pages 173-185, XP002346241, ISSN: 0960-7412, DOI: 10.1046/J.1365-313X.2003.01712.X abstract ----- -/--	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 February 2019	26/02/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mundel, Christophe

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LALLEMENT PIERRE-ALEXANDRE ET AL: "The still mysterious roles of cysteine-containing glutathione transferases in plants", FRONTIERS IN PHARMACOLOGY, vol. 5, 20 August 2014 (2014-08-20), XP002788751, abstract table 2 page 9, left-hand column, line 16 - line 28 -----	1-23
A	DATABASE UniProt [Online] 31 May 2011 (2011-05-31), "SubName: Full=Predicted protein {ECO:0000313¦EMBL:BAK04530.1};", XP002788752, retrieved from EBI accession no. UNIPROT:F2EB08 Database accession no. F2EB08 the whole document -----	1-23