

CLAIMS

1. Grain of a rice plant, the grain comprising an aleurone, a starchy endosperm, a *ROS1a* gene encoding a ROS1a polypeptide and (i) one or more genetic variations
5 which each reduce the activity of at least one *ROS1a* gene in the plant when compared to a corresponding wild-type rice plant, and/or (ii) said aleurone is thickened compared to aleurone from a corresponding wild-type grain.
2. The grain of claim 1, which is further characterised by one or more or all of:
10 (a) the ROS1a polypeptide has DNA glycosylase activity;
(b) the ROS1a polypeptide is a variant of a corresponding wild-type ROS1a polypeptide in that their amino acid sequences are different;
(c) the ROS1a polypeptide has a level of DNA glycosylase activity which is between 2% and about 60% of the level of DNA glycosylase activity of a
15 corresponding wild-type ROS1a polypeptide and/or of ROS1a polypeptide whose amino acids have a sequence set forth in SEQ ID NO: 2;
(d) a level of ROS1a polypeptide between 2% and about 60% is present in the grain compared to the level of ROS1a polypeptide in the corresponding wild-type grain; and
20 (e) the thickened aleurone comprises at least two, at least three, at least four or at least five layers of cells, about 3, about 4, about 5 or about 6 layers of cells, or 2-8, 2-7, 2-6 or 2-5 layers of cells.
3. The grain of claim 1 or claim 2, wherein the one or more genetic variations are
25 each, independently:
(a) a *ROS1a* gene encoding a mutant ROS1a polypeptide with reduced DNA glycosylase activity relative to the wild-type ROS1a polypeptide (SEQ ID NO:2);
(b) a *ROS1a* gene which when expressed produces a reduced level of a wild-type ROS1a polypeptide, for example which comprises a splice-site mutation that
30 results in a reduced level of expression of the *ROS1a* gene, relative to the wild-type *ROS1a* gene whose cDNA sequence is provided as SEQ ID NO:8, or which *ROS1a* gene comprises a mutation in its promoter which results in reduced expression of the *ROS1a* gene relative to the wild-type *ROS1a* gene;
(c) an exogenous nucleic acid construct which encodes a polynucleotide which
35 reduces expression of a *ROS1a* gene in the rice plant, preferably wherein the nucleic acid construct comprises a DNA region encoding the polynucleotide operably linked to

a promoter which is expressed in developing grain of the rice plant at least at a time point between the time of anthesis and 7 days post-anthesis, and

(d) a *ROS1a* gene comprising a premature translational stop codon in its protein coding region such that the gene encodes a truncated polypeptide relative to a wild-type

5 *ROS1a* polypeptide,

preferably wherein the genetic variations comprise an introduced genetic variation.

4. The grain according to any one of claims 1 to 3, wherein the rice plant is characterised by one or more or all of:

10 (a) the rice plant has a level of DNA glycosylase activity in its developing grain which is between 2% and about 60% of the level of DNA glycosylase activity in a corresponding wild-type developing grain;

(b) the activity of at least one *ROS1a* gene in the rice plant is reduced in one or more or all of aleurone, pericarp, nucellar projection, ovary, testa and starchy
15 endosperm of the developing grain;

(c) the activity of a *ROS1a* gene is reduced at least at a time point between the time of anthesis and 7 days post-anthesis, and/or in the egg cell prior to anthesis;

(d) the rice plant is male and female fertile; and

(e) the rice plant exhibits delayed grain maturation.

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5. The grain according to any one or more of claims 1 to 4, which is further characterised by one or more of:

(a) the grain comprises, when compared to a corresponding wild-type grain, one or more or all of the following, each on a weight basis,

25 i) a higher mineral content, preferably the mineral content is the content of one or more or all of zinc, iron potassium, magnesium, phosphorus and sulphur,

ii) a higher antioxidant content,

iii) a higher phytate content,

iv) a higher content of one or more or all of vitamins B3, B6 and B9,

30 v) a higher dietary fibre content and/or insoluble fibre content,

vi) a starch content which is between about 90% and about 100% by weight relative to the starch content of the corresponding wild-type grain;

vii) a higher sucrose content,

viii) a higher monosaccharide content, and

35 ix) a lipid content of at least 90% or at least 100% relative to the lipid content of the corresponding wild-type grain,

- (b) the grain comprises an embryo;
 - (c) the grain is whole grain or cracked grain;
 - (d) the grain has been processed so that it is no longer able to germinate, preferably by heat treatment;
 - 5 (e) the grain has a germination rate which is between about 70 and about 100% relative to the germination rate of a corresponding wild-type grain;
 - (f) the grain comprises an increased proportion of amylose in its total starch content compared to the corresponding wild-type grain; and
 - (g) the grain comprises an increased proportion of oleic acid and/or a decreased
 - 10 proportion of palmitic acid in its total fatty acid content compared to the corresponding wild-type grain.
6. The grain according to any one or more of claims 1 to 5, which is characterised by one or more or all of:
- 15 (a) the grain comprises a *ROS1a* gene which encodes a ROS1a polypeptide which has DNA glycosylase activity, preferably in one or more of aleurone, testa and starchy endosperm of the grain, wherein the ROS1a polypeptide which has DNA glycosylase activity is preferably a mutant ROS1a polypeptide;
- (b) the grain comprises a mutant ROS1a polypeptide having decreased DNA
- 20 glycosylase activity when expressed in the rice plant compared to a corresponding wild-type ROS1a polypeptide, preferably wherein the mutant ROS1a polypeptide comprises one or more amino acid substitutions, deletions or insertions which reduces DNA glycosylase activity compared to the corresponding wild-type ROS1a polypeptide;
- 25 (c) the grain has a reduced total amount of ROS1a polypeptide compared to a corresponding wild-type grain, preferably reduced in one or more of aleurone, testa and starchy endosperm of the grain, provided that the grain comprises at least one *ROS1a* gene which encodes a ROS1a polypeptide which has DNA glycosylase activity; and
- (d) the genetic variation is an exogenous nucleic acid construct which encodes a
- 30 polynucleotide which reduces expression of a *ROS1a* gene in the rice plant, preferably reduced in the rice plant at least at a time point between the time of anthesis and 7 days post-anthesis, provided that the grain comprises at least one *ROS1a* gene which encodes a ROS1a polypeptide which has DNA glycosylase activity.
- 35 7. The grain according to any one of claims 1 to 6, which is pigmented in its outer layer(s).

8. The grain of claim 7, which is;
- (a) brown grain or black grain of a rice plant, the grain comprising (i) an aleurone having a thickness of at least 2 cell layers, or 2-7 cell layers, and (ii) a mutant *ROS1a* gene which encodes a ROS1a polypeptide which comprises one or more amino acid substitutions, insertions or deletions which reduces DNA glycosylase activity when compared to a corresponding wild-type rice ROS1a polypeptide, the reduced DNA glycosylase activity occurring in the rice plant at least at a time point between the time of anthesis and 7 days post-anthesis, provided that at least at a time point between the time of anthesis and 7 days post-anthesis the rice plant has between 2% and about 60% of the level of DNA glycosylase activity in developing grain compared to the wild-type rice plant; or
- (b) brown grain or black grain of a rice plant, the grain comprising (i) an aleurone having a thickness of at least 2 cell layers, or 2-7 cell layers, and (ii) an exogenous nucleic acid construct which encodes a polynucleotide which reduces expression of a *ROS1a* gene in the rice plant, wherein the exogenous nucleic acid construct comprises a DNA region encoding the polynucleotide operably linked to a promoter which is expressed in developing grain of the rice plant at least at a time point between the time of anthesis and 7 days post-anthesis, such that at least at a time point between the time of anthesis and 7 days post-anthesis the rice plant has between 2% and about 60% of the level of DNA glycosylase activity in the developing grain compared to the wild-type rice plant.
9. The grain according to any one of claims 1 to 8, wherein the ROS1a polypeptide comprises amino acids whose sequence is at least 95% identical to SEQ ID NO: 2, or the ROS1a polypeptide(s) comprises amino acids whose sequence is at least 95% identical to SEQ ID NO: 2 and which sequence is different to the amino acid sequence of the corresponding wild-type ROS1a polypeptide.
10. A purified and/or recombinant ROS1a polypeptide whose amino acid sequence is different to the amino acid sequence of a corresponding wild-type ROS1a polypeptide and which has reduced, preferably no, DNA glycosylase activity when compared to the corresponding wild-type ROS1a polypeptide.
11. The polypeptide of claim 10 which comprises amino acids having a sequence which is at least 95% identical to SEQ ID NO: 2.

12. An isolated and/or exogenous polynucleotide encoding a ROS1a polypeptide of claim 10 or claim 11.
- 5 13. An isolated and/or exogenous polynucleotide which, when present in a rice plant, reduces the expression of a *ROS1a* gene.
14. The polynucleotide of claim 13, when used for reducing the expression of a *ROS1a* gene in developing grain of a rice plant at least at a time point between the time
10 of anthesis and 7 days post-anthesis.
15. A nucleic acid construct and/or vector encoding a polynucleotide according to any one of claims 12 to 14, wherein the nucleic acid construct or vector comprises a DNA region encoding the polynucleotide operably linked to a promoter which is
15 expressed in developing grain of a rice plant at least at a time point between the time of anthesis and 7 days post-anthesis.
16. A recombinant cell comprising an exogenous polynucleotide according to any one of claims 12 to 14, or a nucleic acid construct and/or vector of claim 15.
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17. The cell of claim 16, wherein the exogenous polynucleotide, nucleic acid construct or vector is integrated into the genome of the cell, preferably into the nuclear genome.
- 25 18. A cell of a rice plant comprising a *ROS1a* gene encoding a ROS1a polypeptide and a genetic variation which reduces the activity of at least one *ROS1a* gene in the cell when compared to a corresponding wild-type cell.
19. The cell of claim 18 which is an aleurone, pericarp, nucellar projection, ovary,
30 testa or starchy endosperm cell.
20. The cell of claim 19 which is an aleurone cell.
21. A rice plant which produces grain according to any one of claims 1 to 9, a
35 polypeptide of claim 10 or claim 11, a polynucleotide according to any one of claims

12 to 13, a nucleic acid construct and/or vector of claim 15 and/or which comprises a cell according to any one of claims 16 to 20.

22. A population of at least 100 rice plants of claim 21 growing in a field.

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23. A method of producing the cell according to any one of claims 16 to 20, the method comprising a step of introducing an exogenous polynucleotide according to any one of claims 12 to 14, or a nucleic acid construct and/or vector of claim 15, into a cell, preferably a rice cell.

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24. A method of producing a rice plant of claim 21 or transgenic grain therefrom, the method comprising the steps of

i) introducing into a rice cell, an exogenous polynucleotide according to any one of claims 12 to 14, or a nucleic acid construct and/or vector of claim 15,

15 ii) obtaining a transgenic rice plant from a cell obtained from step i), the transgenic rice plant being transgenic for the exogenous polynucleotide, nucleic acid construct or vector, and

iii) optionally harvesting grain from the plant of step ii), the grain being transgenic for the exogenous polynucleotide, nucleic acid construct or vector, and

20 iv) optionally producing one or more generations of transgenic progeny plants from the transgenic grain, the progeny plants being transgenic for the exogenous polynucleotide, nucleic acid construct or vector, thereby producing the rice plant or transgenic grain.

25 25. A method of producing a rice plant of claim 21 or grain therefrom, the method comprising the steps of

i) introducing into a rice cell, a mutation of an endogenous *ROS1a* gene such that the mutated *ROS1a* gene encodes a ROS1a polypeptide as defined in claim 9 or claim 10, or does not encode a ROS1a polypeptide,

30 ii) obtaining a rice plant from a cell obtained from step i), the rice plant comprising the mutation of the endogenous *ROS1a* gene, and

iii) optionally harvesting grain from the plant of step ii), the grain comprising the mutation of the endogenous *ROS1a* gene, and

35 iv) optionally producing one or more generations of progeny plants from the grain, the progeny plants comprising the mutation of the endogenous *ROS1a* gene, thereby producing the rice plant or grain.

26. The method of claim 25, wherein the rice plant or grain comprises at least one *ROS1a* gene which encodes a ROS1a polypeptide which has DNA glycosylase activity.
- 5 27. A method of selecting a rice plant of claim 21 or rice grain according to any one of claims 1 to 9, the method comprising the steps of
- i) screening a population of rice plants or grain each of which were obtained from a mutagenic treatment of progenitor rice cells, grain or plants, for the production of grain according to any one of claims 1 to 9 or for the presence of a mutation in a
 - 10 *ROS1a* gene, or the presence of rice grain according to any one of claims 1 to 9, and
 - ii) selecting from the population of step (i) a rice plant which produces grain according to any one of claims 1 to 9 or which comprises a mutant *ROS1a* gene, or rice grain of step (i) which is rice grain according to any one of claims 1 to 9, thereby selecting the rice plant or grain.
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28. A method of selecting a rice plant of claim 21, the method comprising the steps of
- i) producing one or more progeny plants from rice grain, the rice grain having been derived from a cross of two parental rice plants,
 - 20 ii) screening the one or more progeny plants of step i) for the production of grain according to any one of claims 1 to 9, and
 - iii) selecting a progeny plant which produces the grain, thereby selecting the rice plant.
- 25 29. The method of claim 28, wherein step ii) comprises one or more or all of:
- i) analysing a sample comprising DNA from a progeny plant for the genetic variation,
 - ii) analysing the thickness of aleurone of grain obtained from a progeny plant, and
 - 30 iii) analysing the nutritional content of the grain or a part thereof.
30. The method of claim 28, wherein step iii) comprises one or more or all of:
- i) selecting a progeny plant which is homozygous for the genetic variation, wherein the genetic variation reduces DNA glycosylase activity in the rice plant when
 - 35 compared to a corresponding wild-type rice plant,

ii) selecting a progeny plant whose grain has an increased aleurone thickness compared to a corresponding wild-type grain,

iii) selecting a progeny plant whose grain or a part thereof has an altered nutritional content compared to a corresponding wild-type grain or part thereof.

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31. The method according to any one of claims 28 to 30 which further comprises

i) crossing two parental rice plants, preferably wherein one of the parental rice plants produces grain according to any one of claims 1 to 9, or

10 ii) backcrossing one or more progeny plants from step i) with plants of the same genotype as a first parental rice plant which does not produce grain according to any one of claims 1 to 9 for a sufficient number of times to produce a plant with a majority of the genotype of the first parental rice plant but which produces grain according to any one of claims 1 to 9, and

15 iii) selecting a progeny plant which produces grain according to any one of claims 1 to 9.

32. A rice plant produced using the method according to any one of claims 27 to 31.

20 33. Use of an exogenous polynucleotide according to any one of claims 12 to 14, or a nucleic acid construct and/or vector of claim 15, to produce a recombinant cell, a transgenic rice plant or transgenic grain.

34. The use of claim 33 when used to produce rice grain according to any one of claims 1 to 9.

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35. A method for identifying a rice plant which produces grain according to any one of claims 1 to 9, the method comprising the steps of

i) obtaining a nucleic acid sample from a rice plant, and

30 ii) screening the sample for the presence or absence of a genetic variation which reduces the activity of a *ROS1a* gene in the plant when compared to a corresponding wild-type rice plant.

36. The method of claim 35, wherein the genetic variation is one or both of

35 a) a nucleic acid construct expressing a polynucleotide, or the polynucleotide encoded thereby, which when present in a rice plant reduces the expression of a *ROS1a* gene, and

b) a gene, or mRNA encoded thereby, which expresses a mutant ROS1a polypeptide with reduced ROS1a polypeptide activity.

37. The method of claim 35 or claim 36, wherein the presence of the genetic
5 variation indicates that grain of the rice plant has a thickened aleurone when compared to a corresponding rice plant lacking the genetic variation(s).

38. A method for identifying a rice plant which produces grain according to any one of claims 1 to 9, the method comprising the steps of
10 i) obtaining grain from a rice plant, and
ii) screening the grain or a portion thereof for one or more of
a) a thickened aleurone,
b) the amount of ROS1a polypeptide and/or activity in the grain, and
c) the amount of mRNA encoded by *ROS1a* genes in the grain.

15 39. The method of according to any one of claims 35 to 38 which identifies a rice plant of claim 21 or claim 31.

40. A method of producing a rice plant part, the method comprising,
20 a) growing a rice plant, or at least 100 such rice plants in a field, of claim 21 or claim 32, and
b) harvesting the rice plant part from the rice plant or rice plants.

41. A method of producing rice flour, bran, wholemeal, malt, starch or oil obtained
25 from grain, the method comprising;
a) obtaining grain according to any one of claims 1 to 9, and
b) processing the grain to produce the flour, bran, wholemeal, malt starch or oil.

42. A product produced from grain according to any one of claims 1 to 9, or a rice
30 plant of claim 21 or claim 32, or from a part of said grain or rice plant.

43. The product of claim 42 comprising one or more or all of the *ROS1a* gene, the genetic variation, the exogenous nucleic acid construct and the thickened aleurone.

35 44. The product of claim 42 or claim 43, wherein the part is bran.

45. The product according to any one of claims 42 to 44, wherein the product is a food ingredient, beverage ingredient, food product or beverage product.

46. The product of claim 45, wherein

- 5 i) the food ingredient or beverage ingredient is selected from the group consisting of wholemeal, flour, bran, starch, malt and oil,
- ii) the food product is selected from the group consisting of: leavened or unleavened breads, pasta, noodles, animal fodder, breakfast cereals, snack foods, cakes, pastries and foods containing a flour-based sauce, or
- 10 iii) the beverage product is a packaged beverage or a beverage comprising ethanol.

47. A method of preparing a food or beverage ingredient of claim 45 or claim 46, the method comprising processing grain according to any one of claims 1 to 9, or bran, 15 flour, wholemeal, malt, starch or oil from the grain, to produce the food or beverage ingredient.

48. A method of preparing a food or beverage product of claim 45 or claim 46, the method comprising mixing grain according to any one of claims 1 to 9, or bran, flour, 20 wholemeal, malt, starch or oil from the grain, with another food or beverage ingredient.

49. Use of grain according to any one of claims 1 to 9 or part thereof, or a rice plant of claim 21 or claim 32 or part thereof, as animal feed or food, or to produce feed for animal consumption or food for human consumption.

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50. A composition comprising one or more of a polypeptide of claim 10 or claim 11, a polynucleotide according to any one of claims 12 to 14, a nucleic acid construct and/or vector of claim 15, or a cell according to any one of claims 16 to 20, and one or more acceptable carriers.

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