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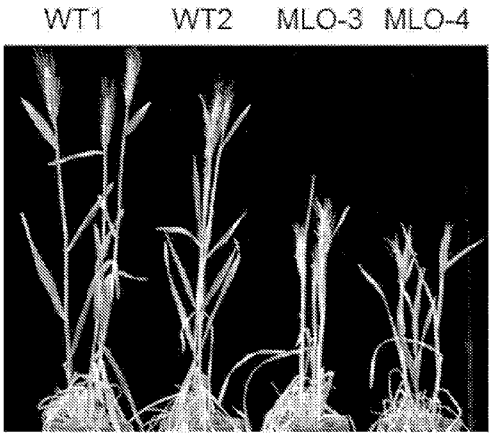

Figure 1

(57) Abstract: The present invention relates to conferring enhanced pathogen resistance in wheat plants using targeted genome modification.

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Modified plants

Field of the invention

The present invention relates to conferring pathogen resistance in wheat plants.

Introduction

In plants, resistance to pathogens is frequently triggered by a recognition event followed by a coordinated complex defence response resulting in localized containment of the intruder.

Powdery mildew (Pm) is one of the most important cereal diseases worldwide. The powdery mildew disease, caused by obligate biotrophic ascomycete fungi of the order *Erysiphales*, is a major impediment for cereal (e.g. wheat and barley) agriculture in temperate climates. Powdery mildew in wheat is caused by the infection of *Blumeria graminis f. sp. tritici* (Bgt) (also called *Erysiphe graminis f. sp. tritici*).

MLO proteins function as negative regulators of plant defence to powdery mildew disease[25]. Loss-of-function *mlo* alleles in barley[26, 40] *Arabidopsis*[27] and tomato[28] lead to broad-spectrum and durable resistance to the fungal pathogen causing powdery mildew.

Resistance responses to the powdery mildew pathogen have been genetically well characterized. In most analyzed cases resistance is specified by race-specific resistance genes following the rules of Flor's gene-for-gene hypothesis. In this type of plant-pathogen interactions, resistance is specified by and dependent on the presence of two complementary genes, one from the host and one from the fungal pathogen. The complementary genes have been termed operationally (pathogen) resistance ("R") gene and avirulence (“Avr”) gene, respectively. Most of the powdery mildew resistance genes (Mlx) act as dominant or semidominant traits.

However, monogenic resistance mediated by recessive (mlo) alleles of the Mlo locus is different. Apart from being recessive, it differs from race-specific resistance to single pathogen strains in that it confers broad spectrum resistance to almost all known

isolates of the pathogen and *mlo* resistance alleles exhibit a defence mimic phenotype in the absence of the pathogen. Thus, the genetic data indicate that the Mlo wild type allele exerts a negative regulatory function on defence responses to pathogen attack (WO98/04586).

Bread wheat (*Triticum aestivum L.*, 2n = 42, AABBDD) is a major staple crop worldwide and provides about 20% of all calories consumed by humans. Because of its economic importance, new traits have always been sought to improve yield, quality and adaptation to biotic and abiotic stresses, mostly through classical breeding. Bread wheat is an allohexaploid, with three similar but not identical copies of most of its genes[5]. Its large genome (17,000 megabases), high ploidy level and high content of repetitive DNA (80% to 90%) make it one of the most challenging species for forward and reverse genetics studies[6].

In wheat, powdery mildew is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), and is one of the most destructive diseases worldwide. Modification of *MLO* genes in wheat may provide the opportunity to breed varieties with broad-spectrum and durable resistance to *Bgt*. In bread wheat, there are three *MLO* homoeologs (*TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*), which are 98% and 99% identical at the nucleotide and protein levels, respectively[29]. *TaMLO-B1* can rescue the resistance of a barley *mlo* mutant to powdery mildew disease, indicating that the function of these *MLO* genes has been conserved during evolution[29]. However, to date, no spontaneous or and induced *mlo* mutants have been reported in bread wheat, probably because of its hexaploid nature and the inherent difficulty in mutating all three *MLO* homoeoalleles. Moreover, no successful progress has been made with transgenic approaches to downregulating *MLO* in wheat. Broad spectrum resistance to powdery mildew is a resistance trait that has not been found in the natural wheat population[4]. Therefore, there is a significant need to develop wheat genotypes that are resistant to Pm.

Recently, genome editing techniques have emerged as alternative methods to conventional mutagenesis methods (such as physical and chemical mutagenesis) or methods using the expression of transgenes in plants to produce mutant plants with improved phenotypes that are important in agriculture. These techniques employ sequence-specific nucleases (SSNs)[1] including zinc finger nucleases (ZFNs)[7], rare-cutting endonucleases, for example transcription activator-like effector nucleases

(TALENs[2]), and the RNA-guided nuclease Cas9 (CRISPR/Cas9)[41,38,3], which generate targeted DNA double-strand breaks (DSBs), which are then repaired mainly by either error-prone non-homologous end joining (NHEJ)[8] or high-fidelity homologous recombination (HR)[1,9]. The SSNs have been used to create targeted knockout plants in various species ranging from the model plants, *Arabidopsis*[10,11] and tobacco[12], to important crops, such as barley[13,14], soybean[15], rice[16-21] and maize[22,23]. Heritable gene modification has been demonstrated in *Arabidopsis*[10,11,24] and rice[18] using the CRISPR/Cas9 system and TALENs. Genome editing of a single MLO gene in bread wheat using a transient protoplast expression system[17] has been demonstrated and it has been shown that introducing mutations in the coding region of all three *MLO* homoeoalleles in wheat confers heritable resistance to powdery mildew fungus[43]. However, the inventors have found that these mutants also show detrimental development related phenotypes compared to wild type plants when grown under disease free conditions.

The invention described herein is thus aimed at providing alternative mutant wheat plants resistant to powdery mildew and related methods which do not show detrimental development related phenotypes compared to wild type plants when grown under disease free conditions, thus providing products and methods of agricultural importance.

Summary of the invention

The inventors have generated mutant wheat lines with mutations inactivating all three MLO homoeoalleles which confer heritable resistance to powdery mildew fungus. These plants do not show senescence like phenotypes which negatively impact on crop yield and quality under non-disease conditions. Thus, the invention relates to these mutant wheat lines and related methods.

In particular, in a first aspect, the invention relates to a wheat plant, plant part or plant cell that has increased resistance to powdery mildew compared to a wild type wheat plant and comparable yield under non-disease conditions compared to a wild type wheat plant wherein said plant comprises a loss of function mutation in the coding regions of two alleles selected from TaMLO-A1, TaMLO-B1 and TaMLO-D1 and reduced expression of the third TaMLO allele.

In another aspect, the invention relates to a wheat plant, plant part of plant cell that has increased resistance to powdery mildew compared to a wild type plant comprising a loss of function mutation in the coding regions of two alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and reduced expression of the third *TaMLO* allele wherein said third *TaMLO* allele does not have a mutation in its coding region.

In one specific aspect, the invention relates to a wheat plant, plant part or plant cell that has increased resistance to powdery mildew compared to a wild type wheat plant and comparable yield under non-disease conditions compared to a wild type wheat plant wherein said plant comprises a loss of function mutation in the coding regions of TaMLO-A1 and TaMLO-D1 and reduced expression of TaMLO-B1 wherein the coding region of TaMLO-B1 does not contain a mutation as compared to the coding region of TaMLO-B1 from a wild type plant.

In one specific aspect, the invention relates to a wheat plant, plant part or plant cell that has increased resistance to powdery mildew compared to a wild type wheat plant and comparable yield under non-disease conditions compared to a wild type wheat plant comprising a Tamlo-a sequence as shown in SEQ ID No. 38, a Tamlo-d sequence as shown in SEQ ID No. 39 and a TaMLO-B1 sequence having a wild type sequence of SEQ ID NO. 2.

In another specific aspect, the invention relates to a wheat plant, plant part or plant cell or part thereof wherein said wheat genotype has the CGMCC Accession Number 10951.

In another aspect, the invention relates to a method for producing a wheat plant, plant part or plant cell with increased resistance to powdery mildew compared to a wild type plant and comparable yield under non-disease conditions compared to a wild type wheat plant using targeted genome modification comprising introducing a loss of function mutation into the coding regions of two MLO alleles selected from TaMLO-A1, TaMLO-B1 and TaMLO-D1 and decreasing expression of the third TaMLO allele.

In another aspect, the invention relates to a plant, plant part or plant cell obtained or obtainable by this method.

Description of figures

The invention is further illustrated in the following non-limiting figures.

Figure 1. *TaMLO* homologous triple mutants When *TaMLO* homologous triple mutants[42] (tamlo-aabbdd) were grown under axenic (disease free) conditions, these triple mutant plants show development related phenotypes, including cell death and senescence-like chlorosis about at 12 weeks.

Figure 2. Targeted knock-out of *TaMLO* genes using TALENs. (a) Sites within a conserved region of exon 2 of wheat *TaMLO* homoeologs targeted by TALENs. The TALEN-targeted sequences in *MLO-A1*, *MLO-B1* and *MLO-D1* are underlined, and the AvaII restriction site in the spacer is GGACC (SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 45). There are three SNPs, two are in the spacer region. The first is C/G/G respectively directly adjacent to the underlined 5' region. The second is A/C/A 3' of the AvaII region following residue C directly adjacent to the AvaII region. The third one lies near the far right of the TALEN binding site (penultimate 3' residue). (b) Mutations in *TaMLO* homologous "triple" mutants are located in the A and D coding sequences. *Tamlo-R (*with genetic profile *tamlo-AaBBDd)* is heterozygous in genome A and D. No mutation was identified at the target site in genome B in T0 plants (SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48). **(c)** Phenotype of homozygous T1 mutant lines. When all the 7 homozygous T1 plants were challenged with conidiospores of a virulent Bgt race, only the homozygous plant R32 confers resistance to powdery mildew. R32 did not display the senescence-like chlorosis, and the plant grew as vigorously as the wild type.

Figure 3. Phenotype of homozygous T1 mutant lines. All the progeny of R32 showed resistance to *Bgt*, and about 1/3 of R26, R40 and R54 offsprings were resistant to the *Bgt*. All the progeny of R51 were susceptible to the *Bgt*. In contrast to fully resistant mutant *tamlo-aabbdd* plants, the resistant mutant plants allow the low-level growth of sporulating *Bgh*.

Figure 4. Transcription level of *mlo* in mutant lines. Transcription of the TaMLO protein of genome B (TaMLO-B1) of these resistant plants is lower compared with wild type.

Figure 5. DNA sequence of the GFP donor cassette. The cassette contains the GFP coding sequence (in bold) and the CaMV 35S terminator sequence (in italics), and is flanked by two T-MLO target sequences (underlined) at both ends.

Figure 6. Vector sequences. (a) The sequence of UBI-attr1-attr2-Nos in vector pYP010: 4047bp. The sequence of Ubi-1 is underlined is and the attr1 and attr2 are in italics. Nos is indicated in bold. (SEQ ID NO. 7) (b) The sequence of TAL-L in vector pZHY500: 2202bp. The sequences of N terminal and C terminal are underlined. TAL-L is labelled in bold. (SEQ ID NO. 8) (c) The sequence of TAL-R in vector pZHY501: 2304bp. The sequences of N terminal and C terminal are indicated. TAL-R is labelled in bold. (SEQ ID NO. 9) (d) The sequence of TALENs (TAL-L + TAL-R) in vector pZHY013. Sequences in italics are attr1 and attr2. The sequences of N terminal and C terminal parts are indicated underlined. TAL-L and TAL-R are in bold. The FokI sequences are in italics and underlined. T2A motif is underlined and in bold. (SEQ ID NO.10).

Figure 7. Genetic mapping.

Figure 8: Phenotypic analysis of *mlo* mutant R32. (A) Thousand kernel weights (TKW) of R32 mutant in Bobwhite background compared to wildtype Bobwhite control (WT). Values are mean ± s.d $^{**}P < 0.01$ (*t*-tests). **(B) (C)** and **(D),** the seed circumference, length and width of R32 mutant plants compared to WT. All the data are from 9 line replicates for R32 and WT.

Figure 9: Phenotypic analysis of *mlo-aabbdd* mutant. (A) Thousand kernel weights (TKW) of *mlo-aabbdd* mutant in Kn199 background compared to wild type Kn199(WT) . **(B) (C)** and **(D),** the seed circumference, length and width of *mlo-aabbdd* mutant plants compared to WT. All the data are from 8 lines replicates for *mlo-aabbdd* and WT.

Detailed description

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, bioinformatics which are within the skill of the art. Such techniques are explained fully in the literature.

As used herein, the words "nucleic acid", "nucleic acid sequence", "nucleotide", "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term "gene", “allele” or “gene sequence” is used broadly to refer to a DNA nucleic acid associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence, or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in combination with regulatory sequences. Thus, according to the various aspects of the invention, genomic DNA, cDNA or coding DNA may be used. In one embodiment, the nucleic acid is cDNA or coding DNA.

The terms “peptide”, "polypeptide" and "protein" are used interchangeably herein and refer to amino acids in a polymeric form of any length, linked together by peptide bonds.

The term “allele” designates any of one or more alternative forms of a gene at a particular locus. Heterozygous alleles are two different alleles at the same locus. Homozygous alleles are two identical alleles at a particular locus. A wild type allele is a naturally occurring allele.

For the purposes of the invention, a “mutant” plant is a plant that has been altered compared to the naturally occurring wild type (WT) plant. Specifically, the endogenous nucleic acid sequences of each of the *MLO* homologs in wheat (wild type nucleic acid sequences *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*) have been altered compared to wild type sequences using mutagenesis methods as described herein. This causes inactivation of the endogenous Mlo genes and thus disables Mlo function. Such plants

have an altered phenotype and show resistance or increased resistance to Pm compared to wild type plants. Therefore, the resistance is conferred by the presence of mutated endogenous *TaMLO-A1, TaMLO-B1* and *TaMLO-D1* genes in the wheat plant genome which has been specifically targeted using targeted genome modification and is not conferred by the presence of transgenes expressed in wheat.

As used herein, wild type nucleic acid sequences of wild type alleles are designated using capital letters, that is *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1.* Mutant *mlo* nucleic acid sequences use non-capitalisation, that is *taml-aa1*, *tamlo-bb1*, *tamlo-dd1*. Wheat plants of the invention are mutant plants compared to wild type plants which comprise and express mutant *mlo* alleles.

mlo mutations that down-regulate or disrupt functional expression of the wild-type Mlo sequence are recessive, such that they are complemented by expression of a wild-type sequence. Thus "*Mlo* function" can be determined by assessing the level of constitutive defence response and/or susceptibility of the plant to a pathogen such as, for example, powdery mildew. Thus, according to the invention, a putative nucleotide sequence with *Mlo* function can be tested upon complementation of a suitable *mlo* mutant. The term "*mlo* function" is used to refer to sequences which confer a *mlo* mutant phenotype on a plant. The capitalisation of "*Mlo*" and non-capitalisation of "*mlo*" is thus used to differentiate between "wild-type and mutant" function.

A *mlo* mutant phenotype according to the invention is characterised by the exhibition of an increased resistance against Pm. In other words, a *mlo* mutant according to the invention confers resistance to the pathogen causing Pm. Moreover, the mutant according to the invention is characterised in that it does not show any negative phenotype compared to the wild type which impacts on crop yield and quality, when grown under disease free conditions. In other words, the mutants of the invention do not show any yield and quality penalties compared to a wild type (wt) plant when grown under disease free conditions.

A negative phenotype compared to the wild type which impacts on crop yield and quality includes senescence-like phenotypes, reduced growth or reduced seed yield compared to a wild type plant. Senescence-like phenotypes can be assessed through

the appearance of chlorosis. The reduction can be 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% or more.

A wild type wheat plant is a plant that does not have any mutant *Mlo* alleles.

The aspects of the invention involve targeted mutagenesis methods, specifically genome editing, and exclude embodiments that are solely based on generating plants by traditional breeding methods. As explained herein, the disease resistant trait is not due to the presence of a transgene.

The inventors have generated mutant wheat lines with mutations inactivating all three MLO homoeoalleles which confer heritable resistance to powdery mildew fungus so that no functional TaMLO-B1, TaMLO-A1 or TaMLO-D1 protein is made. These plants do not show senescence like phenotypes which negatively impact on crop yield and quality. Thus, the invention relates to these mutant wheat lines and related methods.

As shown in Fig. 1, when *TaMLO* homologous triple mutants[42] (genotype: *tamlo-aabbdd*) were grown under axenic (disease free) conditions, these triple mutant plants show development related phenotypes, including cell death and senescence-like chlorosis at about 12 weeks. These phenotypes also occurred, as previously reported, in barley[42] and *Arabidopsis*[44]. The senescence-like phenotypes may negatively influence wheat crop yield and quality.

However, in wheat *mlo* mutants generated using TALENs, we identified one line, Tamlo-R (with genetic profile *tamlo-AaBBDd)*, which is heterozygous in genome A and D, but which does not have a mutation at the target site in genome B in T0 plants (Fig.2). After segregation, in the T1 generation, we identified 7 plants homozygous for the mutation in genome A and D (*tamlo-aaBBdd*), named as R4, R25, R26, R32, R40, R51 and R54. When all the 7 homozygous T1 plants were challenged with conidiospores of a virulent Bgt race, we found that only the homozygous plant R32 confers resistance to powdery mildew (Fig.2). Interestingly, R32 did not display the senescence-like chlorosis, and the plant grew as vigorously as the wild type in disease free conditions.

We also assessed the resistance to powdery mildew of offspring of all the homozygous mutant progeny of line Tamlo-R (R4, R25, R26, R32, R40, R51 and R54). We found that all the progeny of R32 showed resistance to *Bgt*, and about 1/3 of R26, R40 and R54 offspring were resistant to the *Bgt*. All the progeny of R51 were susceptible to the *Bgt* (Fig.3). In contrast to fully resistant mutant *tamlo-aabbdd* plants, the resistant mutant plants allow the low-level growth of sporulating *Bgh* (Fig.3.). This phenotype was similar to the well-known and widely used (in agriculture) barley mlo mutant mlo-11[42]. None of these powdery mildew resistant mutant plants showed developmentally related negative phenotypes, such as cell death or senescence-like chlorosis.

We assessed the level of transcription of mlo for these mutants in genome A, B and D, respectively. We found that the transcription of the TaMLO protein of genome B (*TaMLO-B1*) of these resistant plants was lower compared with wild type (Fig.4). This result is also similar to that described in barley mutant mlo-11[42]. Accumulation of both *Mlo-B* transcript and protein is impaired in the R32 line, but the mutation does not reside in the coding region of *TaMLO-B1*.

Thus, the invention relates to a wheat plant, plant part or plant cell generated by genome editing techniques which has increased resistance to powdery mildew compared to a wild type what plant and which does not show growth or yield penalties under non-disease conditions compared to a wild type plant. Compared to fully resistant mutant *tamlo-aabbdd* plants, such plant shows better growth and/or yield under non-disease conditions. Thus, yield of the plants of the invention is comparable to the yield of wild type plants under non-disease conditions, that is where the plant is not exposed to powdery mildew. This means that there is essentially no reduction in yield or no more than 1-5% reduction in yield.

Specifically, in a first aspect, the invention relates to a wheat plant, plant part or plant cell that has increased resistance to powdery mildew compared to a wild type plant and comparable yield under non-disease conditions compared to a wild type wheat plant wherein said plant comprises a loss of function mutation in the coding regions of two alleles selected from TaMLO-A1, TaMLO-B1 and TaMLO-D1 and reduced expression of the third TaMLO allele or inactivated function of the third TaMLO protein.

In one embodiment, the coding region of said third TaMLO allele does not contain a mutation as compared to the coding region of the TaMLO allele from a wild type plant.

In one embodiment, the coding region of said third TaMLO allele does not contain a mutation as compared to the coding region of TaMLO allele from a wild type plant that renders the protein non-functional or reduces gene expression.

For example, the third TaMLO allele may comprise a mutation that reduces expression of the third TaMLO allele or inactivates function of the third third TaMLO protein wherein said mutation is not in the coding region of said third *TaMLO* allele.

In another aspect, the invention relates to a wheat plant, plant part of plant cell that has increased resistance to powdery mildew compared to a wild type plant comprising a loss of function mutation in the coding regions of two alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and reduced expression of the third *TaMLO* allele wherein said third *TaMLO* allele does not have a mutation in its coding region.

In one embodiment, the invention relates to a wheat plant, plant part or plant cell wherein said plant comprises a loss of function mutation in the coding regions of TaMLO-A1 and TaMLO-D1 and reduced expression of TaMLO-B1 wherein the coding region of TaMLO-B1 does not contain a mutation as compared to the coding region of TaMLO-B1 from a wild type plant.

The loss of function mutations in the two *MLO* alleles result in impaired transcript and/or protein accumulation. Expression of the third *TaMLO* allele is reduced compared to wild type expression, for example by at least 5-50%. In one embodiment, there is essentially no expression. A mutation that reduces expression of or otherwise inactivates the third *TaMLO* allele does not reside in the coding region of said allele, but results in impaired accumulation of the transcript of the third *TaMLO* allele and/or impaired accumulation of the protein encoded by the third *TaMLO* allele. For example, the mutation may be in the regulatory region of the allele (for example in SEQ ID No. 40, 41 or 42 or 5' thereof). Alternatively, the mutation that inactivates the third *TaMLO* allele can be a mutation found in another gene in the pathway which interacts with said *TaMLO* allele, or due to epigenetic factors affecting the sequence of regulatory region. Thus, said reduced expression of the TaMLO allele, for example TaMLO-B1, is caused by

a mutation in the regulatory region of the TaMLO allele, for example TaMLO-B1, a mutation in a gene downstream in the MLO pathogen response pathway or is due to an epigenetic factor.

Thus, the mutant wheat plant according to the invention is a triple mutant and comprises a genotype selected from *tamlo-aaBBdd, tamlo-aabbDD or tamlo-AAbbdd*. The triple mutants do not have a mutation in the coding region in one of the *TaMLO* alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*. Thus, the mutation cannot be found in an exon of said *TaMLO* allele.

The mutations are introduced into the wild type *TaMLO* alleles using targeted genome modification, preferably they are introduced simultaneously.

In one embodiment, said targeted genome modification comprises the use of SSNs. These may be selected from ZFNs, a rare-cutting endonuclease, for example a TALEN or CRISPR/Cas9.

Rare-cutting endonucleases are naturally or engineered proteins having endonuclease activity. These bind to nucleic acid target sequences which have a recognition sequence typically 12-40 bp in length. In one embodiment, the SSN is selected from a TALEN. In another embodiment, the SSN is selected from CRISPR/Cas9. This is described in more detail below.

The loss of function mutation may be a deletion or insertion ("indels") with reference the wild type *TaMLO-A1, TaMLO-B1* and *TaMLO-D1* allele sequence are shown herein. The deletion may comprise 1-20 , for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18 or 20 nucleotides in one or more strand. The insertion may comprise 1-20, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18 or 20 nucleotides in one or more strand.

The plant of the invention includes plants wherein said plant is heterozygous for the each of the mutations. In a preferred embodiment however, said plant is homozygous for the mutations. Progeny that is also homozyous can be generated from these plants according to methods known in the art.

According to the various aspects of the invention, the wild type *TaMLO-A1* allele comprises or consists of SEQ ID NO. 1 or a fragment or a functional variant thereof. The corresponding amino acid sequence is SEQ ID NO. 4. According to the various aspects of the invention, the wild type *TaMLO-B1* allele comprises or consists of SEQ ID NO. 2 or a fragment or a functional variant thereof. The corresponding amino acid sequence is SEQ ID NO. 5. According to the various aspects of the invention, the wild type *TaMLO-D1* allele comprises or consists of SEQ ID NO. 3 or a fragment or a functional variant thereof. The corresponding amino acid sequence is SEQ ID NO. 6.

The term “functional variant of a nucleic acid or protein sequence” as used herein, for example with reference to SEQ ID NOs: 1, 2 or 3 refers to a variant gene sequence or part of the gene sequence which retains the biological function of the full non-variant *TaMLO* allele and hence act to modulate responses to Pm. A functional variant also comprises a variant of the gene of interest encoding a polypeptide which has sequence alterations that do not affect function of the resulting protein, for example in non-conserved residues. Also encompassed is a variant that is substantially identical, i.e. has only some sequence variations, for example in non- conserved residues, to the wild type nucleic acid sequences of the alleles as shown herein and is biologically active.

As used herein, variants of a particular *TaMLO* nucleotide or amino acid sequence according to the various aspects of the invention will have at least about 50%-99%, for example at least 75%, for example at least 85%, 86%, 87%, 88%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to that particular non-variant *TaMLO* nucleotide sequence of the *TaMLO* allele as shown in SEQ ID NO. 1, 2 or 3 or 4, 5 or 6. Sequence alignment programs to determine sequence identity are well known in the art.

Also, the various aspects of the invention the aspects of the invention encompass not only a *TaMLO* nucleic acid sequence, but also fragment thereofs. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence of the protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence act to modulate responses to Pm.

In one embodiment, said mutation is introduced using a TALEN and wherein said TAL effector binds to a sequence in exon 2. In one embodiment, said TAL effector binds to TCGCTGCTGCTCGCCGTgacgcaggaccccatctcCGGGATATGCATCTCCGA (SEQ ID NO. 13).

Specifically, the binding site sequences of the second exon conserved region *TaMLO-A*, *TaMLO-B* and *TaMLO-D* to which these TALENs bind are:

MLO-A:TCGCTGCTGCTCGCCGT**c**acgcaggacccaatctcCGGGATATGCATCTCC**C**A (SEQ ID NO. 14)

MLO-B:TCGCTGCTGCTCGCCGT**g**acgcaggaccccatctcCGGGATATGCATCTCC**G**A (SEQ ID NO. 15)

MLO-D:TCGCTGCTGCTCGCCGT**g**acgcaggacccaatctcCGGGATATGCATCTCC**G**A (SEQ ID NO. 16)

The three SNPs are in bold and underlined. The AvaII restriction site is shown in small letters and underlined.

A TALEN pair has for example the nucleic acid sequence SEQ ID NO. 11. The corresponding amino acid sequence is SEQ ID NO. 12.

In one embodiment, the plant of the invention comprises the mutations in *TaMLO-A1* and/or *TaMLO-D1* as shown in Fig 1. In one embodiment, the mutations are as shown for *tamlo-aaBBdd*. In other words, in said wheat plant, the endogenous *TaMLO-A1* allele is a mutant *Tamlo-a1* allele and comprises SEQ ID NO. 38 the endogenous *TaMLO-B1* allele is a wild type *TaMLO-B1* allele and comprises SEQ ID NO. 2, and the endogenous *TaMLO-D1* allele is a mutant *Tamlo-d1* allele and comprises SEQ ID NO. 39.

In one aspect, the mutant plant is TALEN free.

The wheat plant is selected from the list that includes, but is not limited to, *Triticum aestivum, T. aethiopicum, T. araraticum, T. boeoticum, T. carthlicum, T. compactum, T. dicoccoides, T. dicoccum, T. durum, T. ispahanicum, T. karamyschevii, T. macha, T. militinae, T. monococcum, T. polonicum, T. repens, T. spelta, T. sphaerococcum, T. timopheevii, T. turanicum, T. turgidum, T. urartu, T. vavilovii* and *T. zhukovskyi.*

According to another embodiment the various aspects of the invention described herein, the plant is of the species *Triticum aestivum* or *Triticum turgidum*. According to another preferred embodiment, the plant belongs to the cultivar Bobwhite or the cultivar Don Pedro. More preferably, the cultivars BW208 and BW2003 (Bobwhite), which belong to the wheat species *Triticum aestivum* L. ssp *aestivum,* and the variety Don Pedro, which belongs to the wheat species *Triticum turgidum* L. ssp *durum*, are selected.

Bobwhite is the name of the cultivar obtained from the International Maize and Wheat Improvement Center (CIMMYT). BW208 and BW2003 are different Bobwhite lines. Don Pedro is a hard wheat variety, also from CIMMYT.

In particular, the invention relates to a mutant wheat genotype (*Triticum aestivum*), designated Accession Number CGMCC 10951 deposited under the Budapest Treaty at the China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichen West Road, Chaoyang District, Beijing 100101 on 29 June 2015 by The Institute of Genetics and Developmental Biology Chinese Academy of Sciences, No.1 Beichen West Road, Chaoyang District, Beijing 100101. The depositor's reference is Tamlo-R32. The deposited material was found viable in a test performed on 6 July 2015. The invention thus relates to any what plants, parts thereof, including seeds, having this genotype. This mutant is described herein as *Tamlo-aaBBdd* (Fig. 1).

A triple mutant wheat plant according to the invention shows resistance or increased resistance to Pm compared to a control plant, preferably a wild type plant, because the mutations in impair accumulation of the *TaMLO* allele transcript and/or protein. The wheat plant according to the invention shows increased yield compared to a wild type control plant under biotic stress conditions wherein said stress is Pm.

Resistance can for example be assessed by assessing survival, growth, yield or size of pathogen colonies.

The terms "increase", "improve" or "enhance" are interchangeable. Yield for example is increased by at least a 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%, preferably at least 15%

or 20%, more preferably 25%, 30%, 35%, 40% or 50% or more in comparison to a control plant. The term "yield" in general means a measurable produce of economic value, typically related to a specified crop, to an area, and to a period of time. Individual plant parts directly contribute to yield based on their number, size and/or weight, or the actual yield is the yield per square meter for a crop and year, which is determined by dividing total production (includes both harvested and appraised production) by planted square meters. The term "yield" of a plant may relate to vegetative biomass (root and/or shoot biomass), to reproductive organs, and/or to propagules (such as seeds) of that plant. Thus, according to the invention, yield comprises one or more of and can be measured by assessing one or more of: increased seed yield per plant, increased seed filling rate, increased number of filled seeds, increased harvest index, increased number of seed capsules and/or pods, increased seed size, increased growth or increased branching, for example inflorescences with more branches. Preferably, yield comprises an increased number of seed capsules/pods and/or increased branching. Yield is increased relative to control plants.

A control plant as used herein is a plant, which has not been modified according to the methods of the invention. Accordingly, the control plant does not have a mutant *tamlo* nucleic acid sequence as described herein. In one embodiment, the control plant is a wild type wheat plant. In another embodiment, the control plant is a plant that does not have a mutant *tamlo* nucleic acid sequence as described here, but is otherwise modified. The control plant is typically of the same plant species, preferably the same ecotype or the same or similar genetic background as the plant to be assessed.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, fruit, shoots, stems, leaves, roots (including tubers), flowers, and tissues and organs, wherein each of the aforementioned comprise the gene/nucleic acid of interest. The term "plant" also encompasses plant cells, suspension cultures, protoplasts, callus tissue, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprises the gene/nucleic acid of interest.

The invention also extends to harvestable parts of a mutant plant of the invention as described above such as, but not limited to seeds, leaves, flowers, stems and roots. The invention furthermore relates to products derived, preferably directly derived, from

a harvestable part of such a plant, such as dry pellets or powders, oil, fat and fatty acids, flour, starch or proteins. The invention also relates to food products and food supplements comprising the plant of the invention or parts thereof.

In one aspect, the invention relates to a seed of a mutant wheat plant of the invention. Seeds harvested from a mutant plant that is homozygous for the *mlo* mutations are preferred.

In another embodiment, the present invention provides a regenerable mutant plant as described herein cells for use in tissue culture. The tissue culture will preferably be capable of regenerating plants having essentially all of the physiological and morphological characteristics of the foregoing mutant wheat plant, and of regenerating plants having substantially the same genotype. Preferably, the regenerable cells in such tissue cultures will be callus, protoplasts, meristematic cells, cotyledons, hypocotyl, leaves, pollen, embryos, roots, root tips, anthers, pistils, shoots, stems, petiole, flowers, and seeds. Still further, the present invention provides wheat plants regenerated from the tissue cultures of the invention.

In a preferred embodiment, the mutant wheat plants are produced by simultaneous editing of the target *Mlo* sequences.

The invention also relates to an isolated nucleic acid sequence as defined in SEQ ID NO. 38 or 39. Also within scope of the invention are vectors comprising such sequences and host cells comprising such sequences or such vector.

Method for producing plants

In another aspect, the invention relates to a method for producing a wheat plant, plant part of plant cell resistant to powdery mildew compared to a wild type wheat plant and comparable yield under non-disease conditions compared to a wild type wheat plant using targeted genome modification comprising introducing a loss of function mutation into the coding regions of two Ta*MLO* alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and reducing expression of the third *TaMLO* allele. For example, a mutation that reduces expression of the third *TaMLO* allele may be introduced wherein said mutation is not in the coding region of said third *TaMLO* allele.

In one embodiment, loss of function mutations are introduced in the coding regions of T*aMLO-A1* and *TaMLO-D1* and the expression of *TaMLO-B1* is reduced.

The third mutation results in impaired accumulation of the transcript of the third TaMLO allele and/or impaired accumulation of the protein encoded by the third TaMLO allele but is not in the coding region of said third *TaMLO* allele.

Plants that have a loss of function mutation in one or two *MLO* genes according to the invention can be crossed to obtain a loss of function triple mutant. For example, a plant obtained by a method above that has a loss of function mutation in the *TaMLO-A1* allele can be crossed with a plant obtained by a method above that has a loss of function mutation in *TaMLO-B1* allele or *TaMLO-D1* allele. The resulting double mutant can be crossed with another plant obtained by a method above that has mutation which inactivates the remaining allele.

In one embodiment of the methods described herein, all mutations are introduced simultaneously into the wheat plant using targeted genome modification. Progeny that is homozygous for the mutations can then be generated.

Targeted genome modification or targeted genome editing is a genome engineering technique that uses targeted DNA double-strand breaks (DSBs) to stimulate genome editing through homologous recombination (HR)-mediated recombination events. To achieve effective genome editing via introduction of site-specific DNA DSBs, four major classes of customizable DNA binding proteins can be used: meganucleases derived from microbial mobile genetic elements, ZF nucleases based on eukaryotic transcription factors, rare-cutting endonucleases, for example TALENs, transcription activator-like effectors (TALEs) from Xanthomonas bacteria, and the RNA-guided DNA endonuclease Cas9 from the type II bacterial adaptive immune system CRISPR (clustered regularly interspaced short palindromic repeats). Meganuclease, ZF, and TALE proteins all recognize specific DNA sequences through protein-DNA interactions. Although meganucleases integrate its nuclease and DNA-binding domains, ZF and TALE proteins consist of individual modules targeting 3 or 1 nucleotides (nt) of DNA, respectively. ZFs and TALEs can be assembled in desired combinations and attached

to the nuclease domain of FokI to direct nucleolytic activity toward specific genomic loci.

The step of introducing a mutation comprises contacting a population of wheat plant cells with DNA binding protein targeted to endogenous *TaMLOA, B and D* sequences, for example selected from the DNA binding proteins listed above.

In one embodiment, the method comprises contacting a population of wheat plant cells with one or more rare-cutting endonucleases targeted to endogenous *TaMLO-A, B and D* sequences.

The method may further comprise the steps of selecting, from said population, a cell in which *TaMLOA, B and D* have been inactivated and regenerating said selected plant cell into a wheat plant.

Upon delivery into host cells via the bacterial type III secretion system, TAL effectors enter the nucleus, bind to effector-specific sequences in host gene promoters and activate transcription. Their targeting specificity is determined by a central domain of tandem, 33–35 amino acid repeats. This is followed by a single truncated repeat of 20 amino acids. The majority of naturally occurring TAL effectors examined have between 12 and 27 full repeats.

These repeats only differ from each other by two adjacent amino acids, their repeat-variable di-residue (RVD). The RVD that determines which single nucleotide the TAL effector will recognize: one RVD corresponds to one nucleotide, with the four most common RVDs each preferentially associating with one of the four bases. Naturally occurring recognition sites are uniformly preceded by a T that is required for TAL effector activity. TAL effectors can be fused to the catalytic domain of the FokI nuclease to create a TAL effector nuclease (TALEN) which makes targeted DNA double-strand breaks (DSBs) *in vivo* for genome editing. The use of this technology in genome editing is well described in the art, for example in US 8,440,431, US 8,440,432 and US 8,450,471. Reference 30 describes a set of customized plasmids that can be used with the Golden Gate cloning method to assemble multiple DNA fragments. As described therein, the Golden Gate method uses Type IIS restriction endonucleases, which cleave outside their recognition sites to create unique 4 bp overhangs. Cloning is

expedited by digesting and ligating in the same reaction mixture because correct assembly eliminates the enzyme recognition site. Assembly of a custom TALEN or TAL effector construct and involves two steps: (i) assembly of repeat modules into intermediary arrays of 1–10 repeats and (ii) joining of the intermediary arrays into a backbone to make the final construct.

Another genome editing method that can be used according to the various aspects of the invention is CRISPR. The use of this technology in genome editing is well described in the art, for example in US 8,697,359 and references cited herein. In short, CRISPR is a microbial nuclease system involved in defense against invading phages and plasmids. CRISPR loci in microbial hosts contain a combination of CRISPR-associated (*Cas*) genes as well as non-coding RNA elements capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage (sgRNA). Three types (I-III) of CRISPR systems have been identified across a wide range of bacterial hosts. One key feature of each CRISPR locus is the presence of an array of repetitive sequences (direct repeats) interspaced by short stretches of non-repetitive sequences (spacers). The non-coding CRISPR array is transcribed and cleaved within direct repeats into short crRNAs containing individual spacer sequences, which direct *Cas* nucleases to the target site (protospacer). The Type II CRISPR is one of the most well characterized systems and carries out targeted DNA double-strand break in four sequential steps. First, two non-coding RNA, the pre-crRNA array and tracrRNA, are transcribed from the CRISPR locus. Second, tracrRNA hybridizes to the repeat regions of the pre-crRNA and mediates the processing of pre-crRNA into mature crRNAs containing individual spacer sequences. Third, the mature crRNA:tracrRNA complex directs Cas9 to the target DNA via Watson-Crick base-pairing between the spacer on the crRNA and the protospacer on the target DNA next to the protospacer adjacent motif (PAM), an additional requirement for target recognition. Finally, Cas9 mediates cleavage of target DNA to create a double-stranded break within the protospacer.

Cas9 is thus the hallmark protein of the type II CRISPR-Cas system, and a large monomeric DNA nuclease guided to a DNA target sequence adjacent to the PAM (protospacer adjacent motif) sequence motif by a complex of two noncoding RNAs: CRIPSR RNA (crRNA) and trans-activating crRNA (tracrRNA). The Cas9 protein contains two nuclease domains homologous to RuvC and HNH nucleases. The HNH nuclease domain cleaves the complementary DNA strand whereas the RuvC-like

domain cleaves the non-complementary strand and, as a result, a blunt cut is introduced in the target DNA. Heterologous expression of Cas9 together with an sgRNA can introduce site-specific double strand breaks (DSBs) into genomic DNA of live cells from various organisms. For applications in eukaryotic organisms, codon optimized versions of Cas9, which is originally from the bacterium Streptococcus pyogenes, have been used.

The single guide RNA (sgRNA) is the second component of the CRISPR/Cas system that forms a complex with the Cas9 nuclease. sgRNA is a synthetic RNA chimera created by fusing crRNA with tracrRNA. The sgRNA guide sequence located at its 5′ end confers DNA target specificity. Therefore, by modifying the guide sequence, it is possible to create sgRNAs with different target specificities. The canonical length of the guide sequence is 20 bp. In plants, sgRNAs have been expressed using plant RNA polymerase III promoters, such as U6 and U3.

Cas9 expression plasmids for use in the methods of the invention can be constructed as described in the art. One example is provided as described in the example section herein.

The method for producing a mutant wheat plant according to the invention resistant to Pm using genome editing comprises the use of a SSN. This may be selected from a meganuclease, ZFN, TALEN, or CRISPR/Cas9. In one embodiment, the SSNs is a TALEN.

Thus, in one embodiment, the method comprises the use of TALEN. In this embodiment, the method comprises introducing an expression vector comprising a TALEN into a wheat plant and screening for TALEN-induced targeted mutations in *TaMLO-A1*, *TaMLO-B1* and/or *TaMLO-D1* genes. The method may also comprise the further step of regenerating a plant and selecting or choosing a plant resistant to Pm.

In one embodiment, said vector comprises a pair of TALENs (T-MLO) targeting a conserved region in exon 2 (Fig. 1a, 9 and table 1). The vector construct encodes a pair of TALENs that targets sequences conserved between all three homoeologues *MLO* genes of wheat.

Thus, in one embodiment, the target sequence site in *TaMLO* is TCGCTGCTGCTCGCCGTgacgcaggaccccatctcCGGGATATGCATCTCCGA (SEQ ID NO. 13, Table 1).

Specifically, the binding site sequences of the second exon conserved region *TaMLO-A*, *TaMLO-B* and *TaMLO-D* to which these TALENs bind are:

MLO-A:TCGCTGCTGCTCGCCGT**c**acgcaggacccaatctcCGGGATATGCATCTCC**C**A (SEQ ID NO. 14)

MLO-B:TCGCTGCTGCTCGCCGT**g**acgcaggaccccatctcCGGGATATGCATCTCC**G**A (SEQ ID NO. 15)

MLO-D:TCGCTGCTGCTCGCCGT**g**acgcaggacccaatctcCGGGATATGCATCTCC**G**A (SEQ ID NO. 16)

The three SNPs are in bold and underlined. The AvaII restriction site is shown in small letters and underlined.

A TALEN pair has for example the nucleic acid sequence SEQ ID NO. 11. The corresponding amino acid sequence is SEQ ID NO. 12.

In this embodiment, the TALEN pair recognizes 16 bp and 17 bp, respectively, of contiguous DNA separated by an 18 bp spacer DNA containing an AvaII restriction site as shown above, (Table 1). The TALEN recognition sequences are strictly conserved in *TaMLO-B1* and *TaMLO-D1*, but have one nucleotide mismatch with the cognate *TaMLO-A1* target site (Fig. 2a). In addition, the conserved spacer region in Fig.2a contains two single nucleotide polymorphisms (SNPs) among the three *MLO* homoeo-alleles.

As shown in the examples, in order to detect the mutation at the site targeted by the genetic editing technique, an Ava II enzyme digestion locus was selected from the targeted sites; if mutation occurred, then the Ava II enzyme digestion locus was damaged and cannot be digested. However, non- mutated PCR products are susceptible to digestion.

In one embodiment, the TALENs are assembled by the Golden Gate cloning method and built into a single plasmid as described in the examples.

In one embodiment, screening for TALEN-induced targeted mutations in *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* genes comprises obtaining a DNA sample from a transformed plant and carrying out DNA amplification and optionally restriction enzyme digestion to detect a mutation in *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1.* When the target site is as shown above, the restriction enzyme is AvaII.

PCR fragments amplified from the transformed plants are then assessed using a gel electrophoresis based assay. In a further step, the presence of the mutation may be confirmed by sequencing the *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1* genes.

In another embodiment, the method comprises the use of CRISPR/Cas9. In this embodiment, the method therefore comprises introducing and co-expressing in a wheat plant Cas9 and sgRNA targeted to *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1* and screening for induced targeted mutations in *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* genes. The method may also comprise the further step of regenerating a plant and selecting or choosing a plant resistant to Pm.

Cas9 and sgRNA may be comprises in a single or two expression vectors.

The target sequence in *TaMLO-A1* may be CCGTCACGCAGGACCCAATCTCC (SEQ ID NO. 17, see table 1).

In one embodiment, screening for CRISPR-induced targeted mutations in *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* genes comprises obtaining a DNA sample from a transformed plant and carrying out DNA amplification and optionally restriction enzyme digestion to detect a mutation in *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1.*

In one embodiment, the restriction enzyme is mismatch-sensitive T7 endonuclease. T7E1 enzyme that is specific to heteroduplex DNA caused by genome editing.

PCR fragments amplified from the transformed plants are then assessed using a gel electrophoresis assay based assay. In a further step, the presence of the mutation may be confirmed by sequencing the *TaMLO-A1, TaMLO-B1 and/*or *TaMLO-D1* genes.

As shown in the examples, genomic DNA (i.e. wt and mutant) can be prepared from each sample, and DNA fragments encompassing each target site are amplified by PCR (see Table). The PCR products are digested by restriction enzymes as the target locus includes a restriction enzyme site. The restriction enzyme site is destroyed by CRISPR- or TALEN-induced mutations by NHEJ or HR, thus the mutant amplicons are resistant to restriction enzyme digestion, and result in uncleaved bands. Alternatively, the PCR products are digested by T7E1 (cleaved DNA produced by T7E1 enzyme that is specific to heteroduplex DNA caused by genome editing) and visualized by agarose gel electrophoresis. In a further step, they are sequenced.

In another aspect, the invention relates to a method for conferring resistance to Pm to a wheat plant, plant part or plant cell comprising introducing a loss of function mutation into the coding region of two MLO alleles selected from TaMLO-A1, TaMLO-B1 and TaMLO-D1 and reducing expression of the third TaMLO allele, for example by introducing a further mutation which results in impaired accumulation of the transcript of the third TaMLO allele and/or impaired accumulation of the protein encoded by the third TaMLO allele wherein said mutation is not in the coding region of the third TaMLO allele wherein said mutations are introduced using targeted genome modification.

In one embodiment, ZFN, a rare-cutting endonuclease, for example TALEN, or CRISPR/Cas9 is used. In one embodiment, the method comprises producing a mutant plant as described above.

In the methods above, amplification is preferably carried out using PCR and primers that specifically amplify *TaMLO-A1, TaMLO-B1* and *TaMLO-D1* (table 2) and as shown below:

The following primer pair amplifies the *TaMLO-A1* target site:
MLO-A1-F (SEQ ID NO. 18)
TGGCGCTGGTCTTCGCCGTCATGATCATCGTC
MLO-A1-R (SEQ ID NO. 19)
TACGATGAGCGCCACCTTGCCCGGGAA

The following primer pair amplifies the *TaMLO-B1* target site:

MLO-B1-F (SEQ ID NO. 20)
ATAAGCTCGGCCATGTAAGTTCCTTCCCGG
MLO-B1-R (SEQ ID NO. 21)
CCGGCCGGAATTTGTTTGTGTTTTTGTT

The following primer pair amplifies the *TaMLO-D1* target site:
MLO-D1-F (SEQ ID NO. 22)
TGGCTTCCTCTGCTCCCTTGGTGCACCT
MLO-D1-R (SEQ ID NO. 23)
TGGAGCTGGTGCAAGCTGCCCGTGGACATT

The following primer pair amplifies all three alleles
MLO-F (SEQ ID NO. 24)
GTCTTCGCCGTCATGATCATCGTCTCC
MLO-R (SEQ ID NO. 25)
TGGTATTCCAAGGAGGCGGTCTCTGTCT

In a preferred embodiment, the methods above are carried out by transforming wheat embryos. In a further preferred embodiment, the methods comprise generating stable T2 plants preferably homozygous for the mutation.

In one embodiment, the methods do not comprise transforming wheat protoplasts.

The methods above use plant transformation to introduce an expression vector comprise a SSN into a plant. The term "introduction" or "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The resulting transformed plant cell may then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

The transfer of foreign genes into the genome of a plant is called transformation. Transformation of plants is now a routine technique in many species. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle bombardment as described in the examples, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts, electroporation of protoplasts, microinjection into plant material, DNA or RNA-coated particle bombardment, infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via *Agrobacterium tumefaciens* mediated transformation.

To select transformed plants, the plant material obtained in the transformation is, as a rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility is growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker.

Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2)

transformants selected, and the T2 plants may then further be propagated through classical breeding techniques.

The SSN is preferably introduced into a plant as part of an expression vector. The vector may contain one or more replication systems which allow it to replicate in host cells. Self-replicating vectors include plasmids, cosmids and virus vectors. Alternatively, the vector may be an integrating vector which allows the integration into the host cell's chromosome of the DNA sequence. The vector desirably also has unique restriction sites for the insertion of DNA sequences. If a vector does not have unique restriction sites it may be modified to introduce or eliminate restriction sites to make it more suitable for further manipulation. Vectors suitable for use in expressing the nucleic acids, are known to the skilled person and a non-limiting example is pYP010.

The nucleic acid is inserted into the vector such that it is operably linked to a suitable plant active promoter. Suitable plant active promoters for use with the nucleic acids include, but are not limited to CaMV35S, wheat U6, or maize ubiquitin promoters.

The vector may also comprise a GFP sequence or other marker as explained in the examples and in the figures.

A plant, plant part or plant cell obtained or obtainable by the methods described above is also within the scope of the invention.

In one aspect, the mutant is TALEN free. Thus, according to the method above, the presence of a TALEN can be assessed as described in the examples.

In additional steps, the method may comprise determining the presence of a mutant *tamlo-a1*, *tamlo-b1* and/or *tamlo-d1* nucleic acid or detecting the presence or absence of a TaMLO-A, B or D protein in a wheat plant.

A diagnostic test determining the presence of a mutant *tamlo-a1*, *tamlo-b1* and/or *tamlo-d1* nucleic acid may involve hybridisation of a suitable oligo- or poly-nucleotide, such as a fragment of the Mlo gene. The hybridisation may involve PCR designed to amplify a nucleic acid product from a given allelic version of mlo, with subsequent

detection of an amplified product by any of a number of possible methods including but not limited to gel electrophoresis, capillary electrophoresis and direct hybridisation of nucleotide sequence probes. A diagnostic test may be based on PCR designed to amplify various mutant nucleic acids from the Mlo locus, with a test to distinguish the different possible mutant nucleic acids from the wild type by any of a number of possible methods, including DNA fragment size, restriction site variation (e.g. CAPS - cleaved amplified polymorphic sites) and so on. A diagnostic test may also be based on a great number of possible variants of nucleic acid analysis that will be apparent to those skilled in the art, such as use of a synthetic mlo-derived sequence as a hybridisation probe.

Suitable tests for assessing the presence of a mutant allele according to the invention include but are not limited to among these are Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length polymorphisms (AFLPs), Simple Sequence Repeats (SSRs-which are also referred to as Microsatellites), and Single Nucleotide Polymorphisms (SNPs). In one embodiment, Kompetitive Allele Specific PCR (KASP) genotyping is used.
In one embodiment, the method comprises

a) obtaining a nucleic acid sample from a wheat plant and
b) carrying out nucleic acid amplification of one or more *TaMLO* allele using one or more primer pairs selected from SEQ ID NOs 18 to 25 or SEQ ID NOs. 34-37.

MLO-R32-A1-F: TGATGATGATGATGATGGAACTTGTTCTCG SEQ ID NO. 34
MLO-R32-A1-R: AAGGAGGCGGTCTCTGTCTCCCATTTCTTC SEQ ID NO. 35
MLO-R32-D1-F: TTCATCTCGCTGCTGCTCCATCTCCG SEQ ID NO. 36
MLO-R32-D1-R: AGCCATGATGATGACGCTGTAGGTGACATG SEQ ID NO. 37

In one embodiment, the method comprises determining whether a TaMLO protein accumulates in the plant. Thus, the presence or absence of a TaMLO-A, B or D protein in a plant is detected. If the protein is absent, a mutation which impairs protein accumulation is present in the genome of the plant. In one embodiment, the presence or absence of a TaMLO-B protein in a plant is detected.

Suitable tests for assessing the presence of a protein are known in the art and include, but are not limited to, Gel Electrophoresis (such as Polyacrylamide Protein Gel Electrophoresis or 2D Gel Electrophoresis), colorimetric assays, Western Blotting, Immunoassays (such as ELISA, lateral flow strips or immunostaining) or spectrophotometry.

The invention also relates to an isolated nucleic acid sequence comprising one or more primer selected from SEQ ID NOs. 34-37. The invention also relates to a detection kit comprising one or more primer selected from SEQ ID NOs. 34-37.

The various aspects of the invention described herein clearly extend to any plant cell or any plant produced, obtained or obtainable by any of the methods described herein, and to all plant parts and propagules thereof unless otherwise specified. The present invention extends further to encompass the progeny of a mutant plant cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced by the parent in the methods according to the invention.

While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification, including reference to sequence database identifiers, are incorporated herein by reference in their entirety. Unless otherwise specified, when reference to sequence database identifiers is made, the version number is 1.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

The invention is further described in the following non-limiting examples.

Examples

TALEN design and construction

TALEN target sites were designed using the TAL effector-Nucleotide Targeter 2.0 (TALE-NT) program (https://tale-nt.cac.cornell.edu/). All the target sites had a T at the -1 position, and the corresponding TAL effector arrays were constructed using the Golden Gate method as previously described[33]. Information on all the TAL effecter arrays and target sites is given in Table 1. TALENs were assembled in vectors with a truncated N152/C63 backbone architecture (pZHY500 and pZHY501). The Gateway-compatible entry plasmid, pZHY013, was used as the intermediate vector to create TALEN expression vectors[34]. This plasmid contains two heterodimeric FokI nuclease domains separated by a T2A translational skipping sequence. TAL arrays in the plasmids pZHY500 and pZHY501 were released by digestion with XbaI/BamHI and subcloned into pZHY013 one-by-one[34,35]. One array (left array) was first cloned into pZHY013 as an XbaI/BamHI fragment; the other (right array) was then cloned into the NheI/BglII sites, which have ends compatible with XbaI and BamHI. A Gateway LR reaction was performed to clone the TALEN coding sequences into the destination vector, pYP010 (a derivative of pZHY05134 by replacing the 35S promoter with the maize ubiquitin promoter.

Construction of Cas9 and sgRNA expression vectors

The plasmid pJIT163 was used to construct the Cas9 expression plasmid. It was digested with KpnI and HindIII and fused with the maize *ubiquitin 1* promoter (Ubi) to construct vector pJIT163-Ubi. Full-length *Cas9* (plant codon-optimized) products were digested with BamHI and MfeI and inserted into plasmid pJIT163-Ubi between the

BamHI and MfeI sites to yield the expression vector pJIT163-Ubi-Cas9. The wheat U6 promoters and wheat gRNA scaffolds were synthesized by GenScript and cloned into pEASY-blunt vector (TransGen Biotech). The sequences of Cas9 and the gRNAs are given in a previous publication[17]. Wheat genomic DNA region immediately precede a 5'-NGG PAM, such as 5'-G-N(20)-GG-3' or 5'-N(21)-GG-3' was selected as target.

The CRISPR/Cas9 target site in TaMLO contains two single nucleotide polymorphisms (SNPs) among the three homoeoalleles. We designed a sgRNA (sgMLO-A1) to specifically target *TaMLO-A1*. Our results show that sgRNA-A1-induced mutations only occurred in *TaMLO-A1*, so confirming the specificity of the sgRNA for TaMLO-A1. Therefore, off-target cleavage did not occur in *TaMLO-B1* and *TaMLO-D1*. The results show that CRISPR/Cas9 is active in wheat plants and that transgenic mutant lines can be generated. Other mutants, including a triple mutant AA, BB and DD can be obtained using Cas9/sgRNA by targeting a conserved target site.

Wheat protoplast transformation

Wheat protoplasts were isolated and transformed as previously described[3]. Average transformation efficiencies were 60-80%. Protoplast transformation was carried out with 20μg of TALEN plasmid per transformation, or a mixture of 10μg pJIT163-Ubi-Cas9 plasmid and 10μg pU6-gRNA plasmid.

Biolistic transformation of wheat

Biolistic transformation was performed using a PDS1000/He particle bombardment system (Bio-Rad, Hercules, CA) with a target distance of 6.0 cm from the stopping plate at helium pressure 1100 psi. Plasmid DNAs (T-MLO and pAHC20) were mixed in a 1:1 (1:1:1 for Cas9, sgRNA and pAHC20) molar ratio prior to bombardment. After bombardment, embryos were transferred to callus induction medium. In the third or fourth week, all calli were transferred to selective regeneration medium containing 5 mg/l phosphinothricin (PPT). PPT was present in all subsequent tissue culture procedures including 2 rounds of regeneration (4 weeks) and 2 rounds of rooting (4 weeks). After 10-12 weeks, T0 transgenic plants were obtained, transferred into soil and grown in a management greenhouse[37].

Screening of SSN-induced mutations

Genomic DNA from individual wheat plants was extracted using the high- throughput Automation Workstation Biomek® FX (Beckmen) with the magnetic bead-based DNA extraction kit (GeneOn Biotech). The PCR/RE digestion screen assay and T7E1 assay were used to identify the mutations as previously described[35, 36, 37]. The PCR products amplified with *TaMLO*-specific primers (Table 3) from individual mutant plants were cloned into pUC-T vector (CWBIO) for sequencing. Mutation frequencies (indels (%)) in protoplasts were calculated by measuring band intensities with UVP VisionWorks LS Image Acquisition Analysis Software 7.0[36].

Powdery mildew infection and Microscopic analyses

Wheat plants were grown on soil in controlled environment chambers at 22°C and 16-h photoperiod with light intensity ranging from 400-1,000µmol m^{-2} s^{-1}. Powdery mildew infection and microscopic analyses were performed as previously reported[39] with some modifications. Leaves originating from the main stem (leaves 2, 3, and 4) were cut into 5 cm segments and immediately placed in Petri dishes containing 1% (w/v) distilled water agar and 8.5mM benzimidazole. The leaf segments were incubated at 22°C in continuous light (100µmol m^{-2} s^{-1}) for four hour, then inoculated with virulent strains of *Blumeria graminis* f. sp. *tritici* (*Bgt*) E09, E22 and B13 to give approximately 15 to 20 sporulating colonies per cm^2 and incubated at 22°C in continuous light (100 umol m^{-2} s^{-1}). Seventy-two hours after inoculation, the leaf segments were fixed with 1:1 (v/v) ethanol : acetic acid for 24 h, cleared with lactoglycerol (1:1:1 [v/v] lactic acid : glycerol : H2O) for 48h, and stained for 7 sec with Coomassie blue (0.6% [w/v] Coomassie Brilliant Blue R 250 [Sigma] in methanol) to visualize the fungal structure, finally rinsed in distilled water and mounted in 50% (v/v) glycerol prior to microscopy. Samples were observed and analyzed under an Olympus BX51light microscope, and photographs were taken using software Cellsens Entry 1.21.

Yield index test for *mlo* mutants

Plants were grown in a standard wheat field in 20×10 cm plots (20 plants) under conventional cultivation conditions. There was no powdery mildew disease in the field and no fungicide was used. All phenotypical data including thousand seed weight (TKW), seed circumference, seed length and seed width were measured. Data were from 9 replicates for the mutant R32 and wildtype control (Bobwhite), 8 replicates for the mutat *mlo-aabbdd* and wildtype control (Kn199), respectively.

About 400 filled grains of mutant lines and wildtype plants of one 5-plant sample in every replicate were picked for TKW measurements using Electron balance. About 150 filled mutants and wildtype grains of one treatment in every sample were used to measure the seed circumference, seed length and seed width by Wanshen kaozhong examination analyzer.

Results and discussion

We deployed a pair of TALENs (T-MLO) targeting a conserved region in exon 2 (Fig. 2a). The TALEN pair recognizes 16 bp and 17 bp, respectively, of contiguous DNA separated by an 18 bp spacer DNA containing an AvaII restriction site (Fig. 1a and Table 1). The TALEN recognition sequences are strictly conserved in *TaMLO-B1* and *TaMLO-D1*, but have one nucleotide mismatch with the cognate *TaMLO-A1* target site (Fig. 2a). In addition, the conserved spacer region in Fig.2a contains two single nucleotide polymorphisms (SNPs) among the three *MLO* homoeo-alleles. The TALENs were assembled by the Golden Gate cloning method[30], and built into a single plasmid by a T2A translational skipping sequence driven by the maize ubiquitin promoter. The activity of the resulting T-MLO was first evaluated by transforming the TALEN- carrying plasmid into wheat protoplasts. Analysis of genomic DNA from the transformed protoplasts using a previously developed PCR restriction enzyme digestion assay (PCR/RE)[16] demonstrated the occurrence of mutations at the target site.

We then co-transformed the T-MLO plasmid and pAHC20[31], a plasmid harboring the selectable *bar* gene, into immature wheat embryos by the particle bombardment method. Wheat seedlings were regenerated from herbicide-resistant calli after 6-8 weeks of selection on 5 µg/ml phosphinothricin (PPT). The *MLO* target sites (in *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*) were first amplified from the genomic DNA of these transgenic seedlings (T0 plants) using a conserved primer set (Table 2), and analyzed by the PCR/RE assay to detect potential mutations. In order to identify in which of the *TaMLO* genes the mutations occurred, we designed primers to specifically amplify *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and conducted PCR/RE assays of the PCR amplicons with the specific primers (Table 2). This revealed the revealed that T-MLO-induced mutations as shown in Fig. 1.

We identified Tamlo-R *(*with genetic profile *tamlo-AaBBDd)*, which is heterozygous in genome A and D, but no mutation was identified at the target site in genome B in T0 plants (Fig.2). After segregation, in T1 generation, we identified 7 plants homozygous for the mutation in genome A and D (tamlo-aaBBdd), named as R4, R25, R26, R32, R40, R51 and R54. When all the 7 homozygous T1 plants were challenged with conidiospores of a virulent Bgt race, we found that only the homozygous plant R32 confers resistance to powdery mildew (Fig.2). Interestingly, R32 did not display the senescence-like chlorosis, and the plant grew as vigorously as the wild type.

To confirm this view, we tested the thousand kernel weight (TKW) for the R32 mutant and wild type plant in the yield. The results showed that the R32 (which is in Bobwhite background) has significantly elevated thousand kernel weight (TKW) compared with wildtype in Bobwhite WT control ($P < 0.01$), but there was no such difference between the *mlo*-aabbdd mutant (in Kn199 background) and Kn199 WT control (Figure 8A and Figure 9A). Moreover, there is no obvious change in other parameters including seed circumference, seed length and seed width between mutant (R32 and *mlo-aabbdd*) and wildtype both in Bobwhite and Kn199 (Figure 8 and 9).

We also assessed the resistance to powdery mildew of offspring of all the homozygous mutant progeny of line Tamlo-R (R4, R25, R26, R32, R40, R51 and R54). We found that all the progeny of R32 showed resistance to Bgt, and about 1/3 of R26, R40 and R54 offspring were resistant to the Bgt. All the progeny of R51 were susceptible to the Bgt (Fig.3). In contrast to fully resistant mutant tamlo-aabbdd plants, the resistant mutant plants allow the low-level growth of sporulating Bgh (Fig.3).

We assessed the level of transcription of mlo for these mutants in genome A, B and D, respectively. We find that the transcription of the TaMLO protein of genome B (TaMLO-B1) of these resistant plants was lower compared with wild type (Fig.4).

To date, race-specific resistance controlled by the resistance (*R*) gene is commonly used for developing resistant wheat varieties, but this tends to break down as new *Bgt* races emerge in the field[32]. In contrast, loss-of-function *mlo* mutation-conferred resistance against powdery mildew has not been broken since its introgression into elite barley varieties three decades ago[25]. Therefore, the *mlo-aaBBdd* alleles we generated in the elite wheat cultivars may provide excellent starting materials for

breeding durable and broad-spectrum resistance in bread wheat.

Genetic mapping

Genetic mapping as shown in Figure 7 is being carried out.

Table 1. SSN target loci and sequences

Gene Name	SSN ID	Target Site	Left Binding Site RVDs/ Oligo-F (5′-3′)	Right Binding Site RVDs/Oligo-R(5′-3′)	Detection method
TaMLO	T-MLO	TCGCTGCTGCTCGC CGTgacgcaggaccccatctc CGGGATATGCATCT CCGA SEQ ID NO. 13	HD NN HD NG NN HD NG NN HD NG HD NN HD HD NN NG SEQ ID NO. 49	HD NN NN NI NN NI NG NN HD NI NG NI NG HD HD HD NN SEQ ID NO. 50	PCR/RE : AvaII
TaMLO-A1	sgMLO-A1	CCGTCACGCAGGAC CCAATCTCC SEQ ID No. 17	CTTGGAGATTGGG TCCTGCGTGA SEQ ID No. 26	AAACTCACGCAG GACCCAATCTC SEQ ID No. 27	T7E1

Table 2. PCR primers used and their applications

Primer name	Primer sequence	Experiment
MLO-A1-F MLO-A1-R	TGGCGCTGGTCTTCGCCGTCATGATCATCGTC SEQ ID No. 18 TACGATGAGCGCCACCTTGCCCGGGAA SEQ ID No. 19	Gene specific primer amplifying the *TaMLO-A1* target site
MLO-B1-F MLO-B1-R	ATAAGCTCGGCCATGTAAGTTCCTTCCCGG SEQ ID No. 20 CCGGCCGGAATTTGTTTGTGTTTTTGTT SEQ ID No. 21	Gene specific primer amplifying the *TaMLO-B1* target site
MLO-D1-F MLO-D1-R	TGGCTTCCTCTGCTCCCTTGGTGCACCT SEQ ID No. 22 TGGAGCTGGTGCAAGCTGCCCGTGGACATT SEQ ID No. 23	Gene specific primer amplifying the *TaMLO-D1* target site
MLO-F MLO-R	GTCTTCGCCGTCATGATCATCGTCTCC SEQ ID No. 24 TGGTATTCCAAGGAGGCGGTCTCTGTCT	Amplifying the *TaMLO* target site: This primer can be used to amplify all three alleles

	SEQ ID No. 25	
F1 R1	GTCTTCGCCGTCATGATCATCGTCTCC SEQ ID No. 28 GGTGCTCAGGTAGTGGTTGTC SEQ ID No. 29	Detecting NHEJ-mediated GFP inserts
F2 R2	CTTTGTCGTGAATATAAACCAGACACGAG SEQ ID No. 30 TGGTATTCCAAGGAGGCGGTCTCTGTCT SEQ ID No. 31	Detecting NHEJ-mediated GFP inserts
Ubi-F Ubi-R	CAGTTAGACATGGTCTAAAGGACAATTGAG SEQ ID No. 32 CCAACCACACCACATCATCACAACCAA SEQ ID No. 33	Detecting the absence of TALENs

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Sequence information

SEQ ID NO. 1 Coding sequence of wild type TaMLO-A1:1605bp; The TALEN target site is indicated underlined.

ATGGCGGAGGACGACGGGTACCCCCCGGCGCGGACGCTGCCGGAGACGCCGTCCTGGG
CGGTGGCGCTGGTCTTCGCCGTCATGATCATCGTCTCCGTCCTCCTGGAGCACGCGCTCC
ACAAGCTCGGCCAGTGGTTCCACAAGCGGCACAAGAACGCGCTGGCGGAGGCGCTGGAG
AAGATGAAGGCGGAGCTGATGCTGGTGGGATTCATC<u>TCGCTGCTGCTCGCCGTCACGCAG
GACCCAATCTCCGGGATATGCATCTCCCA</u>GAAGGCCGCCAGCATCATGCGCCCCTGCAAG
GTGGAACCCGGTTCCGTCAAGAGCAAGTACAAGGACTACTACTGCGCCAAAGAGGGCAAG
GTGGCGCTCATGTCCACGGGCAGCCTGCACCAGCTCCACATATTCATCTTCGTGCTAGCC
GTCTTCCATGTCACCTACAGCGTCATCATCATGGCTCTAAGCCGTCTCAAGATGAGAACAT
GGAAGAAATGGGAGACAGAGACCGCCTCCTTGGAATACCAGTTCGCAAATGATCCTGCGC
GGTTCCGCTTCACGCACCAGACGTCGTTCGTGAAGCGGCACCTGGGCCTGTCCAGCACC
CCCGGCGTCAGATGGGTGGTGGCCTTCTTCAGGCAGTTCTTCAGGTCGGTCACCAAGGTG
GACTACCTCACCTTGAGGGCAGGCTTCATCAACGCGCACTTGTCGCAGAACAGCAAGTTC
GACTTCCACAAGTACATCAAGAGGTCCATGGAGGACGACTTCAAAGTCGTCGTTGGCATCA
GCCTCCCGCTGTGGGCTGTGGCGATCCTCACCCTCTTCCTTGATATCGACGGGATCGGCA
CACTCACCTGGGTTTCTTTCATCCCTCTCATCATCCTCTTGTGTGTTGGAACCAAGCTAGAG
ATGATCATCATGGAGATGGCCCTGGAGATCCAGGACCGGTCGAGCGTCATCAAGGGGGC
ACCCGTGGTCGAGCCCAGCAACAAGTTCTTCTGGTTCCACCGCCCCGACTGGGTCCTCTT
CTTCATACACCTGACGCTGTTCCAGAACGCGTTTCAGATGGCACATTTCGTGTGGACAGTG

GCCACGCCCGGCTTGAAGGACTGCTTCCATATGAACATCGGGCTGAGCATCATGAAGGTC
GTGCTGGGGCTGGCTCTCCAGTTCCTGTGCAGCTACATCACCTTCCCCCTCTACGCGCTA
GTCACACAGATGGGATCAAACATGAAGAGGTCCATCTTCGACGAGCAGACAGCCAAGGCG
CTGACCAACTGGCGGAACACGGCCAAGGAGAAGAAGAAGGTCCGAGACACGGACATGCT
GATGGCGCAGATGATCGGCGACGCAACACCCAGCCGAGGCACGTCCCCGATGCCTAGCC
GGGGCTCATCGCCGGTGCACCTGCTTCAGAAGGGCATGGGACGGTCTGACGATCCCCAG
AGCGCACCGACCTCGCCAAGGACCATGGAGGAGGCTAGGGACATGTACCCGGTTGTGGT
GGCGCATCCTGTACACAGACTAAATCCTGCTGACAGGAGAAGGTCGGTCTCTTCATCAGC
CCTCGATGCCGACATCCCCAGCGCAGATTTTTCCTTCAGCCAGGGATGA

SEQ ID NO. 2 Coding sequence of wild type TaMLO-B1:1605bp; The TALEN target site is indicated underlined.

ATGGCGGAGGACGACGGGTACCCCCCAGCGAGGACGCTGCCGGAGACGCCGTCCTGGG
CGGTGGCCCTCGTCTTCGCCGTCATGATCATCGTGTCCGTCCTCCTGGAGCACGCGCTCC
ATAAGCTCGGCCAGTGGTTCCACAAGCGGCACAAGAACGCGCTGGCGGAGGCGCTGGAG
AAGATCAAGGCGGAGCTCATGCTGGTGGGCTTCATC<u>TCGCTGCTGCTCGCCGTGACGCAG
GACCCCATCTCCGGGATATGCATCTCCGA</u>GAAGGCCGCCAGCATCATGCGGCCCTGCAAG
CTGCCCCCTGGCTCCGTCAAGAGCAAGTACAAAGACTACTACTGCGCCAAACAGGGCAAG
GTGTCGCTCATGTCCACGGGCAGCTTGCACCAGCTGCACATATTCATCTTCGTGCTCGCC
GTCTTCCATGTCACCTACAGCGTCATCATCATGGCTCTAAGCCGTCTCAAGATGAGAACCT
GGAAGAAATGGGAGACAGAGACCGCCTCCCTGGAATACCAGTTCGCAAATGATCCTGCGC
GGTTCCGCTTCACGCACCAGACGTCGTTCGTGAAGCGGCACCTGGGCCTCTCCAGCACCC
CCGGCGTCAGATGGGTGGTGGCCTTCTTCAGGCAGTTCTTCAGGTCGGTCACCAAGGTGG
ACTACCTCACCTTGAGGGCAGGCTTCATCAACGCGCATTTGTCGCATAACAGCAAGTTCGA
CTTCCACAAGTACATCAAGAGGTCCATGGAGGACGACTTCAAAGTCGTCGTTGGCATCAGC
CTCCCGCTGTGGTGTGTGGCGATCCTCACCCTCTTCCTTGACATTGACGGGATCGGCACG
CTCACCTGGATTTCTTTCATCCCTCTCGTCATCCTCTTGTGTGTTGGAACCAAGCTGGAGAT
GATCATCATGGAGATGGCCCTGGAGATCCAGGACCGGGCGAGCGTCATCAAGGGGGCGC
CCGTGGTTGAGCCCAGCAACAAGTTCTTCTGGTTCCACCGCCCCGACTGGGTCCTCTTCTT
CATACACCTGACGCTATTCCAGAACGCGTTTCAGATGGCACATTTCGTGTGGACAGTGGCC
ACGCCCGGCTTGAAGAAATGCTTCCATATGCACATCGGGCTGAGCATCATGAAGGTCGTG
CTGGGGCTGGCTCTTCAGTTCCTCTGCAGCTATATCACCTTCCCGCTCTACGCGCTCGTCA
CACAGATGGGATCAAACATGAAGAGGTCCATCTTCGACGAGCAGACGGCCAAGGCGCTGA
CAAACTGGCGGAACACGGCCAAGGAGAAGAAGAAGGTCCGAGACACGGACATGCTGATG
GCGCAGATGATCGGCGACGCGACGCCCAGCCGAGGGGCGTCGCCCATGCCTAGCCGGG
GCTCGTCGCCAGTGCACCTGCTTCACAAGGGCATGGGACGGTCCGACGATCCCCAGAGC
ACGCCAACCTCGCCAAGGGCCATGGAGGAGGCTAGGGACATGTACCCGGTTGTGGTGGC
GCATCCAGTGCACAGACTAAATCCTGCTGACAGGAGAAGGTCGGTCTCGTCGTCGGCACT
CGATGTCGACATTCCCAGCGCAGATTTTTCCTTCAGCCAGGGATGA

SEQ ID NO. 3 Coding sequence of wild type TaMLO-D1:1605bp; The TALEN target site is indicated underlined.

ATGGCGGAGGACGACGGGTACCCCCCGGCGCGGACGCTGCCGGAGACGCCGTCCTGGG
CGGTGGCGCTCGTCTTCGCCGTCATGATCATCGTGTCCGTCCTCCTGGAGCACGCGCTCC
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<u>GACCCAATCTCCGGGATATGCATCTCCGA</u>GAAGGCCGCCAGCATCATGCGGCCCTGCAGC
CTGCCCCCTGGTTCCGTCAAGAGCAAGTACAAAGACTACTACTGCGCCAAAAAGGGCAAG
GTGTCGCTAATGTCCACGGGCAGCTTGCACCAGCTCCACATATTCATCTTCGTGCTCGCCG
TCTTCCATGTCACCTACAGCGTCATCATCATGGCTCTAAGCCGTCTCAAGATGAGGACATG
GAAGAAATGGGAGACAGAGACCGCCTCCTTGGAATACCAGTTCGCAAATGATCCTGCGCG
GTTCCGCTTCACGCACCAGACGTCGTTCGTGAAGCGTCACCTGGGCCTCTCCAGCACCCC
CGGCATCAGATGGGTGGTGGCCTTCTTCAGGCAGTTCTTCAGGTCGGTCACCAAGGTGGA
CTACCTCACCCTGAGGGCAGGCTTCATCAACGCGCATTTGTCGCATAACAGCAAGTTCGAC
TTCCACAAGTACATCAAGAGGTCCATGGAGGACGACTTCAAAGTCGTCGTTGGCATCAGCC
TCCCGCTGTGGTGTGTGGCGATCCTCACCCTCTTCCTTGATATTGACGGGATCGGCACGC
TCACCTGGATTTCTTTCATCCCTCTCGTCATCCTCTTGTGTGTTGGAACCAAGCTGGAGATG
ATCATCATGGAGATGGCCCTGGAGATCCAGGACCGGGCGAGCGTCATCAAGGGGGCGCC
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CGCCCGGCTTGAAGAAATGCTTCCATATGCACATCGGGCTGAGCATCATGAAGGTCGTGC
TGGGGCTGGCTCTTCAGTTCCTCTGCAGCTATATCACCTTCCCGCTCTACGCGCTCGTCAC
ACAGATGGGATCAAACATGAAGAGGTCCATCTTCGACGAGCAGACGGCCAAGGCGCTGAC
AAACTGGCGGAACACGGCCAAGGAGAAGAAGAAGGTCCGAGACACGGACATGCTGATGG
CGCAGATGATCGGCGACGCGACGCCCAGCCGAGGGGCGTCGCCCATGCCTAGCCGGGG
CTCGTCGCCAGTGCACCTGCTTCACAAGGGCATGGGACGGTCCGACGATCCCCAGAGCA
CGCCAACCTCGCCAAGGGCCATGGAGGAGGCTAGGGACATGTACCCGGTTGTGGTGGCG
CATCCAGTGCACAGACTAAATCCTGCTGACAGGAGAAGGTCGGTCTCTTCGTCGGCACTC
GATGCCGACATCCCCAGCGCAGATTTTTCCTTCAGCCAGGGATGA

SEQ ID NO. 4 The amino acid sequence of wild type TaMLO-A1: 534aa.

MAEDDGYPPARTLPETPSWAVALVFAVMIIVSVLLEHALHKLGQWFHKRHKNALAEALEKMKA
ELMLVGFISLLLAVTQDPISGICISQKAASIMRPCKVEPGSVKSKYKDYYCAKEGKVALMSTGSL
HQLHIFIFVLAVFHVTYSVIIMALSRLKMRTWKKWETETASLEYQFANDPARFRFTHQTSFVKRH
LGLSSTPGVRWVVAFFRQFFRSVTKVDYLTLRAGFINAHLSQNSKFDFHKYIKRSMEDDFKVVV
GISLPLWAVAILTLFLDIDGIGTLTWVSFIPLIILLCVGTKLEMIIMEMALEIQDRSSVIKGAPVVEPS
NKFFWFHRPDWVLFFIHLTLFQNAFQMAHFVWTVATPGLKDCFHMNIGLSIMKVVLGLALQFLC
SYITFPLYALVTQMGSNMKRSIFDEQTAKALTNWRNTAKEKKKVRDTDMLMAQMIGDATPSRG

TSPMPSRGSSPVHLLQKGMGRSDDPQSAPTSPRTMEEARDMYPVVVAHPVHRLNPADRRRS
VSSSALDADIPSADFSFSQG

SEQ ID NO. 5 The amino acid sequence of wild type TaMLO-B1: 534aa.

MAEDDGYPPARTLPETPSWAVALVFAVMIIVSVLLEHALHKLGQWFHKRHKNALAEALEKIKAE
LMLVGFISLLLAVTQDPISGICISEKAASIMRPCKLPPGSVKSKYKDYYCAKQGKVSLMSTGSLH
QLHIFIFVLAVFHVTYSVIIMALSRLKMRTWKKWETETASLEYQFANDPARFRFTHQTSFVKRHL
GLSSTPGVRWVVAFFRQFFRSVTKVDYLTLRAGFINAHLSHNSKFDFHKYIKRSMEDDFKVVV
GISLPLWCVAILTLFLDIDGIGTLTWISFIPLVILLCVGTKLEMIIMEMALEIQDRASVIKGAPVVEPS
NKFFWFHRPDWVLFFIHLTLFQNAFQMAHFVWTVATPGLKKCFHMHIGLSIMKVVLGLALQFLC
SYITFPLYALVTQMGSNMKRSIFDEQTAKALTNWRNTAKEKKKVRDTDMLMAQMIGDATPSRG
ASPMPSRGSSPVHLLHKGMGRSDDPQSTPTSPRAMEEARDMYPVVVAHPVHRLNPADRRRS
VSSSALDVDIPSADFSFSQG

SEQ ID NO. 6 The amino acid sequence of wild type TaMLO-D1: 534aa

MAEDDGYPPARTLPETPSWAVALVFAVMIIVSVLLEHALHKLGQWFHKRHKNALAEALEKIKAE
LMLVGFISLLLAVTQDPISGICISEKAASIMRPCSLPPGSVKSKYKDYYCAKKGKVSLMSTGSLH
QLHIFIFVLAVFHVTYSVIIMALSRLKMRTWKKWETETASLEYQFANDPARFRFTHQTSFVKRHL
GLSSTPGIRWVVAFFRQFFRSVTKVDYLTLRAGFINAHLSHNSKFDFHKYIKRSMEDDFKVVVGI
SLPLWCVAILTLFLDIDGIGTLTWISFIPLVILLCVGTKLEMIIMEMALEIQDRASVIKGAPVVEPSN
KFFWFHRPDWVLFFIHLTLFQNAFQMAHFVWTVATPGLKKCFHMHIGLSIMKVVLGLALQFLCS
YITFPLYALVTQMGSNMKRSIFDEQTAKALTNWRNTAKEKKKVRDTDMLMAQMIGDATPSRGA
SPMPSRGSSPVHLLHKGMGRSDDPQSTPTSPRAMEEARDMYPVVVAHPVHRLNPADRRRSV
SSSALDADIPSADFSFSQG

SEQ ID NO. 11 The coding sequence of TALENs (TAL-L + TAL-R) in vector pYP010.

ATGGTGGATCTACGCACGCTCGGCTACAGTCAGCAGCAGCAAGAGAAGATCAAACCGAAG
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GAAACTACAAAGCTCAGCTTACACGATTAAATCATATCACTAATTGTAATGGAGCTGTTCTT
AGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGG
AAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAATAG

SEQ ID NO. 12 The amino acid sequence of the TALENs.

MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVTYQHIIT
ALPEATHEDIVGVGKQWSGARALEALLTDAGELRGPPLQLDTGQLVKIAKRGGVTAMEAVHAS
RNALTGAPLNLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQAL
ETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNGG
GKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAI
ASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTP
DQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQD
HGLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLP
VLCQDHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETV
QRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNNGGKQ
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NPGPRMDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPASMVDLRTLGYSQQQQEKI
KPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQW
SGARALEALLTDAGELRGPPLQLDTGQLVKIAKRGGVTAMEAVHASRNALTGAPLNLTPDQVV
AIASNKGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNKGGKQALETVQRLLPVLCQDHGLT
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KQALETVQRLLPVLCQDHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIA
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QVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNKGGKQALESIVAQLSRPDPALA
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YGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRNKHLNPNEWWKVYPSSVTEFKFLFVSGH
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Nucleic acid sequence of Tamlo-A in lineTamlo-R32 (coding sequence)
SEQ ID NO. 38

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901     AATACCAGTT CGCAAATGGT CAGACAATTT TCCAAATGAA ACCTCTTCTG TTTTGATGCG
961     TTTACAGAGG CAGGCATGAT CAGAGCGAGT GAACTGATGA TATGTTCTTC TCTTTCCCGT
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1081    GGGCCTGTCC AGCACCCCCG GCGTCAGATG GGTGGTGGCC TTCTTCAGGC AGTTCTTCAG
1141    GTCGGTCACC AAGGTGGACT ACCTCACCTT GAGGGCAGGC TTCATCAACG TACGTAATAC
1201    CCCAAAAGCC CCCTCTCCTT CTAGCTCCGT CGGCCATTGC CGCGACGCTT CTGAAATAAG
1261    TACTGTTCCA ACACCAATGA TCACATGCTC TCTCTTTCCA TGATTCTGCG CAGGCGCACT
1321    TGTCGCAGAA CAGCAAGTTC GACTTCCACA AGTACATCAA GAGGTCCATG GAGGACGACT
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2101    GGATGAACTT GTCAGTTAAT AATATGGGTG TCAAGGCACC AAGTGCTGCT GCTGATGAAC
2161    TGCACTGACA GAGATTTACC TGTGTCGCAG GCCACGCCCG GCTTGAAGGA CTGCTTCCAT
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2461    GGCGCTGACC AACTGGCGGA ACACGGCCAA GGAGAAGAAG AAGGTCCGAG ACACGGACAT
2521    GCTGATGGCG CAGATGATCG GCGACGCAAC ACCCAGCCGA GGCACGTCCC CGATGCCTAG
2581    CCGGGGCTCA TCGCCGGTGC ACCTGCTTCA GAAGGGCATG GGACGGTCTG ACGATCCCCA
2641    GAGCGCACCG ACCTCGCCAA GGACCATGGA GGAGGCTAGG GACATGTACC CGGTTGTGGT
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Nucleic acid sequence of Tamlo-D1 in lineTamlo-R32 (coding sequence)
SEQ ID NO. 39

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421     GCCTGCTACA AGCTACTCCC GGAGACGGCC GGGAAAAACA CAAACAGATT CCGGCGGCCG
481     GCCGGAGTTT CTTCTTGTTT CCTTCCTGAT TGGCTTGGCC TAATTGGTGT GTGTTTTTCT
541     GGCAGGGCAA GGTGTCGCTA ATGTCCACGG GCAGCTTGCA CCAGCTCCAC ATATTCATCT
601     TCGTGCTCGC CGTCTTCCAT GTCACCTACA GCGTCATCAT CATGGCTCTA AGCCGTCTCA
661     AAGTGAGTCT GTCAGGCCTA CCTGTTCATG CTTCGGTAAA GCAATAAAAC TACTTGCTAC
721     CAATCCCTAA TCTGCTCCCT CAGGCATAAT ATTGTTCCTT CTTTCCTGCT GCAGATGAGG
781     ACATGGAAGA AATGGGAGAC AGAGACCGCC TCCTTGGAAT ACCAGTTCGC AAATGGTCAG
841     ACAATTTCCG AAATGAAACC TGACTGATGC ATTTACAAAC GCACGCAGGC AGGCATGATC
901     AGAGTGAGTG AACTGATGAT ATGTTTTCTC TCTCTTTCCC GTGCCTCCAG ATCCTGCGCG
961     GTTCCGCTTC ACGCACCAGA CGTCGTTCGT GAAGCGTCAC CTGGGCCTCT CCAGCACCCC
1021    CGGCATCAGA TGGGTGGTGG CCTTCTTCAG GCAGTTCTTC AGGTCGGTCA CCAAGGTGGA
1081    CTACCTCACC CTGAGGGCAG GCTTCATCAA CGTACGTACC AAAACAAATC CTCTCCCTCT
1141    AGCTTCGCCA TTGCTGCGAC GCTTCTGAAA TATGTACCGT TCCGACACCA GCGATCTCAT
1201    GTCTTCTCTT TCCACGATTC TGCGCAGGCG CATTTGTCGC ATAACAGCAA GTTCGACTTC
1261    CACAAGTACA TCAAGAGGTC CATGGAGGAC GACTTCAAAG TCGTCGTTGG CATCAGGTAG
1321    GTTACATTCC ATGGATAGGA TTATAAAATT GCCGTCAGGC TCCATATGAT ATTGCTTAGG
1381    TTCCACATGA TACAATACTA TCAGTTTGCT GCGTCATGGT CTTTGCCCCT GCTGGTCTTC
1441    CTTGCGTGAT CTTGACACAT TTGGCCTCTT TTCGCAGCCT CCCGCTGTGG TGTGTGGCGA
1501    TCCTCACCCT CTTCCTTGAT ATTGACGGTA TGGACCTTGC TAAAACACTT GTAATTTGTC
1561    TCGTAACCAC CGTTCATTTT CTAACCTTCC TTTCCCCTTC TTTCTGCTGG CAGGGATCGG
1621    CACGCTCACC TGGATTTCTT TCATCCCTCT CGTCGTAAGT GCGAATTTCT CCGCCGAAAG
1681    CAACAGCCAG CCCCATTTGA TTGCAATGCG AAACCACACC TTAATTGAAA ATGTCATTGT
1741    CTGTCTTGTC TTTCTCAGAT CCTCTTGTGT GTTGGAACCA AGCTGGAGAT GATCATCATG
1801    GAGATGGCCC TGGAGATCCA GGACCGGGCG AGCGTCATCA AGGGGGCGCC CGTGGTTGAG
1861    CCCAGCAACA AGTTCTTCTG GTTCCACCGC CCCGACTGGG TCCTCTTCTT CATACACCTG
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1921    ACGCTGTTCC AGAATGCGTT TCAGATGGCA CATTTCGTCT GGACAGTGGT ATGTACCAGT
1981    AATTGGCAGT TCAGTTAGGG ATGCAAGGCA CCAAGTAGTG CTGATGAACT GCACTGACGG
2041    AGATTTACTT GTTCGTAGGC CACGCCCGGC TTGAAGAAAT GCTTCCATAT GCACATCGGG
2101    CTGAGCATCA TGAAGGTCGT GCTGGGGCTG GCTCTTCAGT TCCTCTGCAG CTATATCACC
2161    TTCCCGCTCT ACGCGCTCGT CACACAGGTA ATAAAGCCGT TGATGAAGAT GTCTGAACAA
2221    TTGCTCTGGG AGAGGAGAAA CAGCAGCCTT AATCATGTAA TCGGTGTGAT GGGTTGCAGA
2281    TGGGATCAAA CATGAAGAGG TCCATCTTCG ACGAGCAGAC GGCCAAGGCG CTGACAAACT
2341    GGCGGAACAC GGCCAAGGAG AAGAAGAAGG TCCGAGACAC GGACATGCTG ATGGCGCAGA
2401    TGATCGGCGA CGCGACGCCC AGCCGAGGGG CGTCGCCCAT GCCTAGCCGG GGCTCGTCGC
2461    CAGTGCACCT GCTTCACAAG GGCATGGGAC GGTCCGACGA TCCCCAGAGC ACGCCAACCT
2521    CGCCAAGGGC CATGGAGGAG GCTAGGGACA TGTACCCGGT TGTGGTGGCG CATCCAGTGC
2581    ACAGACTAAA TCCTGCTGAC AGGAGAAGGT CGGTCTCTTC GTCGGCACTC GATGCCGACA
2641    TCCCCAGCGC AGATTTTTCC TTCAGCCAGG GATGA
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Tamlo-R32-A upstream sequence (~3000bp); ATG start codon in bold and underlined SEQ ID NO. 40

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1       GTGCGCCACT GCTATATAGC AGTGGCGCAC CACCATCATG GTGCGCCACT AATAGGGATA
61      TTGGCTATAG CCATTTTTCT AGTAGTGTAA GCACAAGAAA TAAAAAAAAT ATGGAAAACC
121     CTCACATCTC ATCTTAAATT CTCAGAGTTA GTAATACGAA ATTCAACGCA AATCAGGGAG
181     TAGGACAACG AGACGAAAGT GATTCCCCCG TAGCTCTTTT ATTTCGCGAG GGCTCTGATC
241     ATGTATAGCT AGCCATGCAT AGACAACATG ACAGGCATGT TTTGGGTGCC CACAGCACAC
301     ACAAGTTGTG AAACAGTACG TGCATGACTG GGCACAGAGC AGGTTAGAGC AAACCTCCAC
361     ATCACCATAA ATTCCGAGTA GCCACTAGAT TCAGCATGCC TGTTTAGGGT TCTGTACAGT
421     ACGTACCTGG CTACTACTGC TTGCCAATTG AAAAATGATT TAGAGCAAAT TCCAGAATGC
481     CATGACACAT CTGCTTTTAT GTCAAACCCA CTGTTCATAA CAATATTTGT GCGGTGGTGT
541     GCATGAGATA AGATCCGGAT GAGGGTGGCG CCCATAAAAA ATGGGCCATT CATGAACCAG
601     CAGCGTCAAC ACGAACGAGC GACGAAGCCG CGGGCTACGA GCGTCACCAC ACAATATGTT
661     AATGGATCGG GCCGGCCATC CCATCGGCCA TCAGGGTGTG CTGCCAAGCA GATCTCCATG
721     CATGATGCAT CACGGGTGCA CCTAGTAGCC ACATAGATCT CCAGACTCCA GGCATGATGC
781     ATCACGGGTG CATCTGTATA TTCAAACCTA CCATTAACTT TGCCCGATCA GAGGAACCGG
841     TCCGGATCCG ATCGTTAATT CGGCGACCAG TGACTTGATC GCGTCTGTTT AAGCACTAGC
901     AGCTCACTGA TCGCATGGAT CGACCGCTGG TAAGAATAGT ACACCCTGTG CATATACAAG
961     TCCGCGAAAA AGTAGCAGCC ACGAATGCAG TCAACGTTTT TATTTGACCT GACTCGCTCG
1021    ATCAGGCCCG TACTCCACTG GTTGAAACGC CCACTTCGCC GGAGCCGCCT GGTCAGACTT
1081    TTCCACGCAC GACCGACCAT TGAGCAGTCA AAGTTCGGAT GCCACGCCGT CGCTCGCATG
1141    CGGACGTACG TGTGCAAGTC GTCGCAACTT GCGTGCTACA GAAATTCAAA ACAAAACAAA
1201    AAAACTTGCG TGGCACAGTA CGAGACTACA AGCGAGTAGA AGCGCACCAC GTATGCCCGT
1261    GTATCTGCAG TAACGGAACC GTGCACGTTT TGGCTAAACG TGCGCATGCA GCAGGGTGCA
1321    CGTCCACGTC CTGCAGGTTT AAGTATATAA TGTAGCTTAC AGTAATTAAC CATGCATGCT
1381    TCGAAATGAA GCACTGCCTG CCGGGCGCCG GCGACCTGAT CCACCACCAC CCGACGCGCG
1441    GCTCGCCGGC GGGAACAGCC AGTCGCGCGC GTGGACCTCT CGCCTCTACC AACGTGTGGC
1501    TACGTGTAAC CGTGCTCCGT AAAACCGTGT TGGTTTTACC TTTACCTTTC TCTCGCCCGC
1561    ACACACGTCG CCGCCAAGAC ATGCGTCGCG TCATTTTCTC CAAATAACTT TGGCGCGAAC
1621    GGGTCTCCGA TCGAGCAGCA CCAAATCAAT CAACCCAACG AAAGTGATCC GACGTCACAA
1681    AATTCGATCC CCCGAGAAAC TGGCAGCACT TTTGCCGTTT TCTTGCCGGT CCCAACGAAC
1741    TCTCCGTCCC TCCTAATTTA ATGTCAAAAA AATATAAAAA AATCTCCGCC TGCGTTGATG
1801    ATCCCAATAA CCAGCAAGCT GTCCTACGGG ATCATTCAGG AGCTTTTAGA GCTGCTACTT
1861    GTCATCTCTT TGATGGAATC GCCGATTCGG AGGTTGCTGA AATTTATGCC TGCAAACGAG
1921    CTTTATAGGT GGCGGCCGAA CTCAACACAT CCAAGCTGTT GTTGGAGACG GACTGTGCTA
1981    ATCTAGCAAA GATGTTGTGT GCGCAAGAGA AAACTCTCTC TGCATTTGGA CCTCTGGTGA
2041    AGGAGATCAA GGAGAGGATG AAAATGTTCC AAGAAGTGAA AATGTCTTGC GTAAGGCGTA
2101    GTGCTAATGC TGCCGCGGAT AAGTTAGCTA AAGTTGGGTT AAGTGATAGA CTGTGTAAGG
2161    TTTGGTTTGC CGTTCCCCCA GATTGTATTC TGGGCATTGT GTCGGACGAG ATTCCTAATT
2221    TCATTTAATT AGTCAATAAA GCGGCAGTAG TTGATCCTCA AAAAAAATAA CCAGCAAGCT
2281    AGCCGGACGC GTCGGTTTTT GTCCTGCCTA AGCTAGGAGT ATCTCCAAGT AACCTACGCG
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2341 GGACAAAACT ATGGCCAGAT AGACACTAGT CAAACGATCG CAACAAGAAA AAAACTAGTC
2401 AAGAAAAATA CTACAGATTA CCTAAAGAAA AAAAATAGAA AACCAAAACA AAAATACTGG
2461 TAAAGTGACC GTCCCCGTCA AAAAATACTT GCCGACCGAC CGGGTGTCCC CCGTCGCCCC
2521 GGCCCGGTGC CGGCCGAGCA CCCCGCCCAG AGCGCCATCA CTGGATCAAC CACCCCGTCC
2581 AACCGCGCGC TACGAAACAT CGGTCGTTTC TCACGGTGCA ATCTCAGCCG GAAACCGGCG
2641 CTCGCGCGCA TCAGCTGTAG CCTGTAGGTC TCGGGCTCCG CAGCGCCGCT GCCGAGCCAC
2701 CCGGCCGGCG CGCACGCACG CACGCGCTTT GACCCGGCCG CCGATAAAAG GCCCCGCGCG
2761 GCAGCTCCCT CCTACCCGGT TGCCACACCC ACAGTCTGCC ACAGCAGCAA CAAGCTAGAC
2821 ATACCTGCGT GCGTACGTAC GTTTTCGTTT TCCTTTCTTG CTCCGGCCGG CCGGCCGGCC
2881 ACGTAGAATA GATACCTGCC CAGGTACGTA CCTCGTTGGC TCAGACGATC GGCGGTTGGA
2941 CTTGGGTGCG CGCCCTGCCC TGCTCCGGCC AAGGAAAGAG GTTGCGCTAA AGACGGGCGG
3001 **ATG**

Tamlo-R32-B upstream sequence (~3500bp); ATG start codon in bold and underlined

SEQ ID NO. 41

1 CCCCGTAGCT CTTTTATTTC GCGAGGGCTT TGATCATGTA TAGCTAGCCA TAAACAACAT
61 GACAGGCATG TTTTGGGTGC CCACAGCACA CACAAGTTGT GAAACAGTAC GTACATGACT
121 GGGCACAGAG CAGGTTAGAG GAAACCTCCA CATCATCATA AATTCTGAGG AGCCACTAGA
181 TTCAGCATGT CTGTTTAGGG TTCTGGCTAC TTGCCAATAA AAAAATATTA TGATTTACTA
241 GCATAGATTC CAGAATGCCA TGACATTTCT GCTTTGATTT CAATCCACTG CTCATAACAG
301 AAGCATATGG CCCGGACTCA TTAACTTGGT CGTTCCTCAT GATTTGTTCT AGTCTCGTTT
361 TATCTCACAA GATGCTTGTT CACAAGGTTG TCAGAATCGC GATTCTGAAT CGGATCGGAG
421 CTCCAATGGC AGGATCACAA ATCATAGAAT CTTCACTATC AGGATCGTGA AAACGTAGAT
481 TCTATGAACC AAAATCATAA AATCAGAGGG GTTAGTTTGA ATCGTAAAAT CGTAGAATCG
541 TACAACATAA TCGCGATTCT GACAACCTTG CTTGTTCATT TGCTGCTATA TATATTAGGA
601 CCATGCATAT TGGTCACACG AGGGCAGCGC TGCAAGTGCA AAGTCGCCGA GACAAGACTG
661 AGCACCGTTT CATGGGCTTG ATCTCTTGGT AAGCAGCCGC CGCCGGACCA TCATCAGCCA
721 AGAAAGACAC ATTCTTGTGC TACTATATTT GTGCGGTTGC GCGCATGAGA TAAGATCCGG
781 ATGAGGATGG CGCGCATAAA AAAATGAGCA ATGTCAAAGC AGTGTACCCT GAGCTTCCTT
841 CCATTCATGA ACCAGTAGCG TCAACTACAG GAACGAGCAA CGAACCGTCA CCTTATATTA
901 GTGGATCGGG CCCATCCATC CCATCAGGGT GTGCCGTCAA GCAGATCTCC ATGCATGCAT
961 CTCGGATTGC ACCTAGTAGC CACATAAACA GAGGCTGATT AGTACTACTA CAAAGGTACC
1021 GGCTAGGCCA AATCATCTCG CCTCGTTGAA ATTCAAACCT GCCATTAACT TTCCCCGATC
1081 AGAAGAAACG GTCCGGATCC GATCGTTAAT TCGGCGACCA GTGACTTGAT CTCGTCCGTT
1141 TAAGCACTAT ACTAGCAGCA GATCACTGAT CACATGGATG GACCGCTGCT AAGAATAGTA
1201 TATCCTTCCT GCATATACAA GTCCGCAAAA AAGTAGCAGC CACACAAATG CAGTCAACGC
1261 TCCATTTGAC TTGACCCGCT CCATCAGGCC CGTACTCCAC TGGTTGAAAC GCCCACTTCG
1321 CCGGAGCGGC GTGGTCGACT TCTCCACGCA GGGGACCGAC CATGAGCAGT CAAACTTGGG
1381 ATGCCACGTC GACCGACGTG TGCAAGTCGT CGCAACTTGC TTGGCACAGT ACGAGACCAC
1441 AAGCGAGCAG GAGTGCGCCA CGTATACGTG ACGGGCCCGT TTGCCTGCAG TGACGGAACC
1501 GTGCACGCTT TGGCTAAATA TAAACGTGCG CATGCAGCAG GGCTTACAAG AACCATTAAG
1561 TAACTTTCAC GTCCACGTCG TACAGTACAT GTTTATATAT AACGTCGTAA ACTACAGTTA
1621 GCGCATGCTC TAGCGGCATA CGGTGCCAGC CGACTGATGG TCCGGCAAGT TTGGGCTGAT
1681 GACCTACCTG ATGATGTAAA CGTTCAGATG GCCAGCGTTT TGCCTGCGCC CGTGTGATTT
1741 ATGGAATCTG GGTGTTCCAT TTAAAAAAAA AAACCCATTC ATGCTTCGAA ATGAAGCATG
1801 GAGGAAGTCG GACGTCACAC AATTCGATCG ATCGACCCAT CGTTTTTCTC GGCCGGGGAA
1861 GAGGCAAGGC GGGCACAGTT TTGCCCTTTT CGATCGTTTG GTCCGTCCCA ACAGATTCTC
1921 CGTCCCCATT AATCAAGTCC AAAACAGGAA TACATGCAGC AATACTCTAT GCTTGTCCAA

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1981    TTAGCAATTA CTCTCACGTC AACCGCTGGC GATTAACAAT GGCTCTCCGT ATGAAAAACT
2041    AACTCGATGG GAGCACCAGG CTAGCCATCG TGCACGCACG TCCCGGCCGG TGAATGTTTC
2101    GACCGTCTGG GTACGAGCCC GACCCGCTCG AAGGTGCCAC GCCCCTGCCT ACCAGGCGCC
2161    GGCGACCTGA TCCACCACCC GACGCGCGGC TCGCCGGCGG GAACAGTCAG TCGCGTTGAC
2221    CTCTCGCCTC TACCAACGTG TGGCTACGTG TAACCGTGGT CCGTAAACCC GTGTTCGTTT
2281    TACCTTACCT TTCTCACGCG CACAATACAT GTTTCGCGTC ATTTTCTCCA CGTAAAACTT
2341    TGGCGCGAAC GGGTCTCCGA TCGAGCAGCA TCAAATCAAT CAACCCAACG AAAGTGATCC
2401    GACGTCACAC AATTCGATTC CCCAAGAAAC GGGGCAGCAC ATTTGCCGTT TCCTTGCCGG
2461    TCCAACGAAC TCTCCGTCCT AATTTAACGT CAGTTTTTTT TCTCCGCCCG CGTTGATGAT
2521    CCCGATAACC AGCAAGCTAG CCAGACGCGT CGGCTTTTGT CCTGCTTAGC TAGGAGTATC
2581    TCCAAGTAAC CTTACCTACG CGGGACAAAA CTATGGCCAG ATATAGATAT ACTAGTCAAA
2641    CGATGGCAAC AAGAACAAAA AAAAACTACT CCCTCCGCTT CTAAATATAA GTTTTTCTAG
2701    AGATTTTACT ATAAACTATA TACGGACGTA TATAGACAAA ATTTAAGTGT ATATTCACTT
2761    ATTTTGCTCT GTATGTAGTT TTTTGTTGGA ATCTCTAAAA AGAAATATAG GAGTATTTAG
2821    GAACAGAGGG AGTAGTCAAG AATAATACTA CGGATTCCCT AAAGGAAAAA ATAGAAAAAA
2881    AATACTACTA GTATTTTTTG AGAAATAATA CTACAAGTAA AGTGACCGTC TCTGTCAGAA
2941    AATACTACGG GACCGACCGG GTGTTCCCCC TCGCCCCGGC CCGGTGCCGG CCGAGCACCC
3001    AGAGTGCCAT CACTGGATCA ACCACCCCGT CCAACCTCGC GCTAGGAAAC ATAGCTCGAT
3061    CCCTCAAACA AAAAAAAAAA GGAAACATAG CTCGTATCAG CCGAAACCCG CCACTCGACA
3121    TTCGTATCAG CTCTAGGCAG GTCTCCCGCT CCGCAGCGCG CCGCTGCCGA GCCACCCGGC
3181    CGGCGCGCAG GCGCGCACGC ACGCGGTTTG ACCCGGCCGC CGCGCGCCCG CGCCGCGCCG
3241    ATAAAAGGCC CCGCGCGGCA GCTCCCTCCC ACCCGGTTGC CACGCCCACA CTTCGCCAAC
3301    ACACAACGTA CCTGCGTACG TACGCTTTCC ATTTCCTTTC TTGCTCCGGC CGGCCGGCCA
3361    CGTAGAATAG ATACCCGGCC AGGTAGGTAC CTCGTTGGCT CAGACGACCG GCGGCTGGGT
3421    CTCCGGACAA GGAAAGAGGT TGCGCTCGGG GACCGATG
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ATG

Tamlo-R32-D upstream sequence (~3500bp); ATG start codon in bold and underlined

SEQ ID NO. 42

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1       GAGGGAAATG TTTTAGAACT GGGCGAGGGC CCGGACTCAT TAACTTGGCT GTTCCTCATG
61      ATCTGTTCTT GTCTCGTTTT ATCTCAGGAG ATGCTTGTTC ATTTGTTGCT ATATAATACT
121     TCCTCCGTTC GGAATTACTT GTCGCAGAAA TGGATGTATC TAGACATATT TTAATTTTAG
181     ATACATTCAT TTTCGAGACA AGTAATTCCG AATGGAGGGA GTACCCATGC ATATTCGTCT
241     CACGAGGGCA GCGCTGCAAA TGCAAAGTCT CGCCGAGACA AGACCGGTCA CCCTTTTCAT
301     GAGCTTGATC TCTTGGTAAG CAGCCCCCGC CGGACCATCA TAATAACTTC ATAAGCCGGG
361     AAAGACCCAT TTGTGGTACG TACTAATACT ATATTTGTGC GGTTGTGCGC ATGAGATAAG
421     ATCCGGTTGA GGGTGGCGCG CATAAAAAAT GGGCTATGTC AAAGCAATAT CCCCTGAGCC
481     TCCATCCATG AACCAGTAGC GTCCGTCAAC TACACGAACG AGCGACGAGG CCGCGCGCTA
541     CGAGCGCCAC CATATACGTA CGTATATATT AGTGGATCGG GCCATTAGCA TAAGATCTCC
601     ATGCATGCAT GTCGGATAGT ACATCTCGAA ATAGTCTTTC GCCCCGCTTT ATCTCGGATG
661     CACCTAGTAG CCACATAGAC AGGCCAAATC ATCGCTTGCT AAAAGAACTG AGCTAGTAGT
721     AGTACTGGCA TCTCTTGATG TGCCTCGTTG AAATTCAAAC CGACCATTAA CTTTCCCCGA
781     TCAGAGGAAC CGGTCCGGAT CCGATCGTTA GTTCGGCGAC GGGCGACTTG ATCCCGTCTG
841     TTTAAGCACT AGTAGTAGCA GATCACTCAT CACATGGACG GACCGCTGCT AATAATTAAT
901     AGTATACCTG CCTGCTGTGC ATATACAAGT CCTGGTAAAA GTAGCAGCCA CACAAATGCA
961     GTCAACGCTT CGTTTGACTT GACTCGCTCA GGCCCGTAGC CGTACTCCAC TGGATCTGGA
1021    TGGAACGCCC GCTTCGCCGG AGCTGCCTGG TCAGACTTCT CCACGCACGC ACGACCGACC
1081    ATGGGCAGTC AAACTTCGGA TGCCACGTCG ACGTCCACGT TGTCGGTCGC ATGCGGACGT
1141    GCGTGTGCAG GTCGTCGCAA CTTGCGTGGT ACAGTACGAG ACTACTCCGT ACAAGCGAGT
1201    AGAAGTGCAC CACGTATACG TGCCGGGCCC GTTTACCTGC AGTAACGGAA CCGTGCACGC
1261    TTTGGCTATA CGTGCGCATG CAGCAGGCTG CACGTCGATG CCGTGCAGGT TTTATAATGT
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1321  AGGAGTATAC TGTAACTACC TTACAATTAA TAACCATGGA TGGATGCTTC GAAATGAAGC
1381  ATGGAGGAAG CCCGACGTCA CACAGTTCGA TCGCCCGATC CCTCGTTTTT CCCGGCCGGG
1441  GAAGAGACAA GAGAAACAGA GCTTTGCCCT TTTCGATCGT CTGGTCTGTC CCAACAGACT
1501  CTCCGTCCTC ATTAATCAAG TCCAAAACAG GAATACATGC AGCAATACTG TATGCTTGCC
1561  AAATTAGCAA TCACTATCAC GTCAACCGGG GGCGATTAAC AATGGCCCCT CCGTATGAAA
1621  AACTAACTCG ATGGGAGCAC CAGGCTAGCC ATCGTACACG CACGTCCCGG CCGGTGAATG
1681  TTTCGACCGT CTGGGTACGA GTCTGACCCG CTCGAAGGTG CCACGCCCCT GCCTGCCGGG
1741  CGCCGGCGAC CTGATCCACC ACCACCCGAC GCGCGGCTCG CCAGCGGGAA CAGTCAGTCG
1801  CGCGCGTGGA CGGCGAGTCT CGCCTCTACC AACGTGTGGC TACGTGTAAC CGTGCTCCGT
1861  AAAACCGTGT TCGTTTTACC TTACCTTTCT CGCGCGCACA CACGTCGCCG CCAATACATG
1921  TTTCGCGTCA TTTTCTCCAC GCAATAACTT TGGCGCGAAC GGGTCTCCGA TCGAGCGGCA
1981  TCAAATCAAT CAACCCAACA AAAGTGATCC GACGTCACAC AATTCGATCC CCCAAGAAAC
2041  GGGGCAGCAC ATTTGCCGTT TTCTTGCCGG TCCCAACGAA CTCTCCGTCC TAATTTAACG
2101  TCAGTTTTTT TTCTCCGCCC GCGTTGATGA TCCCGATAAC GAGCAAGCTA GCCAGACGCG
2161  TCGGTTTTTG TCCTGCCTAG CTAGGAGTAT CTCCAAGTAA CCTACCTACG CGGGACAAAA
2221  CTATGGCCAG ATATAGATAT ACTAGTCAAA CGATGGCAAC AAGAAAAAAA ACTAGTCAAG
2281  AATAATACTC CCTCCATTCT AAATTACTTG TCGCAGGTAT GAATGTATCT AGATGTATTT
2341  TAGTTCTAGA TACATCCATT TCTGCAACGA GTAATTTGAA ACGGAGGGAG TACTACGGAT
2401  TCCCTAAAGA AAAAAATACT ACTAAAAACT AGTACTAGTA GTAAAGTGAC CGTCCCCATC
2461  AAGAAATACT ACGGGACCGA CCGGGTGTCC CCCCTCGCCC CGGCCCGGTG CCGGCCGAGC
2521  ACCCAGAGCG CCATCGCTGG ATCAACCACC CCGTCCAACC TCGCGCTAGG AAACATAGGT
2581  CGTTTCAGCC GAAACCCGCC ACTCGACATT CGTATCAGCT CTAGGCAGGT CTCCCGCTCC
2641  GCAGCGCCGC TGCCGAGCCA CCCGGCCGGC GCGCAGGCCT AGGTTTGACC CGGCCGCCGG
2701  GCGCCCGGCC GATAAAAGGC CCCGCGCGGC AGCTCCCTCC CACCCGGTTG CCACGCACAC
2761  ACTTCGCCAC AGCAGAAACA AGCTAGACAC ACAACGTACC TGCGTACGTA CGCTTTCCTT
2821  CTCCTTGCTT GCTCCGGCCG GCCGGCCACG TAGAATAGAT ACCTGGCCAG GTAGGTACCT
2881  CGTTGGCTCA GACGATCGGT GGTTGGGCTC GGGCGCGCGC CTGTCCGGCT GAGGTGGCCG
2941  CCGTTCGCTC CGGCCAAGGA AAGAGGTTGT GCTCAGGACG GGCGGCGGGG AGCCATG
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CLAIMS:

1. A wheat plant, plant part or plant cell that has increased resistance to powdery mildew compared to a wild type wheat plant and comparable yield under non-disease conditions compared to a wild type wheat plant wherein said plant comprises a loss of function mutation in the coding regions of two alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and reduced expression of the third *TaMLO* allele.
2. A wheat plant, plant part or plant cell according to claim 1 wherein said third *TaMLO* allele does not contain a mutation in the coding region compared to the wild type allele.
3. A wheat plant, plant part or plant cell according to claim 1 wherein said plant comprises a loss of function mutation in the coding regions of *TaMLO-A1* and *TaMLO-D1* and reduced expression of *TaMLO-B1*.
4. A wheat plant, plant part or plant cell according to claim 3 wherein said reduced expression of *TaMLO-B1* is caused by a mutation in the regulatory region of *TaMLO-B1*, a mutation in a gene downstream in the MLO pathogen response pathway or an epigenetic factor.
5. A wheat plant, plant part or plant cell according to claim 1 wherein said mutation is introduced using targeted genome modification.
6. A wheat plant, plant part or plant cell according to claim 5 wherein said mutation is introduced using a rare-cutting endonuclease, for example a TALEN, ZFN or CRISPR/Cas9.
7. A wheat plant, plant part or plant cell according to claim 6 wherein said mutation is introduced using a TALEN and wherein said TAL effector binds to TCGCTGCTGCTCGCCGTgacgcaggaccccatctcCGGGATATGCATCTCCGA (SEQ ID NO. 13)
8. A wheat plant, plant part or plant cell according to claim 1 wherein said mutation is an insertion, deletion or substitution.
9. A wheat plant, plant part or plant cell according to claim 1 wherein said wheat plant is selected from the list that includes, but is not limited to, *Triticum aestivum, T. aethiopicum, T. araraticum, T. boeoticum, T. carthlicum, T. compactum, T. dicoccoides, T. dicoccum, T. durum, T. ispahanicum, T. karamyschevii, T. macha, T. militinae, T. monococcum, T. polonicum, T.*

repens, T. spelta, T. sphaerococcum, T. timopheevii, T. turanicum, T. turgidum, T. urartu, T. vavilovii and *T. zhukovskyi.*

10. A wheat plant, plant part or plant cell according to claim 1 wherein said mutation is in a *TaMLO-A1* allele having a wild type sequence of SEQ ID NO. 1 or a functional variant thereof with at least 75% sequence identity to said wild type sequence.
11. A wheat plant, plant part or plant cell according to claim 1 wherein the mutation is in a *TaMLO-B1* allele having a wild type sequence of SEQ ID NO. 2 or a functional variant thereof with at least 75% sequence identity to said wild type sequence.
12. A wheat plant, plant part or plant cell according to claim 1 wherein the mutation is in a *TaMLO-D1* allele having a wild type sequence of SEQ ID NO. 3 or a functional variant thereof with at least 75% sequence identity to said wild type sequence.
13. A wheat plant, plant part or plant cell according to claim 1 wherein said plant does not comprise a transgene.
14. A wheat plant, plant part or plant cell according to claim 1 comprising a *Tamlo-a* sequence as shown in SEQ ID No. 38, a *Tamlo-d* sequence as shown in SEQ ID No. 39 and a *TaMLO-B1* sequence having a wild type sequence of SEQ ID NO. 2.
15. A wheat plant, plant part or plant cell or part thereof wherein said wheat genotype has been deposited under CGMCC Accession Number 10951.
16. A plant part or plant seed according to a preceding claim.
17. A method for producing a wheat plant, plant part or plant cell with increased resistance to powdery mildew compared to a wild type plant and comparable yield under non-disease conditions compared to a wild type wheat plant using targeted genome modification comprising introducing a loss of function mutation into the coding regions of two *MLO* alleles selected from *TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1* and decreasing expression of the third *TaMLO* allele.
18. A method according to claim 17 wherein said third *TaMLO* allele does not comprise a mutation in the coding region compared to the wild type allele.
19. A method according to claim 17 comprising introducing a loss of function mutation in the coding regions of *TaMLO-A1, TaMLO-D1* and reducing expression of *TaMLO-B1*.

20. A method according to claim 19 wherein *TaMLO-B1* comprises a mutation in the regulatory region.
21. A method according to claims 17 and 20 wherein said mutation is introduced using a rare-cutting endonuclease, for example a TALEN, ZFN or CRISPR/Cas9.
22. A method according to claim 21 wherein screening for induced targeted mutations in a *TaMLO-A1*, *TaMLO-B1* and/or *TaMLO-D1* allele comprises obtaining a nucleic acid sample from a said plant and carrying out nucleic acid amplification and optionally restriction enzyme digestion to detect a mutation in a *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1* allele.
23. A method according to claim 22 comprising confirming the presence of the mutation by sequencing the *TaMLO-A1, TaMLO-B1* and/or *TaMLO-D1* nucleic acid.
24. A method according to claim 21 comprising the further step of regenerating a plant and screening for a plant resistant to powdery mildew.
25. A method for conferring resistance to powdery mildew to a wheat plant comprising producing a plant according to claim 17.
26. The method according to claim 17 wherein said plant does not contain a transgene.
27. A plant, plant part or plant cell obtained or obtainable by a method of claim 17.
28. A plant part according to claim 27 wherein said plant part is a seed.
29. An isolated *Tamlo* nucleic acid sequence as defined in SEQ ID NO. 38 or 39.

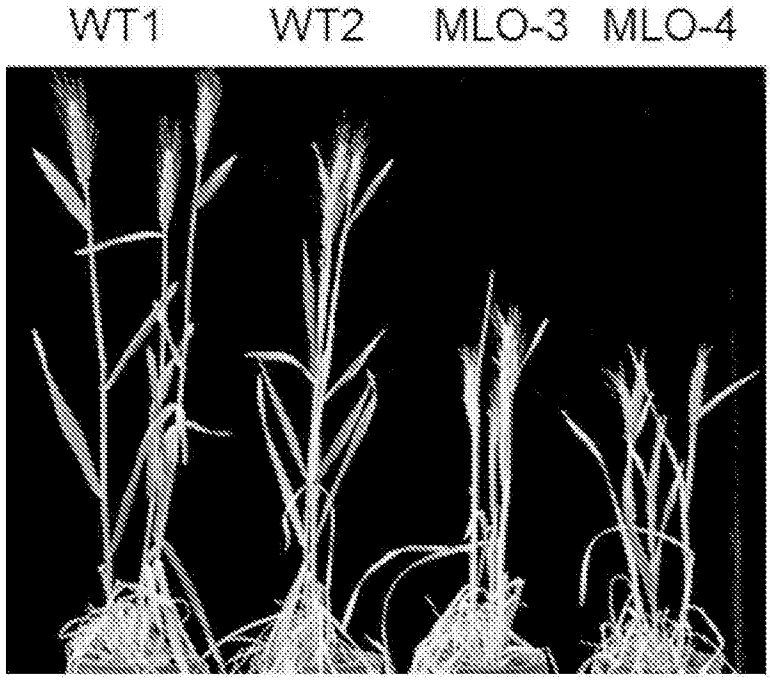

Figure 1

Figure 2A

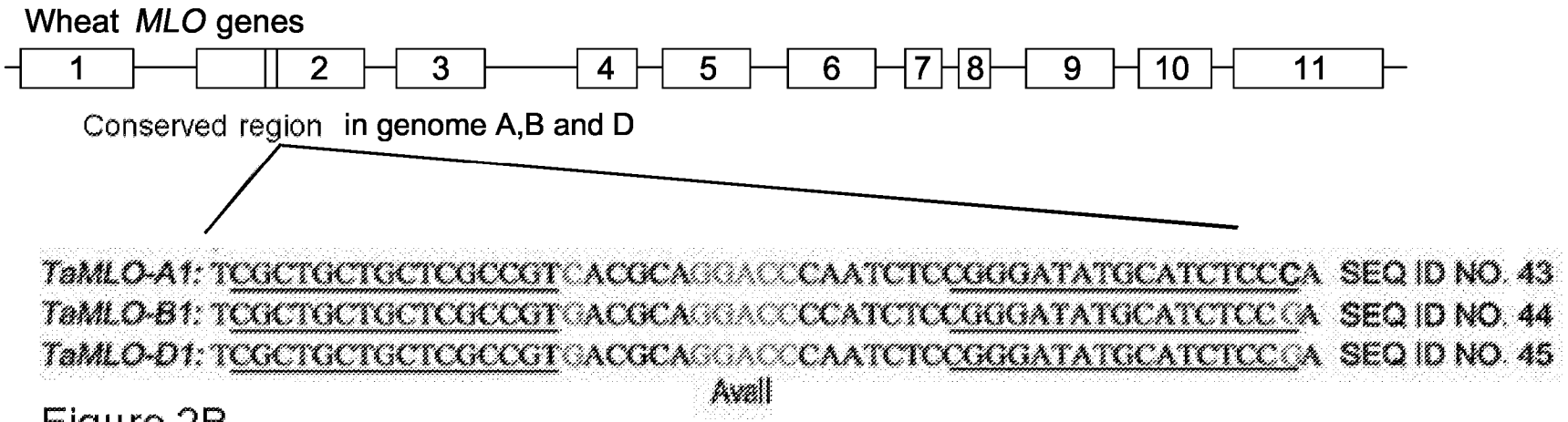

Figure 2B

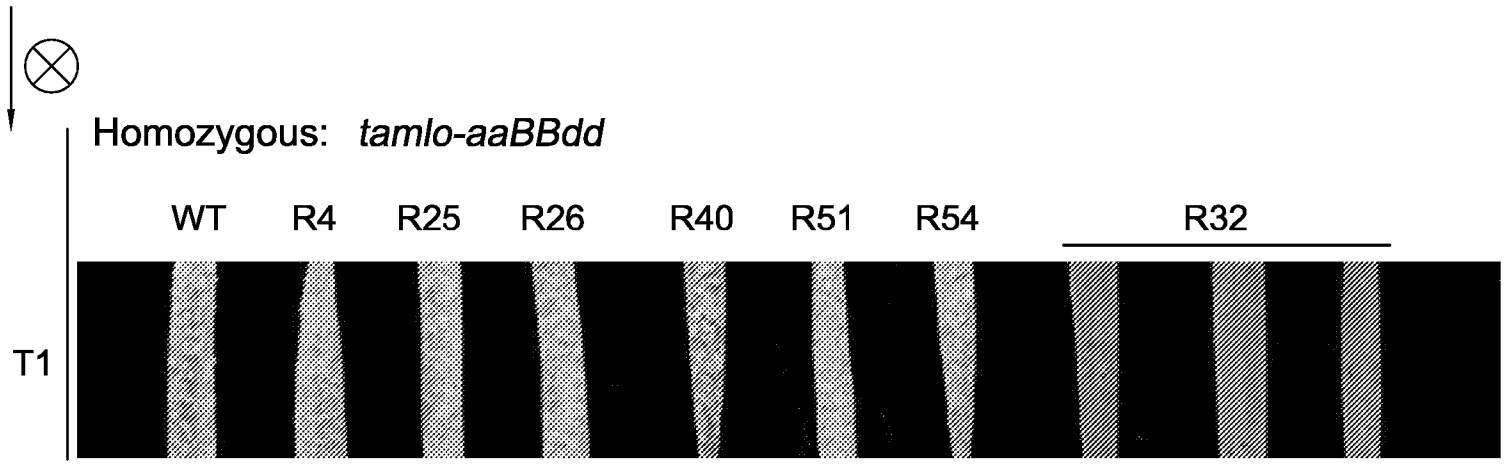

Figure 2

Figure 2C

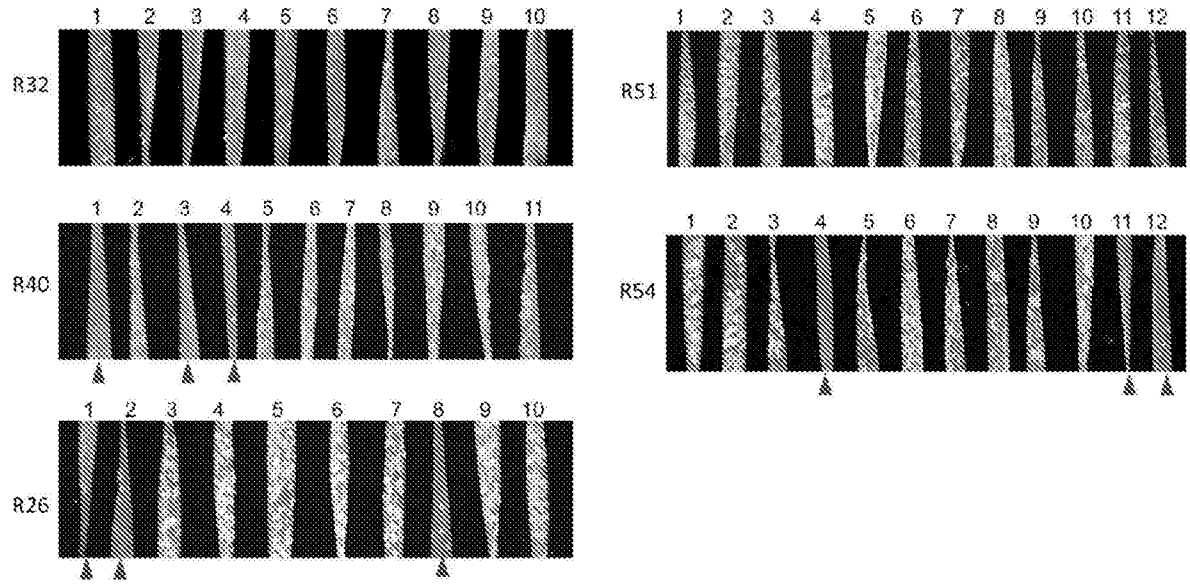

Figure 3

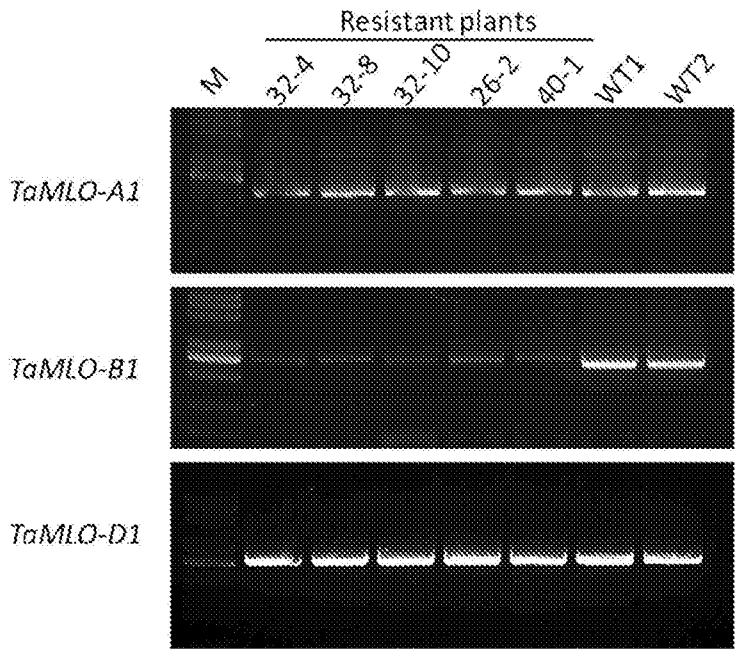

Figure 4

Figure 5

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SEQ ID NO. 49

Figure 6A

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Figure 6A continued

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Figure 6B

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Figure 6C

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Figure 6C continued

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Figure 6D

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Figure 6D continued

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Figure 6D continued

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Figure 6D continued

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Figure 7

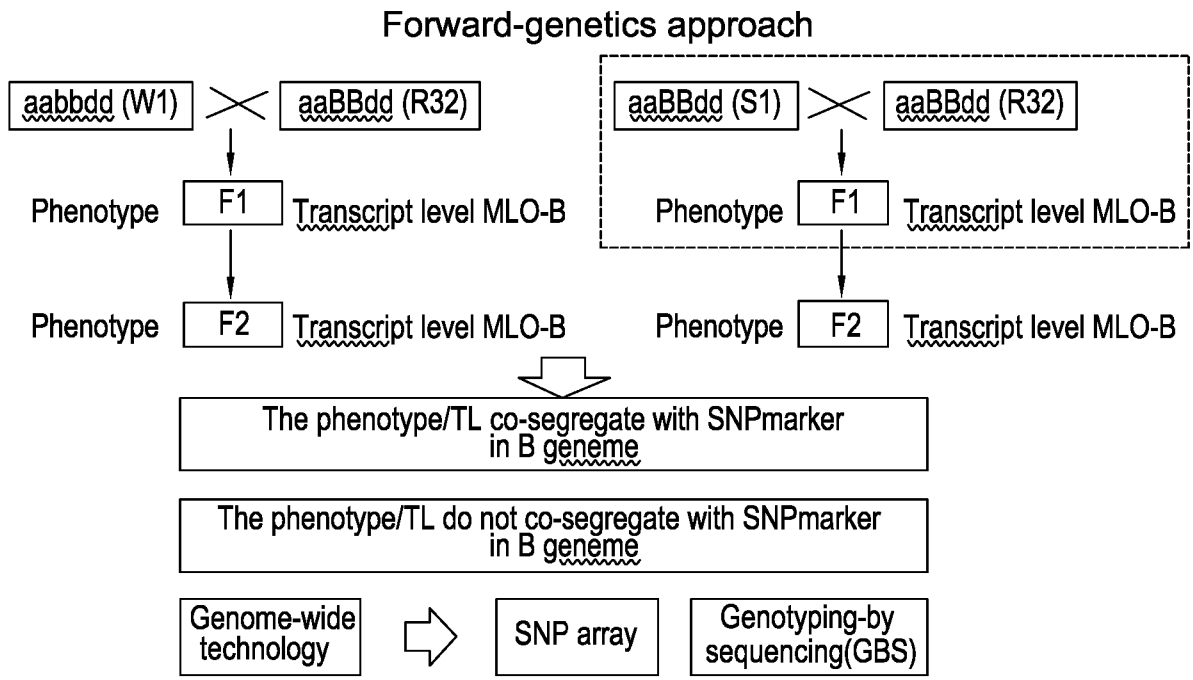
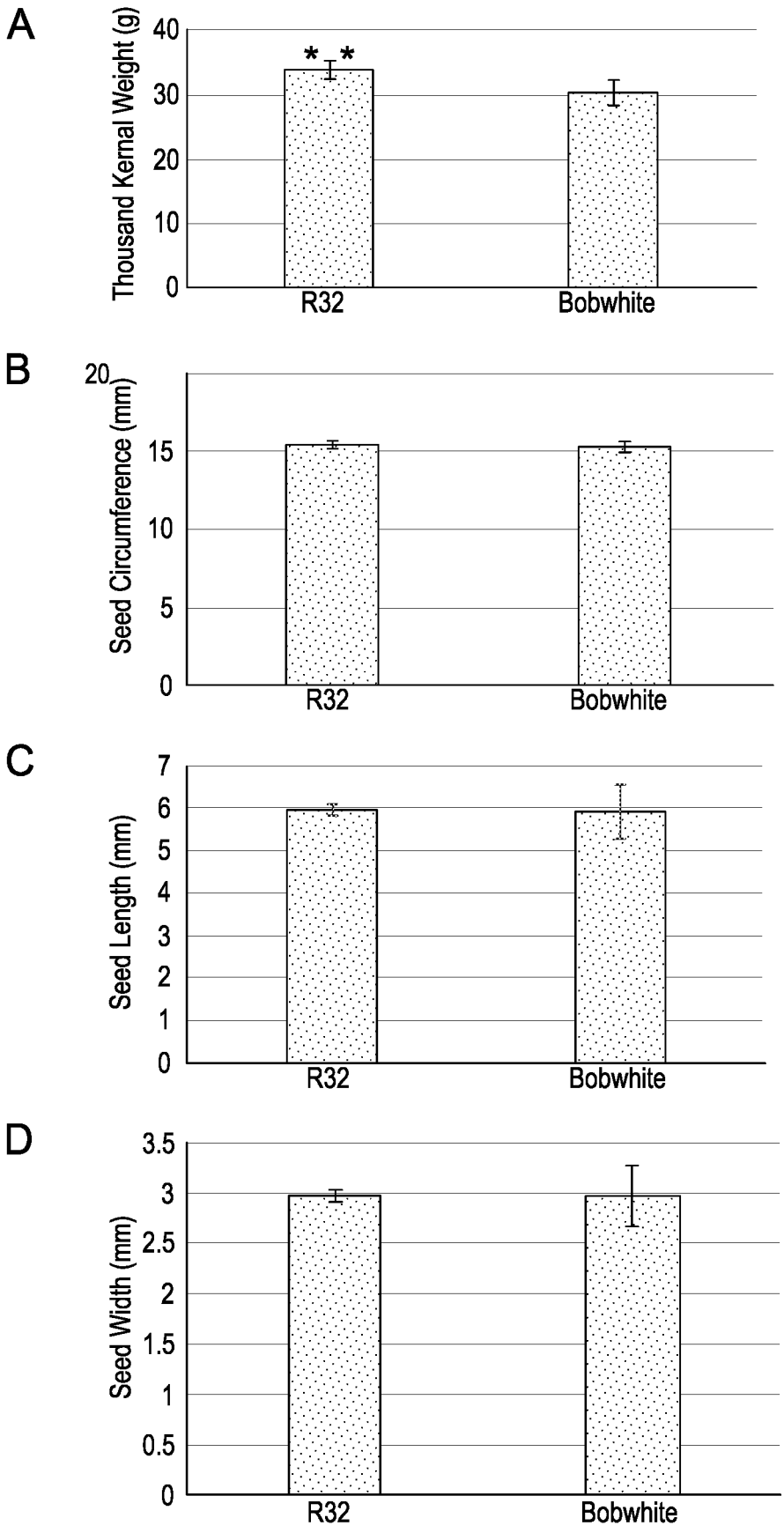


Figure 8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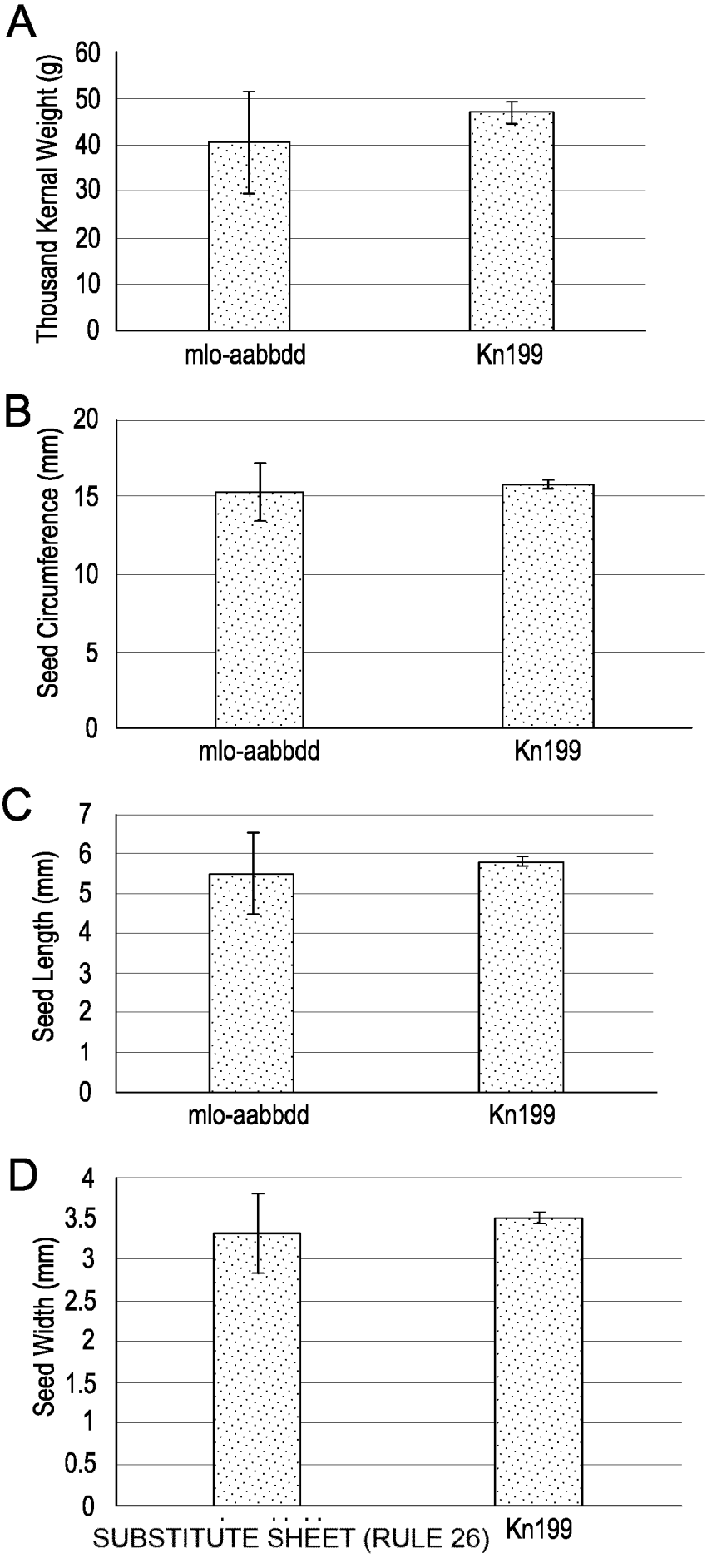
40
30
20
10
0
* *
R32
Bobwhite
B
Seed Circumference (mm)
20
15
10
5
0
R32
Bobwhite
C
Seed Length (mm)
7
6
5
4
3
2
1
0
R32
Bobwhite
D
Seed Width (mm)
3.5
3
2.5
2
1.5
1
0.5
0
R32
Bobwhite

Figure 9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Thousand Kernal Weight (g)
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50
40
30
20
10
0
mlo-aabbdd
Kn199
B
Seed Circumference (mm)
20
15
10
5
0
mlo-aabbdd
Kn199
C
Seed Length (mm)
7
6
5
4
3
2
1
0
mlo-aabbdd
Kn199
D
Seed Width (mm)
4
3.5
3
2.5
2
1.5
1
0.5
0
Kn199