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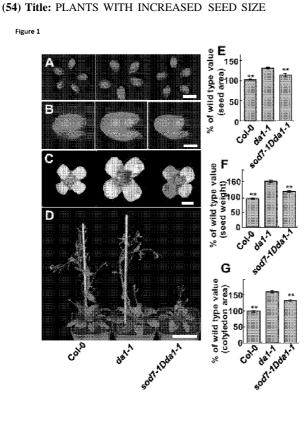
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(57) Abstract: The invention relates to genetically modified plants with an altered seed phenotype, in particular increased seed size. The invention relates to a plant that does not produce a functional NGAL2 polypeptide or functional NGAL2 and NGAL3 polypeptides. NGAL2 and NGAL3 are members of the RAV family and comprise a B3 DNA- binding domain and a transcriptional repression motif.

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PLANTS WITH INCREASED SEED SIZE

Field of the invention

5 The invention relates to transgenic plants with improved growth and yield-related traits, in particular increased seed size. Also within the scope of the invention are related methods, uses, isolated nucleic acids and vector constructs.

Introduction

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The ever-increasing world population and the dwindling supply of arable land available for agriculture fuels research towards increasing the efficiency of agriculture and providing food security. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogeneous genetic components that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to modify the germplasm of animals and plants. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has the capacity to deliver crops or plants having various improved economic, agronomic or horticultural traits, including increased yield. There are a number of methods that can be used, for example genome editing (using CRISPR or TALEN) or mutagenesis.

A trait of particular economic interest is increased seed size. Seed size is an important agronomic trait which increased crop yield, and is also a key ecological trait that influences many aspects of a species' regeneration strategy, such as seedling survival rates and seed dispersal syndrome (Harper et al., 1970; Westoby et al., 2002; Moles et al., 2005; Fan et al., 2006; Orsi and Tanksley, 2009; Gegas et al., 2010). Although the size of seeds is one of the most important agronomic traits in plants, the genetic and molecular mechanisms that set the final size of seeds are almost unknown. In higher plants, seed development starts with a double fertilization process, in which one of the two haploid pollen nuclei fuses with the haploid egg cell to produce the diploid embryo,

while the other sperm nucleus fuses with the diploid central cell to form the triploid endosperm (Lopes and Larkins, 1993). The integuments surrounding the ovule are maternal tissues and form the seed coat after fertilization. Therefore, the size of the seed is the result of the growth of the embryo, the endosperm and the maternal tissues. However, the genetic and molecular mechanisms setting the limits of seed growth are almost unknown in plants.

Several factors that function maternally to regulate seed size have been identified in Arabidopsis. For example, TRANSPARENT TESTA GLABRA 2 (TTG2) influences 10 seed growth by increasing cell elongation in the maternal integuments (Garcia et al., 2005; Ohto et al., 2009), while APETALA2 (AP2) may control seed growth by limiting cell elongation in the maternal integuments (Jofuku et al., 2005; Ohto et al., 2005; Ohto et al., 2009). By contrast, AUXIN RESPONSE FACTOR 2 (ARF2) acts maternally to control seed growth by restricting cell proliferation (Schruff et al., 2006). Similarly, the 15 ubiquitin receptor DA1 acts synergistically with the E3 ubiquitin ligases DA2 and EOD1/BB to control seed size by limiting cell proliferation in the maternal integuments (Li et al., 2008; Xia et al., 2013). Mutations in the suppressor of da1-1 (SOD2), which encodes the ubiquitin-specific protease (UBP15), suppress the large seed phenotype of da1-1 (Du et al., 2014). DA1 physically associates with UBP15/SOD2 and modulates the stability of UBP15. These studies show that the ubiquitin pathway plays an 20 important part in the maternal control of seed size. KLU/CYTOCHROME P450 78A5 (CYP78A5) regulates seed size by increasing cell proliferation in the maternal integuments of ovules (Adamski et al., 2009). KLU has also been suggested to generate mobile plant-growth substances that promote cell proliferation (Anastasiou et al., 2007; Adamski et al., 2009). By contrast, overexpression of CYP78A6/EOD3 increases both cell proliferation and cell elongation in the integuments, resulting in large seeds (Fang et al., 2012). Seed size is also determined by zygotic tissues. Several factors have been described to influence seed size via the zygotic tissues in including HAIKU1(IKU1), IKU2, MINISEED3 (MINI3) and SHORT Arabidopsis, 30 HYPOCOTYL UNDER BLUE1 (SHB1) (Garcia et al., 2003; Luo et al., 2005; Zhou et al., 2009; Wang et al., 2010; Kang et al., 2013), iku and mini3 mutants form small seeds due to precocious cellularization of the endosperm (Garcia et al., 2003; Luo et al., 2005; Wang et al., 2010). SHB1 associates with MINI3 and IKU2 promoters and regulates expression of MINI3 and IKU2 (Zhou et al., 2009; Kang et al., 2013). ABA INSENSITIVE5 (ABI5) has been recently described to repress the expression of SHB1 (Cheng et al., 2014), and MINI3 has been reported to activate expression of the

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cytokinin oxidase (CKX2) (Li et al., 2013), suggesting the roles of phytohormones in regulating endosperm growth. In addition, the endosperm growth is influenced by parent of-origin effects (Scott et al., 1998; Xiao et al., 2006).

5 The invention is aimed at providing plants with improved yield traits that are beneficial to agriculture.

Summary of the invention

10 In a first aspect, the invention relates to a plant generated that does not produce a functional NGAL2 polypeptide or does not produce functional NGAL2 and NGAL3 polypeptides.

In another aspect, the invention relates to a method for altering a plant phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding

- 15 a NGAL2 polypeptide or reducing or abolishing the activity of a NGAL2 or reducing or abolishing the expression of a nucleic acid sequences encoding NGAL2 and NGAL3 polypeptides or reducing or abolishing the activity of a NGAL2 and NGAL3 polypeptide relative to a control plant.
- In another aspect, the invention relates to a method for making a plant with an altered phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 polypeptide or reducing or abolishing the activity of a NGAL2 or reducing or abolishing the expression of a nucleic acid sequences encoding NGAL2 and NGAL3 polypeptides or reducing or abolishing the activity of a NGAL2 and NGAL3 polypeptide relative to a control plant..
- 25 In another aspect, the invention relates to a plant obtained or obtainable any method described above.

In another aspect, the invention relates to an isolated nucleic acid comprising a sequence comprising or consisting of SEQ ID NO: 1 or 2 or a functional variant or homologue thereof.

30 In another aspect, the invention relates to a vector comprising an isolated nucleic acid described above.

In another aspect, the invention relates to a silencing nucleic acid construct targeting sequence comprising or consisting of SEQ ID NO: 1, 2 or 3 or a functional variant, part or homologue thereof.

35 Figures

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

Figure 1. Isolation of a suppressor of *da1-1* (sod7-1D).

(A) Seeds from wild-type, da 1-1 and sod7-1D da1-1 plants (from left to right). (B) Mature embryos of the wild type, da 1-1 and sod7-1D da1-1 (from left to right). (C) Flowers from wild-type, da 1-1 and sod7-1D da1-1 plants (from left to right). (D) 30-dayold plants of the wild type, da 1-1 and sod7-1D da1-1 (from left to right). (E) Projective area of wild-type, da1-1 and sod7-1D da1-1 seeds. (F) Weight of wild-type, da1-1 and sod7-1D da 1-1 seeds. (G) Cotyledon area of 10-d-old wild-type, da 1-1 and sod7-1D da1-1 seedlings. Values (E-G) are given as mean \pm SD relative to the respective wildtype values, set at 100%. **, P<0.01 compared with da 1-1 (Student's t-test). Bars = 0.5

Figure 2. Seed and organ size in the sod7-1D mutant.

mm in (A), 0.2 mm in (B), 1 mm in (C) and 5 cm in (D).

(A and B) Seeds of Col-0 (A) and sod7-1D (B). (C and D) Mature embryos of Col-0 (C) and sod7-1D (D). (E and F) 10-day-old seedlings of Col-0 (E) and sod7-1D (F). (G) Projective area of Col-0 and sod7-1D seeds. (H) Weight of Col-0 and sod7-1D seeds. (I) Cotyledon area of 10-day-old Col-0 and sod7-1D seedlings. Values (G-I) are given

as mean ± SD relative to the respective wild-type values, set at 100%. **, P<0.01 compared with the wild type (Student's t-test). Bars = 0.5 mm in (A) and (B), 0.2 mm in (C) and (D), and 1 mm in (E) and (F).

20 Figure 3. Cloning of the SOD7 gene.

> (A) Structure of the T-DNA insertion in the sod7-1D mutant. (B) Expression levels of At3g1 1580 (SOD7) and At3g1 1590 in *da 1-1* and *sod7-1D da1* seedlings.

(C) The SOD7 protein contains a B3 DNA binding domain (second domain in lighter shading) and a transcriptional repression motif (small light box in darker shading, marked with an arrow). (D) Projective area of Col-0, 35S:GFP-SOD7#3 and 35S:GFP-25 SOD7#5 seeds. (E) Cotyledon area of 10-day-old Col-0, 35S:GFP-SOD7#3 and 35S:GFP-SOD7#5 seedlings. (F) Expression levels of SOD7 in Col-0. 35S:GFP-SOD7#3 and 35S:GFP-SOD7#5 seedlings. Values (D-F) are given as mean ± SD relative to the respective wild-type values, set at 100%. **, P<0.01 compared with the wild type (Student's t-test).

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Figure 4. Expression pattern and subcellular localization of SOD7.

(A-K) SOD7 expression activity was monitored by pSOD7:GUS transgene expression. Histochemical analysis of GUS activity in the developing leaves (A, B and C), the developing sepals (D, E), the developing petals (F, G), the developing stamens (H, I), and the developing carpels (J, K). (L) GFP florescence of SOD7-GFP in a young ovule of pSOD7:SOD7-GFP transgenic plants. (M-O) GFP fluorescence of SOD7-GFP (M),

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DAPI staining (N), and merged (O) images are shown. Epidermal cells in *pSOD7:SOD7-GFP* leaves were used to observe GFP signal. (P-R) GFP fluorescence of GFP-SOD7 (P), DAPI staining (Q), and merged (R) images are shown. Epidermal cells in *35S:GFP-SOD7* leaves were used to observe GFP signal. Bars = 100 μ m in (A-K), 10 μ m in (L), and 2 μ m in (M-R).

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Figure 5. SOD7 acts redundantly with NGAL3 to control seed size.

(A) The SOD7 gene structure. The start codon (ATG) and the stop codon (TGA) are shown. Closed boxes indicate the coding sequence, and the line between boxes indicates intron. The T-DNA insertion site (*sod7-ko1*) in the SOD7 gene was indicated.
(B) The *NGAL3* gene structure. The start codon (ATG) and the stop codon (TGA) are shown. Closed boxes indicate the coding sequence, and the line between boxes indicates intron. The T-DNA insertion site (*ngal3-ko1*) in the NGAL3 gene was indicates indicates intron. The T-DNA insertion site (*ngal3-ko1*) in the NGAL3 gene was indicated.
(C) Seeds from Col-0, *sod7-ko1*, *ngal3-ko1* and *sod7-ko1* ngal3-ko1 plants (from left to right). (D) Mature embryos of Col-0, *sod7-ko1*, *ngal3-ko1* and *sod7-ko1* ngal3-ko1 (from left to right). (E) 25-day-old plants of Col-0, *sod7-ko1*, *ngal3-ko1* and *sod7-ko1* ngal3-ko1 (from left to right). (F) Flowers of Col-0, *sod7-ko1*, *ngal3-ko1* and *sod7-ko1* ngal3-ko1 (from left to right). (G) Projective area of Col-0, *sod7-ko1*, *ngal3-ko1* and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) Cotyledon area of Col-0, *sod7-ko1*, ngal3-ko1 and *sod7-ko1* ngal3-ko1 seeds. (I) are given as mean ± SD relative to the respective wild-type values, set at 100%. **, P<0.01 compared with the wild type

0) (Student's t-test). Bars = 0.5 mm in (C), 0.2 mm in (D), 5 cm in (E), and 1 mm in (F).

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Figure 6. SOD7 acts maternally to determine seed size.
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(A) Projective area of Col-OxCol-0 (C/C) F1, **Col**-o×sod7-ko1ngal3-ko1(C/d) F1, sod7ko1ngal3-ko1×Col-0 (d/C) F1 and sod7-ko1ngal3-koUsod7-ko1ngal3-ko1(d/d)

F1 seeds. Values are given as mean ± SD relative to the respective wild-type values, set at 100%. (B) Projective area of Col-OxCol-0 (C/C) F2, Col-0*sod7-ko1 ngal3-ko1 (C/d) F2, sod7-ko1 ngal3-ko1*Col-0 (d/C) F2 and sod7-ko1 ngal3-ko1*sod7-ko1 ngal3-ko1 (d/d) F2 seeds. Values are given as mean ± SD relative to the respective wild-type values, set at 100%. (C and D) Mature ovules of Col-0 (C) and sod7-ko1 ngal3-ko1 (D). (E) Outer integument length of mature Col-0 (lighter bar to the left) and sod7-ko1 ngal3-ko1 (darker bar to the right) ovules. Values are given as mean ± SD. (F) The number of cells in the outer integuments of Col-0 and sod7-ko1 ngal3-ko1 at 0, 6 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD. (F) The length of cells in the outer integuments of Col-0 and 8 DAP. Values are given as mean ± SD.

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mean ± SD. **, P<0.01 compared with the wild type (Col-0) (Student's t-test). Bars = 50 μ m in (C) and (D).

Figure 7. klu-4 is epistatic to sod7-ko1 ngal3-ko1 with respect to seed size.

(A) Seed area of Col-0, *klu-4, sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* (from left to right). Values are given as mean ± SD relative to the respective wild-type values, set at 100%. (B) Seed weight of Col-0, *klu-4, sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* (from left to right). Values are given as mean ± SD relative to the respective wild-type values, set at 100%. (C) The outer integument length of Col-0, *klu-4, sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* (from left to right). ngal3-ko1 and 8 DAP. Values are given as mean ± SD. (D) The number of cells in the outer integuments of Col-0, *klu-4, sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* and *klu-4 sod7-ko1 ngal3-ko1* (from left to right) at 0 and 8 DAP. Values are given as mean ± SD. **, P<0.01 compared with their respective controls (Student's t-test).

Figure 8. SOD7 directly binds to the promoter of KLU and represses the expression of KLU.

(A) Expression dynamics of SOD7 and KLU in pER8-SOD7 transgenic plants treated with β -estradiol for 0, 4 and 8 hours. Means were calculated from three biological samples. Values are given as mean ± SD. **, P<0.01, compared with the expression levelof KLU and SOD7 at 0 hour, respectively (Student's t-test). (B) A 2-kb promoter 20 region of *KLU* upstream of its ATG codon contains a CACTTG sequence. PF1 and PF2 represent PCR fragments used for ChIP-quantitative PCR analysis. A and A-m indicate the wild-type probe and the mutated probe used in the EMSA essay, respectively. (C) ChIP-qPCR analysis shows that SOD7 binds to the promoter fragment PF1 of KLU. Chromatin from 35S:GFP and 35S:GFP-SOD7 transgenic plants was 25 immunoprecipitated by anti-GFP, and the enrichment of the fragments was determined by quantitative real-time PCR. The ACTIN7 promoter was used as a negative control. The fold enrichment was normalized to the ACTIN7 amplicon, set at 1. Means were calculated from three biological samples. Values are given as mean ± SD. **, P<0.01, compared with 35S:GFP transgenic plants (Student's t-test). (D) Direct interaction 30 between SOD7 and the KLU promoter determined by EMSA. The biotin-labeled probe A and MBP-SOD7 formed the DNA-protein complex, but the mutated probe A-m and MBP-SOD7 did not form the DNA-protein complex. The retarded DNA-protein complex was reduced by competition using the unlabeled probe A.

Figure 9. The organ size phenotype of 35S:GFP-SOD7 transgenic plants. 35 Overexpression of SOD7 results in small plants compared with the wild type. Bar = 5 cm.

Figure 10. Phylogenetic tree of the RAV family members in Arabidopsis.

Figure 11. SOD7 acts redundantly with NGAL3 to influence organ size.

Petal area of *Col-0, sod7-ko1, ngal3-ko1* and *sod7-ko1 ngal3-ko1*. (B) The seventh leaf area of Col-0, *sod7-ko1, ngal3-ko1* and *sod7-ko1 ngal3-ko1*. Values (A and B) are given as meant SD relative to the respective wild-type values, set at 100%. **, P<0.01 and *, P<0.05 compared with the wild type (Col-0).

Figure 12: Conserved domains in NGAL2, NGAL3 and homologs. a) B box motif. b) Repressor motif

Figure 13: Alignment of sequences. The following sequences are shown (from top to

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bottom): RMZM2G053008, HvMLOC 57250, Os12g0157000, GmLoc100778733, Bra004501, Bra000434, Bra040478, Bra014415, Bra003482, Bra007646, GmLoc100781489, GRMZM2G024948_T01, os02g0683500, HvMLOC 66387, os04g0581400, GRMZM2G102059 T01, Os10g0537100, GRMZM2G142999 T01, GRMZM2G125095 T01, os03g0120900, GRMZM2G098443 T01,

- 15 GRMZM2G082227_T01 , Os1 1g01 56000, GRMZM2G328742_T01 , GmLoc100802734 GmLod 00795470, GmLod 0081 8164, Bra017262, At2g36080/NGAL1, Bra005301, At3g1 1580/SOD7, BraLOCI 03849927, Bra034828, At5g06250/NGAL3, Bra005886, GmLod 02660503, HvMLOC_38822, os01g0693400, HvMLOC44012, HvMLOC_7940 HvMLOC_75135, TRAECDM81004, HvMLOC_56567, TRAES3BF098300010CFD_t1
- HvMLOC_63261, TRAES3BF062700040CFD_t1, TRAES3BF062600010CFD_t1,
 Bra038346, GmLoc732601, GmLod 00789009, GmLod 00776987, GmLod 00801 107.
 Conserved B3 domain and repressor motif are boxed.

Figure 14: Genome editing experiments to knock out rice genes Os1 1g01560000 and Os12g01 57000 in rice. gRNA stands for guide RNA, target site linked with gRNA scaffold will recruit CAS9 enzyme to target site in the genome and cause gene-editing.

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.
- 35 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", 5 "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), naturally occurring, mutated, synthetic DNA or RNA molecules, and analogues of the DNA or RNA generated using nucleotide analogues. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences 10 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" 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 15 may include cDNAs in combination with regulatory sequences.

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.

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For the purposes of the invention, "transgenic", "transgene" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

the nucleic acid sequences encoding proteins useful in the methods of the (a) invention, or

genetic control sequence(s) which is operably linked with the nucleic acid (b) sequence according to the invention, for example a promoter, or

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both (a) and (b) (c)

are not located in their natural genetic environment or have been modified by genetic intervention techniques, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a

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genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette - for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above - becomes a transgenic expression cassette when this expression cassette is modified by nonnatural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815 both incorporated by reference.

- In certain embodiments, a transgenic plant for the purposes of the invention is thus understood as meaning, as above, that the nucleic acids used in the method of the invention are not at their natural locus in the genome of said plant, it being possible for the nucleic acids to be expressed homologously or heterologously. Thus, the plant can express a silencing construct transgene. However, as mentioned, in certain embodiments, transgenic also means that, while the nucleic acids according to the different embodiments of the invention are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified, for example by mutagenesis.
- Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place. According to the invention, the transgene is stably integrated into the plant and the plant is preferably homozygous for the transgene.

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The various aspects of the invention use genetic engineering methods. Thus, the plants have been generated using genetic engineering methods, for example transgene expression, mutagenesis, gene targeting, gene silencing or genome editing as detailed below. Thus, the various aspects of the invention can involve recombinant DNA technology. The plants of the invention are thus mutant plants which have been genetically engineered, that is manipulated by human intervention. The plants of the

various aspects of the invention do not relate to natural variants which have not been manipulated by genetic engineering methods. The plant may be a transgenic plant in some embodiments, for example a plant which comprises a nucleic acid construct expressing a silencing construct.

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In preferred embodiments exclude embodiments that are solely based on generating plants by traditional breeding methods.

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The inventor has identified a B3 domain transcriptional repressor termed AtNGAL2, encoded by the suppressor of *Atda1-1 (AtSOD7)*, which acts maternally to control seed size by restricting cell proliferation in the integuments of ovules and developing seeds.

The inventor previously identified the ubiquitin receptor DA1 as a negative regulator of seed size in Arabidopsis (Li et al., 2008). The da1-1 mutant formed large seeds due to 15 increased cell proliferation in the maternal integuments (Li et al., 2008; Xia et al., 2013). To identify novel components in the DA1 pathway or other seed size regulators, the inventor initiated a T-DNA activation tagging screen for modifiers of da 1-1 (Fang et al., 2012). A dominant suppressor of da1-1 (sod7-1D) was isolated from seeds produced from approximate 16,000 T1 plants (Fig.IA). Seeds of the sod7-1D da1-1 20 double mutant were significantly smaller and lighter than da1-1 seeds (Figures 1A, E and F). The results show that the sod7-1D mutation suppressed the seed and organ size phenotypes of da 1-1. The SOD7 gene was isolated and found to encode a NGATHA like protein (NGAL2) containing a B3 DNA-binding domain and a transcriptional repression motif (Figure 3C) (Alvarez et al., 2009; Ikeda and Ohme-25 Takagi, 2009; Trigueros et al., 2009). SOD7 belongs to the RAV gene family that consists of 13 members in Arabidopsis (Figure 10) (Swaminathan et al., 2008). Several members of the RAV family contain the putative transcriptional repression motifs, including NGA1, NGA2, NGA3, NGA4, NGAL1, NGAL2/SOD7 and NGAL3 (Figure 10) (Ikeda and Ohme-Takagi, 2009). The transcriptional repression motifs in 30 NGA1, NGAL1 and NGAL2/SOD7 have been known to possess the repressive activity (Ikeda and Ohme-Takagi, 2009), indicating that they are transcriptional repressors. SOD7 exhibits the highest similarity to Arabidopsis NGAL3/DEVELOPMENT-RELATED PcG TARGET IN THE APEX 4 (DPA4) (Figure 10), which has known roles in the regulation of leaf serrations (Engelhorn et al., 2012), but no previously identified 35 function in seed size control.

The inventor has shown that overexpression of *AtSOD7* significantly decreases seed size of wild-type plants, while the disruption of *AtSOD7* increases seed size. The inventors have shown that disruption of *AtNGAL3*, a close homolog of *AtSOD7* also increases seed size. Moreover, the simultaneous disruption of *AtSOD7* and *AtNGAL3* further increases seed size in a synergistic manner. Genetic analyses carried out by the inventor indicate that *AtSOD7* acts in a common pathway with the seed size regulator *AtKLU* to control seed growth, but does so independently of *AtDA1*. Further results show that *AtSOD7* directly binds to the promoter of *AtKLU in vitro* and in *vivo* and represses expression of *AtKLU*. Therefore, the inventor's findings show that *AtSOD7* (aka *AtNGAL2*) is a target for seed size improvement in crops. The plants of the invention are characterised by increased embryo size, for example. Increased seed size, and also increase in seed yield and the plants of the invention are thus characterised by increased seed yield.

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Thus, the invention relates to a plant wherein said plant does not produce a functional NGAL2 and/or NGAL3 polypeptide. For example, the plant does not produce a full length transcript of a nucleic acid sequence encoding a NGAL2 and/or NGAL3 protein. In another embodiment, the plant produces a full length transcript of a nucleic acid sequence encoding a NGAL2 and/or NGAL3, but the resulting protein is not functional. In a preferred embodiment, said plant does not produce a functional NGAL2 polypeptide and also does not produce a functional NGAL3 polypeptide. Such plants are double knock-out or knock-down mutants (loss of function mutants) and methods according to the invention as described below relate to making such double mutants.

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The plants of the invention are mutant plants which have been genetically modified and are not naturally occurring varieties. Thus, the plants have been generated using genetic engineering methods, for example mutagenesis, gene targeting, gene silencing or genome editing as detailed below. Thus, the various aspects of the invention can
involve recombinant DNA technology. The plant may be a transgenic plant in some embodiments, for example a plant which comprises a transgene to silence gene expression of *SOD7* and/or *NGAL3*. In other embodiments, the plant does not carry a transgene, but is a mutant plant wherein the endogenous nucleic acid sequence encoding a NGAL2 and/or NGAL3 polypeptide or the endogenous *SOD7* and/or *NGAL3* promoter sequence has been manipulated to either reduce or abolish expression of a nucleic acid sequence encoding a NGAL2 polypeptide

or reduce or abolish the activity of a NGAL2 and/or NGAL3 polypeptide. The plants of the various aspects of the invention do not relate to natural variants which have not been manipulated by genetic engineering methods.

- 5 In one aspect, the invention relates to a plant generated by genetic engineering methods wherein the expression of a nucleic acid sequence encoding a NGAL2 and/or NGAL3 polypeptide and/or the activity of a NGAL2 and/or NGAL3 polypeptide is reduced or abolished relative to a control plant. In one embodiment, expression of a nucleic acid sequence encoding a NGAL2 polypeptide or the activity of a NGAL2 10 polypeptide is reduced or abolished. In another embodiment, expression of a nucleic acid sequence encoding a NGAL3 polypeptide or the activity of a NGAL3 polypeptide is reduced or abolished. In a preferred embodiment the presence of function of both proteins is affected, in other words, the plant is characterised in that expression of a nucleic acid sequence encoding a NGAL2 polypeptide or the activity of a NGAL2 15 polypeptide is reduced or abolished and also expression of a nucleic acid sequence encoding a NGAL3 polypeptide or the activity of a NGAL3 polypeptide is reduced or abolished in said plant.
- For example, said plant can have reduced or abolished expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reduced or abolished expression of a nucleic acid sequence encoding a NGAL3 polypeptide. In another embodiment, said plant can have reduced or abolished activity of a NGAL2 polypeptide and reduced or abolished activity of a NGAL3 polypeptide. In another embodiment, said plant can have reduced or abolished expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reduced or abolished activity of a NGAL3 polypeptide. In another embodiment, said plant can have reduced or abolished expression of a nucleic acid sequence encoding a NGAL3 polypeptide and reduced or abolished expression of a nucleic acid sequence encoding a NGAL3 polypeptide and reduced or abolished activity of a NGAL2 polypeptide.
- 30 A NGAL2 or NGAL3 polypeptide as described in the various aspects of the invention has a characteristic domain structure as explained below.

A NGAL2 OR NGLA3 polypeptide as described in the various aspects of the invention comprises a B3 DNA binding domain which has the structure shown in figure 12.

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In one embodiment, the domain is: SNNNNNNGGSGDDVACHFQRFDLHRLFIGWRGE

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(SEQ ID NO:6) or a domain with at least 80%, at least 95% or at least 95% sequence identity thereto.

A NGAL2 OR NGAL3 polypeptide as described in the various aspects of the invention also comprises a transcriptional repression motif shown in figure 12.

In one embodiment, the domain is: VRLFGVNLE (SEQ ID NO:7) or a domain with at least 95% sequence identity thereto.

In one embodiment, the NGAL2 protein is AtNGAL2, a functional variant, part or homologue thereof. AtNGAL2 is encoded by AtSOD7. The term AtSOD7 refers to the wild type AtSOD7 nucleic acid sequence comprising or consisting of SEQ ID NO. 1 (CDNA) or SEQ ID NO 2 (genomic DNA). The protein encoded by AtSOD7 is termed *At*NGAL2 SEQ ID NO.3. In one embodiment, said functional homologue is not AtNGAL3.

In one embodiment, the NGAL3 protein is AtNGAL3, a functional variant, part or homologue thereof. The term *AtNGAL3* refers to the wild type *AtNGAL3* nucleic acid sequence comprising or consisting of SEQ ID NO. 4. The protein encoded by *AtNGAL3* is termed *At*NGAL3 SEQ ID NO.5.

The term "functional" refers to the biological function of the NGAL2 or NGAL3, that is their function in controlling organ size, in particular seed size. The terms "functional variant" or "functional part "as used herein, for example with reference to SEQ ID NOs: 1, 2 or 3, or SEQ ID NOs: 4 or 5 refers to a variant gene or polypeptide sequence or part of the gene or polypeptide sequence which retains the biological function of the full non-variant SOD7/NGAL2 or NGAL2/NGAL3 sequence, that is regulation of seed size. Such sequences complement the *Atsod7-1D* mutant or *Atngal3* mutant respectively.

Thus, it is understood, as those skilled in the art will appreciate, that the aspects of the invention, encompass not only targeting a *AtSOD7* and/or *AtNGAL3* nucleic acid, for example a nucleic acid sequence comprising or consisting of SEQ ID NO: 1 or SEQ ID NO: 2, or SEQ ID NO: 4 respectively or a polypeptide comprising or consisting of SEQ ID NO: 3, or SEQ ID NO: 5, or a promoter of a AtSOD7 and/or *AtNGAL3* nucleic acid.
The aspects of the invention encompass also functional variants of AtNGAL2 or AtNGAL3 that do not affect the biological activity and function of the resulting protein.

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Alterations in a nucleic acid sequence which result in the production of a different amino acid at a given site that do however not affect the functional properties of the encoded polypeptide, are well known in the art. For example, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also produce a functionally equivalent product. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. 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 sequences as shown herein and is biologically active.

15 Generally, variants of a particular SOD7/NGAL3 nucleotide sequence or NGAL2/NGAL3 polypeptide as described herein will have at least about 60%, preferably at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to that particular non-variant nucleotide sequence, as determined by sequence alignment programs described elsewhere herein.

Furthermore, the various the aspects of the invention encompass not only a *AtSOD7* and/or *AtNGAL3* nucleic acid, for example a nucleic acid sequence comprising or consisting of SEQ ID NO: 1 or SEQ ID NO: 2, or SEQ ID NO: 4 respectively or a polypeptide comprising or consisting of SEQ ID NO: 3, or SEQ ID NO: 5, or their functional variants but also homologues of *AtSOD7* and/or *AtNGAL3* in *Arabidopsis* or other plants. Also within the scope of the invention are functional variants of such homologues as defined above.

The term homologue as used herein also designates an *AtSOD7* and/or *AtNGAL3* orthologue from other plant species. A homologue of AtNGAL2 or AtNGAL3 polypeptide respectively has, in increasing order of preference, at least 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% overall sequence identity to the amino acid represented by SEQ ID NO: 3 or 5 respectively. Preferably, overall sequence identity is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, most preferably 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99%.

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In another embodiment, the homologue of a AtSOD7 or AtNGAL3 nucleic acid sequence respectively has, in increasing order of preference, at least 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% overall 15 sequence identity to the nucleic acid represented by SEQ ID NO: 1 or 2 or 4 respectively. Preferably, overall sequence identity is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, most preferably 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99%. The overall 20 sequence identity is determined using a global alignment algorithm known in the art, such as the Needleman Wunsch algorithm in the program GAP (GCG Wisconsin Package, Accelrys).

In a preferred embodiment, the NGAL2 or NGAL3 homologue is from a plant that is not Arabidopsis.

In one embodiment, an AtNGAL2 or a homologue thereof or AtNGAL3 or a homologue thereof comprises a B3 domain having the sequence as defined above

30 In one embodiment, an AtNGAL2 or a homologue thereof or AtNGAL3 or a homologue thereof comprises a transcriptional repression motif having the sequence as defined above

Examples of homologues are shown in figure 13 and in SEQ ID NO: 49-145. In certain 35 embodiments, if a plant has more than one AtNGAL2 and/or AtNGAL3 homologue, then all homologues are knocked out or knocked down. Suitable homologues can be

identified by sequence comparisons and identifications of conserved domains. There are predictors in the art that can be used to identify such sequences. The function of the homologue can be identified as described herein and a skilled person would thus be able to confirm the function, for example when overexpressed in a plant or knocked out in a plant or when expressed in a plant or by expressing the homologous nucleic acid sequence in an Arabidopsis gain of function mutant.

Thus, the nucleotide sequences of the invention and described herein can also be used to isolate corresponding sequences from other organisms, particularly other plants, for example crop plants. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences described herein. Topology of the sequences and the characteristic domains structure can also be considered when identifying and isolating homologues. Sequences may be isolated based on their sequence identity to the entire sequence or 15 to fragments thereof. In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen plant. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labelled with a detectable group, or 20 any other detectable marker. Thus, for example, probes for hybridization can be made by labelling synthetic oligonucleotides based on the ABA-associated sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in 25 Sambrook, et al., (1989) Molecular Cloning: A Library Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions 30 under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). 35 Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing).

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Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Duration of hybridization is generally less than about 24 hours, usually about 4 to 12. Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

According to the invention, preferred homologues of *AtSOD7* and *AtNGAL3* peptides are selected from crop plants, for example cereal crops. Preferred homologues of AtNGAL2 and AtNGAL3 and their polypeptide sequences are also shown in Fig. 13.

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A plant according to the various aspects of the invention, including the transgenic plants, methods and uses described herein may be a monocot or a dicot plant.

A dicot plant may be selected from the families including, but not limited to Asteraceae,
 Brassicaceae (e.g. Brassica napus), Chenopodiaceae, Cucurbitaceae, Leguminosae (Caesalpiniaceae, Aesalpiniaceae Mimosaceae, Papilionaceae or Fabaceae),
 Malvaceae, Rosaceae or Solanaceae. For example, the plant may be selected from lettuce, sunflower, Arabidopsis, broccoli, spinach, water melon, squash, cabbage, tomato, potato, yam, capsicum, tobacco, cotton, okra, apple, rose, strawberry, alfalfa,
 bean, soybean, field (fava) bean, pea, lentil, peanut, chickpea, apricots, pears, peach, grape vine, bell pepper, chilli or citrus species.

A monocot plant may, for example, be selected from the families *Arecaceae, Amaryllidaceae* or *Poaceae.* For example, the plant may be a cereal crop, such as 30 maize, wheat, rice, barley, oat, sorghum, rye, millet, buckwheat, or a grass crop such as Lolium species or Festuca species, or a crop such as sugar cane, onion, leek, yam or banana.

Also included are biofuel and bioenergy crops such as rape/canola, sugar cane, sweet sorghum, *Panicum virgatum* (switchgrass), linseed, lupin and willow, poplar, poplar hybrids, Miscanthus or gymnosperms, such as loblolly pine. Also included are crops for

silage (maize), grazing or fodder (grasses, clover, sanfoin, alfalfa), fibres (e.g. cotton, flax), building materials (e.g. pine, oak), pulping (e.g. poplar), feeder stocks for the chemical industry (e.g. high erucic acid oil seed rape, linseed) and for amenity purposes (e.g. turf grasses for golf courses), ornamentals for public and private gardens (e.g. snapdragon, petunia, roses, geranium, Nicotiana sp.) and plants and cut flowers for the home (African violets, Begonias, chrysanthemums, geraniums, Coleus spider plants, Dracaena, rubber plant).

10 Preferably, the plant is a crop plant. By crop plant is meant any plant which is grown on a commercial scale for human or animal consumption or use. In a preferred embodiment, the plant is a cereal.

Most preferred plants are maize, rice, wheat, oilseed rape/canola, sorghum, soybean, sunflower, alfalfa, potato, tomato, tobacco, grape, barley, pea, bean, field bean, lettuce, cotton, sugar cane, sugar beet, broccoli or other vegetable brassicas or poplar.

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, callus tissue, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprises the gene/nucleic acid of interest.

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According to the various aspects of the invention, including the plants and methods of the invention, abolishing, inactivating, repressing, reducing or down-regulating the activity of a NGAL2 and/or NGAL3 polypeptide can be achieved through different means. Such means that are within the scope of the various aspects of the invention are methods for abolishing or reducing translation or transcription of the *SOD7* and/or *NGAL3* gene, destabilizing *SOD7* and/or *NGAL3* transcript stability, destabilizing NGAL2 and/or NGAL3 polypeptide stability or abolishing or reducing the activation or activity of the NGAL2 and/or NGAL3 or polypeptide. Thus, in one embodiment, endogenous *SOD7* and/or *NGAL3* gene or its promoter carry a functional mutation so that no full length transcript is made. In another embodiment, the *SOD7* and/or *NGAL3* gene is silenced in said plant using gene silencing techniques. In another embodiment,

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the *SOD7* and/or *NGAL3* nucleic acid sequence has been altered to introduce a mutation which results in a NGAL2/NGAL3 protein with reduced or abolished activity. These embodiments and the techniques used are described in more detail below.

- 5 In another aspect, the invention relates to a method for altering a plant phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 and/or NGAL3 polypeptide and/or reducing or abolishing the activity of a NGAL2 and/or NGAL3 polypeptide relative to a control plant.
- 10 In another aspect, the invention relates to a method for making a plant with an altered phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 and/or NGAL3 polypeptide and/or reducing or abolishing the activity of a NGAL2 and/or NGAL3 polypeptide relative to a control plant.
- 15 As previously described, such methods above use genetic engineering methods.

In this aspect, a wild type plant may be targeted to simultaneously knock out or down both *SOD7* and *NGAL3* function. Alternatively, the method may comprise the following steps

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- a) Knocking out or down SOD7 function in a first plant;
- b) knocking out or down NGAL3 function in a second plant and
- c) crossing plants regenerated from said first plant with plants regenerated from said second plant.
- In one embodiment of these methods, expression of a nucleic acid sequence encoding a NGAL2 polypeptide or the activity of a NGAL2 polypeptide is reduced or abolished. In another embodiment, expression of a nucleic acid sequence encoding a NGAL3 polypeptide or the activity of a NGAL3 polypeptide is reduced or abolished. In a preferred embodiment, the method comprises reducing or abolishing expression of a nucleic acid sequence encoding a NGAL2 polypeptide or the activity of a NGAL2 polypeptide and reducing or abolishing expression of a nucleic acid sequence encoding a NGAL3 polypeptide or the activity of a NGAL2 polypeptide to create a double loss of function mutant.
- 35 For example, the method comprises reducing or abolishing expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reducing or abolishing expression of a

nucleic acid sequence encoding a NGAL3 polypeptide. In another embodiment, the method comprises reducing or abolishing activity of a NGAL2 polypeptide and reducing or abolishing activity of a NGAL3 polypeptide. In another embodiment, the method comprises reducing or abolishing expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reducing or abolishing activity of a NGAL3 polypeptide. In another embodiment the method comprises reducing or abolishing activity of a NGAL3 polypeptide. In another embodiment the method comprises reducing or abolishing expression of a nucleic acid sequence encoding a nucleic acid sequence encoding a NGAL3 polypeptide or reducing or abolishing activity of a NGAL3 polypeptide.

- 10 According to these methods, the phenotype is preferably selected from increased organ size, for example increased seed size or increased seed weight. Increased seed size leads to an increase in yield and the methods of the invention also increased yield.
- 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" as described herein relates to yield-related traits and 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, the term yield refers to organ size, in particular seed size and can be measured by assessing seed size or seed weight or cotyledon size.
- 25 The terms "increase", "improve" or "enhance" are interchangeable. Yield or seed size for example is increased by at least a 2%, 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.
- 30 A control plant as used herein according to all of the aspects of the invention is a plant which has not been modified according to the methods of the invention. Accordingly, the control plant has not been genetically modified to alter either expression of a nucleic acid encoding a NGAL2 or NGAL3 polypeptide or to alter the activity of a NGAL2 or NGAL3 polypeptide as described herein. In one embodiment, the control plant is a wild type plant that has not been genetically altered. In another embodiment, the control plant is a transgenic plant that does not have altered expression of a nucleic

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acid encoding a NGAL2 or NGAL3 polypeptide or altered activity of a NGAL2 or NGAL3 polypeptide, but has been genetically altered in other ways, for example by expressing a desirable transgene to confer certain traits.

- 5 The reduction, decrease, down-regulation or repression of the activity of the NGAL2 and/or NGAL3 polypeptide or corresponding *SOD7* and/or *NGAL3* nucleic acid sequences according to the aspects of the invention is at least 10%, 20%, 30%, 40% or 50% in comparison to the control plant.
- 10 For example, the plant is a reduction (knock down) or loss of function (knock out) mutant wherein the function of the SOD7 and/or NGAL3 nucleic acid sequence is reduced or lost compared to a wild type control plant. To this end, a mutation is introduced into the SOD7 and/or NGAL3 nucleic acid sequence or the corresponding promoter sequence which disrupts the transcription of the gene leading to a gene 15 product which is not functional or has a reduced function. The mutation may be a deletion, insertion or substitution. The expression of active protein may thus be abolished by mutating the nucleic acid sequences in the plant cell which encode the NGAL2 or NGAL3 polypeptide and regenerating a plant from the mutated cell. The nucleic acids may be mutated by insertion or deletion of one or more nucleotides. 20 Techniques for the inactivation or knockout of target genes are well-known in the art. These techniques include gene target using vectors that target the gene of interest and which allow integration allows for integration of transgene at a specific site. The targeting construct is engineered to recombine with the target gene, which is accomplished by incorporating sequences from the gene itself into the construct. 25 Recombination then occurs in the region of that sequence within the gene, resulting in the insertion of a foreign sequence to disrupt the gene. With its sequence interrupted, the altered gene will be translated into a nonfunctional protein, if it is translated at all. Other techniques include genome editing (targeted genome engineering) as described below. Using either of these techniques, in preferred embodiment, conserved domains 30 which confer function of NGAL2 or NGAL3 respectively are modified.

A skilled person will know further approaches can be used to generate such mutants. In one embodiment, insertional mutagenesis is used, for example using T-DNA mutagenesis (which inserts pieces of the T-DNA from the *Agrobacterium tumefaciens* T-Plasmid into DNA causing either loss of gene function or gain of gene function mutations), site-directed nucleases (SDNs) or transposons as mutagens. Insertional

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mutagenesis is an alternative means of disrupting gene function and is based on the insertion of foreign DNA into the gene of interest (see Krysan et al, The Plant Cell, Vol. 11, 2283-2290, December 1999).

In one embodiment, as discussed in the examples, T-DNA may be used as an insertional mutagen which disrupts SOD7 and/or NGAL3 gene expression. T-DNA not only disrupts the expression of the gene into which it is inserted, but also acts as a marker for subsequent identification of the mutation. Since the sequence of the inserted element is known, the gene in which the insertion has occurred can be recovered, using various cloning or PCR-based strategies. The insertion of a piece of T- DNA on the order of 5 to 25 kb in length generally produces a disruption of gene function. If a large enough population of T-DNA transformed lines is generated, there are reasonably good chances of finding a transgenic plant carrying a T-DNA insert within any gene of interest. Transformation of spores with T-DNA is achieved by an Agrobacterium-mediated method which involves exposing plant cells and tissues to a suspension of Agrobacterium cells.

The details of this method are well known to a skilled person. In short, plant transformation by Agrobacterium results in the integration into the nuclear genome of a sequence called T-DNA, which is carried on a bacterial plasmid. The use of T-DNA transformation leads to stable single insertions. Further mutant analysis of the resultant transformed lines is straightforward and each individual insertion line can be rapidly characterized by direct sequencing and analysis of DNA flanking the insertion. Gene expression in the mutant is compared to expression of the SOD7 and/or NGAL3 nucleic acid sequence in a wild type plant and phenotypic analysis is also carried out. Other techniques for insertional mutagenesis include the use of transposons.

In another embodiment, mutagenesis is physical mutagenesis, such as application of ultraviolet radiation, X-rays, gamma rays, fast or thermal neutrons or protons. The targeted population can then be screened to identify a *SOD7* or *NGAL3* loss of function mutant.

In another embodiment of the various aspects of the invention, the plant is a mutant plant derived from a plant population mutagenised with a mutagen. The mutagen may 35 be fast neutron irradiation or a chemical mutagen, for example selected from the following non-limiting list: ethyl methanesulfonate (EMS), methylmethane sulfonate

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(MMS), N-ethyl-N-nitrosurea (ENU), triethylmelamine (1°E M), N-methyl-N-nitrosourea (MNU), procarbazine, chlorambucil, cyclophosphamide, diethyl sulfate, acrylamide monomer, melphalan, nitrogen mustard, vincristine, dimethylnitosamine, N-methyl-N'-nitro-Nitrosoguanidine (MNNG), nitrosoguanidine, 2-aminopurine, 7,12 dimethyl-benz(a)anthracene (DMBA), ethylene oxide, hexamethylphosphoramide, bisulfan, diepoxyalkanes (diepoxyoctane (DEO), diepoxybutane (BEB), and the like), 2-methoxy-6-chloro-9 [3-(ethyl-2-chloroethyl)aminopropylamino]acridine dihydrochloride (ICR-170) or formaldehyde.

10 In one embodiment, the method used to create and analyse mutations is targeting induced local lesions in genomes (TLLING), reviewed in Henikoff et al, 2004. In this method, seeds are mutagenised with a chemical mutagen, for example EMS. The resulting M1 plants are self-fertilised and the M2 generation of individuals is used to prepare DNA samples for mutational screening. DNA samples are pooled and arrayed 15 on microtiter plates and subjected to gene specific PCR. The PCR amplification products may be screened for mutations in the SOD7 and/or NGAL3 target gene using any method that identifies heteroduplexes between wild type and mutant genes. For example, but not limited to, denaturing high pressure liquid chromatography (dHPLC), constant denaturant capillary electrophoresis (CDCE), temperature gradient capillary 20 electrophoresis (TGCE), or by fragmentation using chemical cleavage. Preferably the PCR amplification products are incubated with an endonuclease that preferentially cleaves mismatches in heteroduplexes between wild type and mutant sequences. Cleavage products are electrophoresed using an automated sequencing gel apparatus, and gel images are analyzed with the aid of a standard commercial image-processing 25 program. Any primer specific to the SOD7 or NGAL3 nucleic acid sequence may be utilized to amplify the SOD7 or NGAL3 nucleic acid sequence within the pooled DNA sample. Preferably, the primer is designed to amplify the regions of the SOD7 and/or NGAL3 gene where useful mutations are most likely to arise, specifically in the areas of the SOD7 and/or NGAL3 gene that are highly conserved and/or confer activity as 30 explained elsewhere. To facilitate detection of PCR products on a gel, the PCR primer may be labelled using any conventional labelling method.

Rapid high-throughput screening procedures thus allow the analysis of amplification products for identifying a mutation conferring the reduction or inactivation of the expression of the *SOD7* and/or *NGAL3* gene as compared to a corresponding non-mutagenised wild type plant. Once a mutation is identified in a gene of interest, the

seeds of the M2 plant carrying that mutation are grown into adult M3 plants and screened for the phenotypic characteristics associated with the target gene SOD7 or NGAL3. Loss of function or reduced function mutants with increased seed size compared to a control can thus be identified.

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Plants obtained or obtainable by such method which carry a functional mutation in the endogenous SOD7 and/or NGAL3 locus are also within the scope of the invention

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In another embodiment, RNA-mediated gene suppression or RNA silencing may be used to achieve silencing of the SOD7 and/or NGAL3 nucleic acid sequence. "Gene silencing" is a term generally used to refer to suppression of expression of a gene via sequence-specific interactions that are mediated by RNA molecules. The degree of reduction may be so as to totally abolish production of the encoded gene product, but more usually the abolition of expression is partial, with some degree of expression 15 remaining. The term should not therefore be taken to require complete "silencing" of expression.

Transgenes may be used to suppress endogenous plant genes. This was discovered originally when chalcone synthase transgenes in petunia caused suppression of the 20 endogenous chalcone synthase genes and indicated by easily visible pigmentation changes. Subsequently it has been described how many, if not all plant genes can be "silenced" by transgenes. Gene silencing requires sequence similarity between the transgene and the gene that becomes silenced. This sequence homology may involve promoter regions or coding regions of the silenced target gene. When coding regions 25 are involved, the transgene able to cause gene silencing may have been constructed with a promoter that would transcribe either the sense or the antisense orientation of the coding sequence RNA. It is likely that the various examples of gene silencing involve different mechanisms that are not well understood. In different examples there may be transcriptional or post-transcriptional gene silencing and both may be used 30 according to the methods of the invention.

The mechanisms of gene silencing and their application in genetic engineering, which were first discovered in plants in the early 1990s and then shown in Caenorhabditis elegans are extensively described in the literature.

RNA-mediated gene suppression or RNA silencing according to the methods of the invention includes co-suppression wherein over-expression of the target sense RNA or mRNA, that is the SOD7 and/or NGAL3 sense RNA or mRNA, leads to a reduction in the level of expression of the genes concerned. RNAs of the transgene and 5 homologous endogenous gene are co-ordinately suppressed. Other techniques used in the methods of the invention include antisense RNA to reduce transcript levels of the endogenous target gene in a plant. In this method, RNA silencing does not affect the transcription of a gene locus, but only causes sequence-specific degradation of target mRNAs. An "antisense" nucleic acid sequence comprises a nucleotide sequence that 10 is complementary to a "sense" nucleic acid sequence encoding a NGAL2 and/or NGAL3 protein, or a part of the protein, i.e. complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA transcript sequence. The antisense nucleic acid sequence is preferably complementary to the endogenous SOD7 and/or NGAL3 gene to be silenced. The complementarity may be located in the 15 "coding region" and/or in the "non-coding region" of a gene. The term "coding region" refers to a region of the nucleotide sequence comprising codons that are translated into amino acid residues. The term "non-coding region" refers to 5' and 3' sequences that flank the coding region that are transcribed but not translated into amino acids (also referred to as 5' and 3' untranslated regions).

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Antisense nucleic acid sequences can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid sequence may be complementary to the entire SOD7 and/or NGAL3 nucleic acid sequence, but may also be an oligonucleotide that is antisense to only a part of the nucleic acid sequence (including 25 the mRNA 5' and 3' UTR). For example, the antisense oligonucleotide sequence may be complementary to the region surrounding the translation start site of an mRNA transcript encoding a polypeptide. The length of a suitable antisense oligonucleotide sequence is known in the art and may start from about 50, 45, 40, 35, 30, 25, 20, 15 or 10 nucleotides in length or less. An antisense nucleic acid sequence according to the 30 invention may be constructed using chemical synthesis and enzymatic ligation reactions using methods known in the art. For example, an antisense nucleic acid sequence (e.g., an antisense oligonucleotide sequence) may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical 35 stability of the duplex formed between the antisense and sense nucleic acid sequences, e.g., phosphorothioate derivatives and acridine-substituted nucleotides

may be used. Examples of modified nucleotides that may be used to generate the antisense nucleic acid sequences are well known in the art. The antisense nucleic acid sequence can be produced biologically using an expression vector into which a nucleic acid sequence has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Preferably, production of antisense nucleic acid sequences in plants occurs by means of a stably integrated nucleic acid construct comprising a promoter, an operably linked antisense oligonucleotide, and a terminator.

10 The nucleic acid molecules used for silencing in the methods of the invention hybridize with or bind to mRNA transcripts and/or insert into genomic DNA encoding a polypeptide to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense 15 nucleic acid sequence which binds to DNA duplexes, through specific interactions in the major groove of the double helix. Antisense nucleic acid sequences may be introduced into a plant by transformation or direct injection at a specific tissue site. Alternatively, antisense nucleic acid sequences can be modified to target selected cells and then administered systemically. For example, for systemic administration, 20 antisense nucleic acid sequences can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid sequence to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid sequences can also be delivered to cells using vectors.

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RNA interference (RNAi) is another post-transcriptional gene-silencing phenomenon which may be used according to the methods of the invention. This is induced by double-stranded RNA in which mRNA that is homologous to the dsRNA is specifically degraded. It refers to the process of sequence-specific post-transcriptional gene silencing mediated by short interfering RNAs (siRNA). The process of RNAi begins when the enzyme, DICER, encounters dsRNA and chops it into pieces called smallinterfering RNAs (siRNA). This enzyme belongs to the RNase III nuclease family. A complex of proteins gathers up these RNA remains and uses their code as a guide to search out and destroy any RNAs in the cell with a matching sequence, such as target mRNA.

Artificial and/or natural microRNAs (miRNAs) may be used to knock out gene expression and/or mRNA translation. MicroRNAs (miRNAs) miRNAs are typically single stranded small RNAs typically 19-24 nucleotides long. Most plant miRNAs have perfect or near-perfect complementarity with their target sequences. However, there are natural targets with up to five mismatches. They are processed from longer noncoding RNAs with characteristic fold-back structures by double-strand specific RNases of the Dicer family. Upon processing, they are incorporated in the RNA-induced silencing complex (RISC) by binding to its main component, an Argonaute protein. miRNAs serve as the specificity components of RISC, since they base-pair to target nucleic acids, mostly mRNAs, in the cytoplasm. Subsequent regulatory events include target mRNA cleavage and destruction and/or translational inhibition. Effects of miRNA overexpression are thus often reflected in decreased mRNA levels of target genes. Artificial microRNA (amiRNA) technology has been applied in Arabidopsis thaliana and other plants to efficiently silence target genes of interest. The design principles for have been generalized and integrated into a Web-based amiRNAs tool (http://wmd.weigelworld.org).

Thus, according to the various aspects of the invention a plant may be transformed to introduce a RNAi, shRNA, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, amiRNA or 20 cosuppression molecule that has been designed to target the expression of an SOD7 and/or NGAL3 nucleic acid sequence and selectively decreases or inhibits the expression of the gene or stability of its transcript. Preferably, the RNAi, snRNA, dsRNA, shRNA siRNA, miRNA, amiRNA, ta-siRNA or cosuppression molecule used according to the various aspects of the invention comprises a fragment of at least 17 25 nt, preferably 22 to 26 nt and can be designed on the basis of the information shown in SEQ ID NO: 1. Guidelines for designing effective siRNAs are known to the skilled person. Briefly, a short fragment of the target gene sequence (e.g., 19-40 nucleotides in length) is chosen as the target sequence of the siRNA of the invention. The short fragment of target gene sequence is a fragment of the target gene mRNA. In preferred 30 embodiments, the criteria for choosing a sequence fragment from the target gene mRNA to be a candidate siRNA molecule include 1) a sequence from the target gene mRNA that is at least 50-100 nucleotides from the 5' or 3' end of the native mRNA molecule, 2) a sequence from the target gene mRNA that has a G/C content of between 30% and 70%, most preferably around 50%, 3) a sequence from the target 35 gene mRNA that does not contain repetitive sequences (e.g., AAA, CCC, GGG, TTT, AAAA, CCCC, GGGG, TTTT), 4) a sequence from the target gene mRNA that is

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accessible in the mRNA, 5) a sequence from the target gene mRNA that is unique to the target gene, 6) avoids regions within 75 bases of a start codon. The sequence fragment from the target gene mRNA may meet one or more of the criteria identified above. The selected gene is introduced as a nucleotide sequence in a prediction program that takes into account all the variables described above for the design of optimal oligonucleotides. This program scans any mRNA nucleotide sequence for regions susceptible to be targeted by siRNAs. The output of this analysis is a score of possible siRNA oligonucleotides. The highest scores are used to design double stranded RNA oligonucleotides that are typically made by chemical synthesis. In addition to siRNA which is complementary to the mRNA target region, degenerate siRNA sequences may be used to target homologous regions. siRNAs according to the invention can be synthesized by any method known in the art. RNAs are preferably chemically synthesized using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer. Additionally, siRNAs can be obtained from commercial RNA oligonucleotide synthesis suppliers.

siRNA molecules according to the aspects of the invention may be double stranded. In one embodiment, double stranded siRNA molecules comprise blunt ends. In another embodiment, double stranded siRNA molecules comprise overhanging nucleotides (e.g., 1-5 nucleotide overhangs, preferably 2 nucleotide overhangs). In some embodiments, the siRNA is a short hairpin RNA (shRNA); and the two strands of the siRNA molecule may be connected by a linker region (e.g., a nucleotide linker or a nonnucleotide linker). The siRNAs of the invention may contain one or more modified nucleotides and/or non-phosphodiester linkages. Chemical modifications well known in 25 the art are capable of increasing stability, availability, and/or cell uptake of the siRNA. The skilled person will be aware of other types of chemical modification which may be incorporated into RNA molecules.

In one embodiment, recombinant DNA constructs as described in US 6635805, 30 incorporated herein by reference, may be used.

The silencing RNA molecule is introduced into the plant using conventional methods, for example a vector and Agrobacterium-mediated transformation. Stably transformed plants are generated and expression of the SOD7 and/or NGAL3 gene compared to a wild type control plant is analysed.

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Silencing of the SOD7 and/or NGAL3 nucleic acid sequence may also be achieved using virus-induced gene silencing.

Thus, in one embodiment of the invention, the plant expresses a nucleic acid construct
comprising a RNAi, shRNA snRNA, dsRNA, siRNA, miRNA, ta-siRNA, amiRNA or cosuppression molecule that targets the *SOD7* or *NGAL3* nucleic acid sequence as described herein and reduces expression of the endogenous *SOD7* or *NGAL3* nucleic acid sequence. A gene is targeted when, for example, the RNAi, snRNA, dsRNA, siRNA, shRNA miRNA, ta-siRNA, amiRNA or cosuppression molecule selectively
decreases or inhibits the expression of the gene compared to a control plant. Alternatively, a RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, amiRNA or cosuppression molecule targets A *SOD7* or *NGAL3* nucleic acid sequence when the RNAi, shRNA snRNA, dsRNA, siRNA, miRNA, ta-siRNA, amiRNA or cosuppression molecule targets a *SOD7* or *NGAL3* nucleic acid sequence when the RNAi, shRNA snRNA, dsRNA, siRNA, miRNA, ta-siRNA, amiRNA or cosuppression

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Gene silencing may also occur if there is a mutation on an endogenous gene and/or a mutation on an isolated gene/nucleic acid subsequently introduced into a plant. The reduction or substantial elimination may be caused by a non-functional polypeptide. For example, the polypeptide may bind to various interacting proteins; one or more mutation(s) and/or truncation(s) may therefore provide for a polypeptide that is still able to bind interacting proteins (such as receptor proteins) but that cannot exhibit its normal function (such as signalling ligand).

A further approach to gene silencing is by targeting nucleic acid sequences complementary to the regulatory region of the gene (e.g., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. Other methods, such as the use of antibodies directed to an endogenous polypeptide for inhibiting its function *in planta,* or interference in the signalling pathway in which a polypeptide is involved, will be well known to the skilled man. In particular, it can be envisaged that manmade molecules may be useful for inhibiting the biological function of a target polypeptide, or for interfering with the signalling pathway in which the target polypeptide is involved.

In one embodiment, the suppressor nucleic acids may be anti-sense suppressors of expression of the NGAL2 or NGAL3 polypeptides. In using anti-sense sequences to down-regulate gene expression, a nucleotide sequence is placed under the control of a promoter in a "reverse orientation" such that transcription yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene.

- 5 An anti-sense suppressor nucleic acid may comprise an anti-sense sequence of at least 10 nucleotides from the target nucleotide sequence. It may be preferable that there is complete sequence identity in the sequence used for down-regulation of expression of a target sequence, and the target sequence, although total complementarity or similarity of sequence is not essential. One or more nucleotides 10 may differ in the sequence used from the target gene. Thus, a sequence employed in a down-regulation of gene expression in accordance with the present invention may be a wild-type sequence (e.g. gene) selected from those available, or a variant of such a sequence.
- 15 The sequence need not include an open reading frame or specify an RNA that would be translatable. It may be preferred for there to be sufficient homology for the respective anti-sense and sense RNA molecules to hybridise. There may be down regulation of gene expression even where there is about 5%, 10%, 15% or 20% or more mismatch between the sequence used and the target gene. Effectively, the homology should be sufficient for the down-regulation of gene expression to take place.

Suppressor nucleic acids may be operably linked to tissue-specific or inducible promoters. For example, integument and seed specific promoters can be used to specifically down-regulate a *SOD7* or *NGAL3* nucleic acids in developing ovules and seeds to increase final seed size.

Nucleic acid which suppresses expression of a NGAL2 or NGAL3 polypeptide as described herein may be operably linked to a heterologous regulatory sequence, such as a promoter, for example a constitutive, inducible, tissue-specific or developmental specific promoter. The construct or vector may be transformed into plant cells and expressed as described herein. Plant cells comprising such vectors are also within the scope of the invention.

In another aspect, the invention relates to a silencing construct to silence expression of *NGAL2* or *NGAL3* obtainable or obtained by a method as described herein and to a plant cell comprising such construct. Accordingly, the invention also relates to the use

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of a nucleic acid sequence comprising or consisting of SEQ ID NO: 1, 2 or 3 or a part thereof or a homologue of SEQ ID NO: 1, 2 or 3 or a part thereof in silencing expression of *NGAL2* or *NGAL3*. Host cells transformed with such construct are also within the scope of the invention.

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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) including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the RNA-guided nuclease Cas9 (CRISPR/Cas9), which generate targeted DNA double-strand breaks (DSBs), which are then repaired mainly by either error-prone non-homologous end joining (NHEJ) or high-fidelity homologous recombination (HR). The SSNs have been used to create targeted knockout plants in various species ranging from the model plants, *Arabidopsis* and tobacco, to important crops, such as barley, soybean, rice and maize. Heritable gene modification has been demonstrated in *Arabidopsis* and rice using the CRISPR/Cas9 system and TALENs.

20 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 25 from microbial mobile genetic elements, ZF nucleases based on eukaryotic transcription factors, 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 30 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 Fokl to direct nucleolytic activity toward specific genomic loci.

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.

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These repeats only differ from each other by two adjacent amino acids, their repeatvariable 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 Fokl nuclease to create a TAL effector nuclease (TALEN) which makes targeted DNA 15 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, 20 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 25 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, 30 CRISPR is a microbial nuclease system involved in defense against invading phages and plasmids. CRISPR loci in microbial hosts contain a combination of CRISPRassociated (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. 35 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

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(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 into mature crRNAs 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.

35 Using these techniques, it is possible to specifically target conserved domains to abolish the function of the NGAL2 and/or NGAL3 polypeptide.

For example, the conserved B3 domain or repression motif may be targeted.

Thus, in another embodiment of the invention directed to a mutant plant, plant cell, plant or a part thereof characterised in that the activity of a NGAL2 polypeptide is altered and said plant expresses a nucleic acid comprising a mutant SEQ ID NO. 1 or 2 and encoding a mutant NGAL2 polypeptide, a functional homologue or variant thereof, for example one which carries a mutation in the B3 or repressor domain.

10 Thus, in another embodiment of the invention directed to a mutant plant, plant cell, plant or a part thereof characterised in that the activity of a NGAL3 polypeptide is altered and said plant expresses a nucleic acid comprising a mutant SEQ ID NO. 4 and encoding a mutant NGAL3 polypeptide, a functional homologue or variant thereof which carries a mutation in the B3 or repressor domain.

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In a preferred embodiment, the invention directed to a mutant plant, plant cell, plant or a part thereof characterised in that the activity of a NGAL2 and a NGAL3 polypeptide is altered and said plant expresses a nucleic acid comprising a mutant SEQ ID NO. 1 or 2 and encoding a mutant NGAL2 polypeptide, a functional homologue or variant thereof, for example one which carries a mutation in the B3 or repressor domain and said plant expresses a nucleic acid comprising a mutant SEQ ID NO. 4 and encoding a mutant NGAL3 polypeptide which carries a mutation in the B3 or repressor domain.

Mutations in the promoter region of SOD7 and/or NGAL3 resulting in a loss of function are also within the scope of the invention.

Constructs designed using the genome editing technologies to knock out or knock down *NGAL2* or *NGAL3*, for example as shown herein, are also within the scope of the invention as well as host cells comprising these constructs. In one embodiment, the constructs comprise or consist of a sequence selected from SEQ ID NO: 155, 156, 157 or 158. Accordingly, in a further aspect of the invention, there is provided a nucleic acid construct comprising a sequence selected from SEQ ID NO: 155, 156, 157 or 158. In a further aspect of the invention, there is provided a nucleic acid construct comprising a sequence, wherein the target sequence is selected from SEQ ID NO: 150, 160, 161, 162 and 163. Preferably, the target sequence comprises at least

two CRISPR target sequences, preferably SEQ ID No 159 and 160 or SEQ ID No 161 and 162, or SEQ ID No 161 and 163 or SEQ ID No 159 and 163.

In another embodiment of the methods of the invention, inactivating, repressing or 5 down-regulating the activity of NGAL2 and/or NGAL3 can be achieved by manipulating the expression of SOD7 and/or NGAL3 inhibitors in a plant, for example transgenic plant. For example, a gene expressing a protein that inhibits the expression of the SOD7 and/or NGAL3 gene or activity of the SOD7 and/or NGAL3 protein can be introduced into a plant and over-expressed. The inhibitor may interact with the 10 regulatory sequences that direct SOD7 and/or NGAL3 gene expression to downregulate or repress SOD7 and/or NGAL3 gene expression. For example, the inhibitor may be a transcriptional repressor. Alternatively, it may interact and repress transcriptional regulators, for example transcription factors, that positively regulate expression of the SOD7 and/or NGAL3 gene. Alternatively, the inhibitor it may directly 15 interact with the NGAL2 and/or NGAL3 protein to inhibit its activity or interact with modulators of the NGAL2 and/or NGAL3 protein. For example, the activity of the NGAL2 and/or NGAL3 protein may be inactivated, repressed or down-regulated by manipulating post-transcriptional modifications, of the NGAL2 and/or NGAL3 protein resulting in a reduced or lost activity.

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In one embodiment, the methods of the invention comprise comparing the activity of the NGAL2 and/or NGAL3 polypeptide and/or expression of the SOD7 and/or NGAL3 gene with the activity of the NGAL2 and/or NGAL3 polypeptide and/or expression of the SOD7 and/or NGAL3 gene in a control plant.

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In another aspect, the invention relates to a plant obtainable or obtained by a method as described herein.

In another aspect, the invention relates to an expression cassette comprising an

isolated nucleic acid sequence comprising or consisting of a sequence as shown in

be a promoter. The invention also relates to a vector comprising such expression

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SEQ ID NO: 1 or 2 a functional part, variant, homologue or orthologue thereof operably linked to a regulatory element. In another aspect, the invention relates to an expression cassette comprising an isolated nucleic acid sequence comprising or consisting of a sequence as shown in SEQ ID NO: 4 or a functional part, variant, homologue or orthologue thereof operably linked to a regulatory element. The regulatory element may

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cassette. The invention also relates to a composition comprising the two expression cassettes above.

In the methods described here, plants can be regenerated from plants transformed or genetically altered as described above and the phenotype, specifically the seed phenotype is analysed by known methods.

Transformation methods are known in the art. The nucleic acid sequence is introduced into said plant through a process called transformation. The term "introduction" or 10 "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 15 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 20 polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. 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 gun bombardment, 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 5 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 10 transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker such as the ones described above. 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, 15 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 generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and nontransformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

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 primary transformed or transfected 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.

The invention also extends to harvestable parts of a plant of the invention as described above such as, but not limited to seeds, leaves, fruits, flowers, stems, roots, rhizomes, tubers and bulbs. 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, starch or proteins. The invention also relates to food products and food supplements comprising the plant of the invention or parts thereof.

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.

20 All documents mentioned in this specification are incorporated herein by reference in their entirety, including references to gene and protein accession numbers.

"and/or" where used herein is to be taken as specific disclosure of each of the multiple specified features or components with or without the other at each combination unless
otherwise dictated. For example "A, B and/or C" is to be taken as specific disclosure of each of (i) A, (ii) B, (iii) C, (iv) A and B, (v) B and C or (vi) A and B and C, 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.

35 Examples

PCT/GB2016/050245

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METHODS

Plant materials and growth conditions

Arabidopsis thaliana Columbia (Col-0) was used as wild-type line. The da 1-1, sod7-1 D, sod7-ko1 and ngal3-ko1 were in the Col-0 background. sod7-1D was identified as a 5 suppressor of da 1-1 by using T-DNA activation tagging method. The sod7-ko1 (SM_3_34191) and ngal3-ko1 (SM_3_36641) were identified in AtIDB (www.atidb.org) and obtained from Arabidopsis Stock Centre NASC collection. T-DNA insertions were confirmed by PCR and sequencing by using the primers described in Table 1. Arabidopsis plants were grown under long-day conditions (16 h light/8 h dark) at 22°C. 10 Activation tagging screening The activation tagging plasmid pJFAT260 was introduced into the da1-1 mutant plants using Agrobacterium tumefaciens strain GV3101 (Fan et al., 2009; Fang et al., 2012), and T1 plants were selected by using the herbicide Basta. Seeds produced from T1 plants were used to isolate modifiers of da 1-1.

15 Morphological and cellular analysis

To measure seed size, we photographed dry seeds of the wild type and mutants under a Leica microscope (LEICA S8APO) using Leica CCD (DFC420). The projective area of wild-type and mutant seeds was measured by using Image J software. Average seed weight was determined by weighing mature dry seeds in batches of 100 using an electronic analytical balance (METTLER TOLEDO AL104, China). The weights of five 20 sample batches were measured for each seed lot. Fully expanded cotyledons, petals (stage 14) and leaves were scanned to produce digital images for area measurement. To measure cell number and cell size, petals, leaves, ovules and seeds were placed in a drop of clearing solution [30ml H20, 80g Chloral hydrate (Sigma, C8383), 10 ml 25 100% Glycerol (Sigma, G6279)]. Cleared Samples were imaged under a Leica microscope (LEICA DM2500) with differential interference contrast (DIC) optics and photographed with a SPOT FLEX Cooled CCD Digital Imaging System. Area measurement was made by using Image J software.

30 Cloning of the SOD7 gene

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The flanking sequences of the T-DNA insertion of the sod7-1D mutant were identified by the thermal asymmetric interlaced PCR (TAIL-PCR) according to a previously reported method (Liu et al., 1995). Briefly, TAIL-PCR utilizes three nested specific primers (OJF22, OJF23 and OJF24) within the T-DNA region of the pJFAT260 vector and a shorter arbitrary degenerate primer (AD1). Thus, the relative amplification efficiencies of specific and non-specific products can be thermally controlled. TAIL-

PCR products were sequenced using the primer OJF24. The specific primers OJF22, OJF23 and OJF24 and an arbitrary degenerate (AD1) primer are described in Table 1.

Constructs and plant transformation

5 The 35S:GFP-SOD7, pSOD7:SOD7-GFP and pSOD7:GUS constructs were made using a PCR-based Gateway system. The coding sequence (CDS) of SOD7 was amplified using the primers SOD7CDS-F and SOD7CDS-R (Table 1). PCR products were cloned into pCR8/TOPO TA cloning vector. The SOD7 CDS was then subcloned into the binary vector pMDC43 with the GFP gene to generate the transformation 10 plasmid 35S:GFP-SOD7. The SOD7 genomic sequence containing 2040-bp promoter sequence and 2104-bp SOD7 gene was amplified using the primers SOD7G-F and SOD7G-R (Table 1). PCR products were cloned into pCR8/TOPO TA cloning vector. The SOD7 genomic sequence was then subcloned into the binary vectors pMDC107 with the GFP gene to generate the transformation plasmid pSOD7:SOD7-GFP. The 15 2262-bp SOD7 promoter sequence was amplified using the primers SOD7P-F and SOD7P-R (Table 1). PCR products were cloned into pCR8/TOPO TA cloning vector. The SOD7 promoter was then subcloned into the binary vectors pGWB3 with the GUS gene to generate the transformation plasmid pSOD7:GUS. The plasmids 35S:GFP-SOD7, pSOD7:SOD7-GFP and pSOD7:GUS were introduced into Col-0 or sod7-ko1 20 ngal3ko1 plants using Agrobacterium tumefaciens GV3101, respectively, and transformants were selected on hygromycin (30µg/ml)-containing medium. The SOD7 cDNA was cloned into the Apal and Spel sites of the binary vector pER8 to generate a chemically inducible construct pER8-SOD7. The specific primers for the pER8-SOD7 construct were SOP7ER-F and SOD7ER-R. The plasmid pER8-SOD7 was introduced 25 into Col-0 plants using Agrobacterium tumefaciens GV3101, and transformants were selected on hygromycin (30µg/ml)-containing medium. GUS staining Samples (pSOD7:GUS) were stained in a GUS staining solution (1 mM X-gluc, 50 Mm NaP04 buffer, 0.4 mM each K3Fe(CN)6/K4Fe(CN)6, and 0.1% (v/v) Triton X-100) and incubated at 37°C for 3 hours. After GUS staining, chlorophyll was removed by 70% 30 ethanol. RT-PCR and quantitative real-time RT-PCR. Total RNA was extracted from Arabidopsis seedlings using an RNAprep pure Plant kit (TIANGEN). mRNA was reverse transcribed into cDNA using SuperScriptIII reverse transcriptase (Invitrogen). cDNA samples were standardized on ACTIN2 transcript amount using the primers ACTIN2-F and ACTIN2-R (Table 1). Quantitative real-time RT-PCR analysis was 35 performed with a Lightcycler 480 machine (Roche) using the Lightcycler 480 SYBR Green I Master (Roche). ACTIN2 mRNA was used as an internal control, and relative

amounts of mRNA were calculated using the comparative threshold cycle method. The primers used for RT-PCR and quantitative real-time RT-PCR are described in Table 1.

The chromatin immunoprecipitation (ChIP) assay

5 The chromatin immunoprecipitation (ChIP) assay was performed as described previously with minor modifications (Gendrel et al., 2005). Briefly, 35S:GFP and 35S:GFP-SOD7 transgenic seeds were grown on 1/2 MS plates for 10 days. The seedlings were cross-linked by 1% formaldehyde for 15 min in vacuum and stopped by 0.125 M Glycine. Samples were ground in liquid nitrogen, and nuclei were isolated.
10 Chromatin was immunoprecipitated by anti-GFP (Roche, 11814460001) and protein A+G beads (Millpore Magna ChIP Protein A+G Magnetic Beads, 16-663). DNA was precipitated by glycogen, NaOAc and ethanol, washed by 70% ethanol, and dissolved in 60 μ I of water. Gene-specific primers (PF1-F, PF1-R, PF-2F, PF2-R, ACTIN7-ChIP-F, and ACTIN7-ChIP-R) were used to quantify the enrichment of each fragment (Table 1).

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The DNA electrophoretic mobility shift assay (EMSA)

The coding sequence of SOD7 was cloned into the Ndel and BamHI sites of the pMAL-C2 vector to generate the construct MBP-SOD7. MBP-SOD7 fusion proteins were expressed in Escherichia coli BL21 (DE3) (Biomed) and purified by Amylose resins(New England Biolabs). The biotin-labeled and unlabeled probes were synthesized as forward and reverse strands. The forward and reverse strands were then incubated in a solution (50mM Tris-HCI, 5mM EDTA and 250mM NaCI) at 95 °C for 10min and renatured to double stranded probes at room temperature. The gel-shift assay was performed according to the method described previously (Smaczniak et al., 2012).

Results

30 sod7-1D suppresses the seed size phenotype of da 1-1

We previously identified the ubiquitin receptor DA1 as a negative regulator of seed size in Arabidopsis (Li et al., 2008). The *da1-1* mutant formed large seeds due to increased cell proliferation in the maternal integuments (Li et al., 2008; Xia et al., 2013). To identify novel components in the DA1 pathway or other seed size regulators, we initiated a T-DNA activation tagging screen for modifiers of *da1-1* (Fang et al., 2012). A dominant suppressor of *da1-1* (sod7-1 D) was isolated from seeds produced from

approximate 16,000 T1 plants (Fig.IA). Seeds of the *sod7-1D da1-1* double mutant were significantly smaller and lighter than *da 1-1* seeds (Figures 1A, E and F). The embryo constitutes the major volume of a mature seed in Arabidopsis. *sod7-1D da1-1* embryos were smaller than *da 1-1* embryos (Figure 1B). The size of *sod7-1D da1-1* cotyledons was significantly reduced, compared with that of *da 1-1* cotyledons (Figure 1G). In addition, *sod7-1D da1-1* double mutant formed smaller leaves and flowers than *da1-1* (Figures 1C and 1D). Thus, these results show that the *sod7-1D* mutation suppressed the seed and organ size phenotypes of *da 1-1*.

10 *sod7-1D* produces small seeds

We isolated the single *sod7-1D* mutant among F2 progeny derived from a cross between the wild type (Col-0) and *sod7-1D da1-1*. The *sod7-1D* seeds were significantly smaller and lighter than wild-type seeds (Figures 2A, B, G and H). We further isolated and visualized embryos from mature wild-type and *sod7-1D* seeds. The *sod7-1D* embryos were obviously smaller than wild-type embryos (Figures 2C and D). The changes in seed size were also reflected in the size of seedlings (Figures 2E and F). The 10-d old *sod7-1D* cotyledons were significantly smaller than wild-type cotyledons (Figure. 2E, F and I). In addition, the *sod7-1D* mutants exhibited small leaves and flowers compared with the wild type. The decreased size of *sod7-1D* leaves and petals was not caused by smaller cells, indicating that the *sod7-1D* mutation results in a decrease in cell number. In fact, the average area of epidermal cells in *sod7-1D* petals was larger than that in wild-type petals, suggesting a possible compensation mechanism between cell number and cell size.

25 SOD7 encodes a B3 domain transcriptional repressor NGAL2

To determine whether the seed and organ size phenotypes of *sod7-1D* was caused by the T-DNA insertion, we firstly analyzed the genetic linkage of the mutant phenotypes with Basta resistance, which is conferred by the selectable marker of the activation tagging vector (Fan et al., 2009). In a T2 population, 181 plants with *sod7-1D da1-1* phenotypes were resistant, whereas 55 plants with *da 1-1* phenotypes were sensitive, indicating that the insertion is cosegregated with the *sod7-1D* phenotypes. To clone the SOD7 gene, we isolated the T-DNA flanking sequences using thermal asymmetric interlaced PCR (Liu et al., 1995). DNA sequencing revealed that the T-DNA had inserted approximately 5.6 kb upstream of the At3g1 1580 and about 3.7 kb upstream of the At3g1 1590 gene (Figure 3A). To determine which gene is responsible for the *sod7-1D* phenotypes, we examined the mRNA levels of these two genes. The mRNA

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of the At3g1 1590 gene accumulated at a similar level in sod7-1D da1-1 and da1-1, suggesting that At3g1 1590 is not the SOD7 gene (Figure 3B). By contrast, expression level of the At3g1 1580 gene in sod7-1D da1-1 plants was dramatically higher than that in da1-1 plants, suggesting that At3g1 1580 is the SOD7 gene (Figure 3B). To further confirm whether the sod7-1D phenotypes were caused by ectopic At3g1 1580 expression, we overexpressed the At3q1 1580 gene (35S:GFP-SOD7) in wild-type plants (Col-0) and isolated 37 transgenic plants. Most transgenic lines showed small seeds and organs (Figures 3D-F), similar to those observed in the sod7-1D single mutant, indicating that At3g1 1580 is the SOD7 gene. The SOD7 gene encodes a 10 NGATHA like protein (NGAL2) containing a B3 DNA-binding domain and a transcriptional repression motif (Figure 3C) (Alvarez et al., 2009; Ikeda and Ohme-Takagi, 2009; Trigueros et al., 2009). SOD7 belongs to the RAV gene family that consists of 13 members in Arabidopsis (Figure 10) (Swaminathan et al., 2008). Several members of the RAV family contain the putative transcriptional repression motifs, including NGA1, NGA2, NGA3, NGA4, NGAL1, NGAL2/SOD7 and NGAL3 (Figure 10) (Ikeda and Ohme-Takagi, 2009). The transcriptional repression motifs in NGA1, NGAL1 and NGAL2/SOD7 have been known to possess the repressive activity (Ikeda and Ohme-Takagi, 2009), indicating that they are transcriptional repressors. SOD7 exhibits the highest similarity to Arabidopsis NGAL3/DEVELOPMENT-RELATED PcG TARGET IN THE APEX 4 (DPA4) (Figure 10), which has known roles in the regulation of leaf serrations (Engelhorn et al., 2012), but no previously identified function in seed size control.

Expression pattern and subcellular localization of SOD7

To monitor SOD7 expression pattern during development, the pSOD7:GUS and 25 pSOD7:SOD7-GFP vectors were constructed and transformed to wild-type plants, respectively. The tissue-specific expression patterns of SOD7 were examined using a histochemical assay for GUS activity. In seedlings, relatively higher GUS activity was detected in younger leaves than in older leaves (Figures 4A -C). In flowers, GUS 30 activity was observed in sepals, petals, stamens and carpels (Figures 4D-K). GUS activity was stronger in younger floral organs than in older ones (Figures 4D-K). Expression of SOD7 was also detected in ovules (Figure.4L). Thus, these analyses indicate that SOD7 is a temporally and spatially expressed gene. As SOD7 encodes a B3 domain transcriptional repressor, we speculated that SOD7 is localized in the nucleus. To determine subcellular localization of SOD7, we observed GFP 35 inflorescence in pSOD7:SOD7-GFP transgenic plants. As shown in Figures 4M-0,

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GFP signal was only detected in nuclei. We also expressed a GFP-SOD7 fusion protein under the control of the 35S promoter in wild-type plants. Transgenic lines overexpressing *GFP-SOD7* formed smaller seeds than the wild type (Figure 3D), indicating that the *GFP-SOD7* fusion protein is functional. As shown in Figures 4P-R, GFP fluorescence in *35S:GFP-SOD7* transgenic plants was exclusively observed in nuclei. Thus, these results show that SOD7 is a nuclear-localized protein.

SOD7/NGAL2 acts redundantly with NGAL3 to control seed size

In order to further investigate the function of SOD7 in seed size control, we isolated T-10 DNA inserted loss-of-function mutants for SOD7 and NGAL3, the most closely related family member. sod7-ko1 (SM 3 34191) was identified with T-DNA insertion in the first exon of the SOD7 gene (Figure 5A). ngal3-ko1 (SM 3 36641) had T-DNA insertion in the first exon of the NGAL3 gene (Figure 5B). The T-DNA insertion sites were confirmed by PCR using T-DNA specific and flanking primers and sequencing PCR 15 products. sod7-ko1 and ngal3-ko1 mutants had no detectable full-length transcripts of SOD7 and NGAL3, respectively. Seeds from sod7-ko1 and ngal3-ko1 mutants were slightly larger and heavier than seeds from wild-type plants (Figures 5C, G and H). The cotyledon area of sod7-ko1 and ngal3-ko1 mutants was increased, compared with that of the wild type (Figure 5I). Considering that SOD7 shares the highest similarity with 20 NGAL3, we speculated that SOD7 may act redundantly with NGAL3 to influence seed size. To test this, we generated the sod7-ko1 ngal3-ko1 double mutant. As shown in Figures 5C, D, G and H, the seed size and weight phenotypes of sod7-ko1 mutant were synergistically enhanced by the disruption of NGAL3, indicating that SOD7 functions redundantly with NGAL3 to control seed size. We further measured the cotyledon area of 10-d-old seedlings. A synergistic enhancement of cotyledon size of 25 sod7-ko1 by the ngal3-ko1 mutation was also observed (Figure 5I). In addition, the sod7-ko1 ngal3-ko1 double mutant formed larger leaves and flowers than their parental lines (Figures 5E and F; 11). Thus, these results indicate that SOD7 and NGAL3 act redundantly to control seed and organ growth.

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SOD7 acts maternally to control seed size

As the size of a seed is determined by the zygotic and/or maternal tissues (Garcia et al., 2005; Xia et al., 2013; Du et al., 2014), we asked whether SOD7 functions maternally or zygotically. We therefore performed reciprocal cross experiments between the wild type and *sod7-ko1 ngal3-ko1*. The effect of *sod7-ko1 ngal3-ko1* on seed size was observed only when *sod7-ko1 ngal3-ko1* was used as maternal plants

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(Figure 6A). The size of seeds from *sod7-ko1 ngal3-ko1* plants pollinated with wild-type pollen was similar to that from the self-pollinated *sod7-ko1 ngal3-ko1* plants (Figure 6A). By contrast, the size of seeds from wild-type plants pollinated with *sod7-ko1 ngal3-ko1* mutant pollen was similar to that from the self-pollinated wild-type plants (Figure 6A). These results indicate that *sod7-ko1 ngal3-ko1* acts maternally to influence seed size. We further investigated the size of Col-O/Col-0 F2, *Col-0/sod7-ko1 ngal3-ko1* F2, *sod7-ko1 ngal3-ko7*/Col-O F2 and *sod7-ko1 ngal3-ko11 sod7-ko1 ngal3-ko1* seeds. As shown in Figure 6B, *sod7-ko1 ngal3-ko11 sod7-ko1 ngal3-ko1* F2 seeds. As shown in Figure 6B, *sod7-ko1 ngal3-ko11 sod7-ko1 ngal3-ko1* F2 and *sod7-ko1 ngal3-ko7*/Col-O F2 seeds was similar to that of wild-type seeds. Thus, these results indicate that the embryo and endosperm genotypes for SOD7 do not determine seed size, and SOD7 is required in the sporophytic tissue of the mother plant to control seed growth.

15 SOD7 regulates cell proliferation in the maternal integuments

The reciprocal crosses showed that SOD7 functions maternally to influence seed size. The integuments surrounding the ovule are maternal tissues, which could set the growth potential of the seed coat after fertilization. Consistent with this idea, several studies showed that the integument size influences the final size of seeds in Arabidopsis (Garcia et al., 2005; Schruff et al., 2006; Adamski et al., 2009; Xia et al., 20 2013; Du et al., 2014). We therefore asked whether SOD7 acts through the maternal integuments to determine seed size. To test this, we characterized mature ovules of the wild type and sod7-ko1 ngal3-ko1. As shown in Figures 6C and D, the sod7-ko1 ngal3-ko1 ovules were obviously larger than wild-type ovules. The outer integument 25 length of sod7-ko1 ngal3-ko1 ovules was significantly increased, compared with that of wild-type ovules (Figure 6E). As the size of the integument is determined by cell proliferation and cell expansion, we examined the number and size of outer integument cells in wild-type and sod7-ko1 ngal3-ko1 ovules. As shown in Figure 6F, the number of outer integument cells in sod7-ko1 ngal3-ko1 ovules was increased, compared with 30 that in wild-type ovules. By contrast, the length of outer integument cells in sod7-ko1 ngal3-ko1 ovules was similar to that in wild-type ovules (Figure 6G). These results showed that SOD7 is required for cell proliferation in the maternal integuments of ovules. After fertilization, cells in the integument mainly undergo expansion but still have division. We further examined the number and size of outer integument cells in wild-type and sod7-ko1 ngal3-ko1 seeds at 6 and 8 day after pollination (DAP). In wild-35 type seeds, the number of outer integument cells at 6 DAP was comparable with that at

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8 DAP (Figure 6F), indicating that cells in the outer integuments of wild-type seeds completely stop dividing by 6 DAP. Similarly, cells in the outer integuments of *sod7-ko1 ngal3-ko1* seeds also cease division by 6 DAP. The number of outer integument cells in *sod7-ko1 ngal3-ko1* seeds was significantly increased, compared with that in wild-type seeds (Figure 6F). By contrast, the length of outer integument cells in *sod7-ko1 ngal3-ko1* seeds was not increased in comparison to that in wild-type seeds (Figure 6G). Thus, these analyses indicate that *SOD7* is required for cell proliferation in the maternal integuments of ovules and developing seeds.

10 <u>SOD7 acts in a common pathway with *KLU* to control seed size, but does so independently of *DM*</u>

The Arabidopsis klu mutants formed small seeds due to the decreased cell proliferation in the integuments, while plants overexpressing KLU/CYP78A5 produced large seeds as a result of the increased cell proliferation in the integuments (Adamski et al., 2009), suggesting that SOD7 and KLU could function antagonistically in a common pathway to control seed growth. To test for genetic interactions between SOD7 and KLU, we generated the klu-4 sod7-ko1 ngal3-ko1 triple mutant and measured the size of seeds from wild-type, klu-4, sod7-ko1 ngal3-ko1 and klu-4 sod7-ko1 ngal3-ko1 plants. As shown in Figures 7A and B, the average size and weight of klu-4 sod7-ko1 ngal3-ko1 seeds were similar to those of the klu-4 single mutant, indicating that klu-4 is epistatic to sod7-ko1 ngal3-ko1 with respect to seed size and weight. We further investigated the mature ovules from wild-type, klu-4, sod7-ko1 ngal3-ko1 and klu-4 sod7-ko1 ngal3ko1 plants. The outer integument length of klu-4 sod7-ko1 ngal3-ko1 ovules was comparable with that of klu-4 ovules (Figure 7C). Similarly, the outer integument length of klu-4 sod7-ko1 ngal3-ko1 seeds was indistinguishable from that of klu-4 seeds at 8 DAP (Figure 7C). In addition, the size of klu-4 sod7-ko1 ngal3-ko1 petals was similar to that of klu-4 petals).

Thus, these genetic analyses show that klu-4 is epistatic to sod7-ko1 ngal3-ko1 with
respect to seed and organ size, indicating that SOD7 and KLU act antagonistically in a common pathway to control seed and organ growth. To further understand the cellular basis of epistatic interactions between SOD7 and KLU, we investigated the outer integument cell number of ovules and developing seeds from wild-type, klu-4, sod7-ko1 ngal3-ko1 and klu-4 sod7-ko1 ngal3-ko1 plants. The number of outer integument cells in klu-4 sod7-ko1 ngal3-ko1 ovules was similar to that in klu-4 ovules (Figure 7D). Similarly, the number of outer integument cells in klu-4 sod7-ko1 seeds was

comparable with that in klu-4 seeds (Figure 7D). These results indicate that klu-4 is epistatic to sod7-ko1 ngal3-ko1 with respect to the number of outer integument cells. We also observed that cells in the outer integuments of klu-4 and klu-4 sod7-ko1 ngal3ko1 seeds were slightly longer than those in wild-type seeds, suggesting a possible compensation mechanism between cell proliferation and cell expansion. Together, these findings show that SOD7 functions antagonistically in a common pathway with KLU to control cell proliferation in the maternal integuments.

Considering that sod7-1D was identified as a suppressor of da 1-1 in seed size, we 10 further asked whether SOD7 and DA1 could act in the same genetic pathway. To test this, we measured the size of wild-type, da 1-1, sod7-1D and sod7-1D da1-1 seeds. The genetic interaction between sod7-1D and da 1-1 was essentially additive for seed size, compared with that of sod7-1D and da 1-1 single mutants, indicating that SOD7 might function independently of DM to control seed size. We further crossed sod7-ko1 15 ngal3-ko1 with da 1-1 and generated the sod7-ko1 ngal3-ko1 da 1-1 triple mutant and measured its seed size. The genetic interaction between sod7-ko1 ngal3-ko1 and da1-1 was also additive for seed size, compared with their parental lines, further supporting that SOD7 functions to control seed growth separately from DM.

20 SOD7 directly binds to the promoter of KLU and represses the expression of KLU

Considering that SOD7 acts antagonistically in a common pathway with KLU to control seed size, we asked whether the transcription repressor SOD7 could repress the expression of KLU. We therefore investigated the expression of KLU in the chemicallyinducible SOD7 {pER8-SOD7) transgenic plants. After the pER8-SOD7 transgenic plants were treated with the inducer (β -estradiol), the expression of SOD7 was 25 strongly induced at 4 and 8 hours (Figure 8A). As expected, the expression of KLU was dramatically repressed at 4 and 8 hours (Figure 8A). Thus, these results indicate that SOD7 represses the expression of KLU and also suggest that KLU might be a direct target of SOD7.

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To determine whether SOD7 can directly bind to the promoter of the KLU gene, we performed a chromatin immunoprecipitation (ChIP) assay with 35S:GFP and 35.GFP-SOD7 transgenic plants. It has been reported that the CACCTG sequence is recognized by the B3 domain of RAV1, one member of the RAV family (Kagaya et al., 1999; Yamasaki et al., 2004). We therefore analyzed the promoter sequence of KLU and did not find an intact CACCTG sequence within 2 kb promoter region of KLU.

However, we found a similar sequence (CACTTG) in the promoter region of *KLU* (Figure 8B), which could be the potential SOD7-binding site. To test this, we examined the enrichment of a *KLU* promoter fragment (PF1) containing the CACTTG sequence by ChIP analyses and found that the fragment PF1 was strongly enriched in the chromatin-immunoprecipitated DNA with anti-GFP antibody (Figures 8B and C). By contrast, we did not detect significant enrichment of an ACTIN7 promoter sequence and the KLU promoter fragment PF2, which do not contain the CACTTG sequence (Figures 8B and C). This result shows that SOD7 associates with the promoter of *KLU* in vivo. We further expressed SOD7 as a MBP fusion protein (MBP-SOD7) and performed the DNA electrophoretic mobility shift assays (EMSA). As shown in Figures 8B and D, MBP-SOD7 was able to bind to the biotin-labeled probe A containing the CACTTG sequence, and the binding was reduced by the addition of an unlabeled probe A. By contrast, MBP-SOD7 failed to bind to a probe A-m with mutations in the CACTTG sequence (Figures 8B and D). Taken together, these results show that SOD7 directly binds to the promoter of *KLU* and represses *KLU* expression.

Discussion

Seed size is crucial for plant fitness and agricultural purposes, but little is known about the genetic and molecular mechanisms that set the final size of seeds in plants. In this study, we show that SOD7 acts maternally to control seed size by restricting cell proliferation in the integuments of ovules and developing seeds. *SOD7* encodes a B3 domain transcriptional repressor NGAL2 and acts redundantly with its closest homolog NGAL3 to control seed size. Genetic analyses indicate that SOD7 functions in a common pathway with the maternal factor *KLU* to control seed growth, but does so independently of DA1. Further results reveal that SOD7 directly binds to the promoter region of *KLU* and represses *KLU* expression. Thus, our findings identify SOD7 as a negative factor for seed size and define the genetic and molecular mechanisms of *SOD7* and KLU in seed size control.

30 SOD7 acts maternally to regulate seed size

The *sod7-1D* gain-of-function mutant was identified as a suppressor of the large seed phenotype of *da 1-1*. However, genetic analyses showed that *SOD7* functions independently of DA1 to control seed growth. The *sod7-1D* single mutant produced small seeds and organs (Figure 2), while the simultaneous disruption of *SOD7* and the closely related family member *NGAL3* resulted in large seeds and organs (Figure 5), indicating that *SOD7* is a negative regulator of seed and organ size. Several previous

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studies suggest that there is a possible link between seed size and organ growth. For instance, art2, da 1-1, da2A and eod3-1D mutants produced large seeds and organs (Schruff et al., 2006; Li et al., 2008; Fang et al., 2012; Xia et al., 2013), whereas klu and sod2/ubp15 mutants formed small seeds and organs (Anastasiou et al., 2007; Adamski et al., 2009; Du et al., 2014). However, seed size is not invariably associated with organ size. For example, eod8/med25 mutants with large organs formed normalsized seeds (Xu and Li, 201 1), while ap2 mutants with normal-sized organs produced large seeds (Jofuku et al., 2005; Ohto et al., 2005). Thus, these findings suggest that seeds and organs not only share common mechanisms but also possess distinct pathways to control their respective size.

Reciprocal cross experiments showed that SOD7 acts maternally to restrict seed growth, and the endosperm and embryo genotypes for SOD7 do not determine seed size (Figure 6). The integuments surrounding the ovule are maternal tissues and form the seed coat after fertilization. Arabidopsis art2, ap2, da1-1, da2-1 and eod3-1D mutants with large integuments formed large seeds (Jofuku et al., 2005; Ohto et al., 2005; Schruff et al., 2006; Li et al., 2008; Fang et al., 2012; Xia et al., 2013), while klu-4 and ubp15/sod2 mutants with small integuments produced small seeds (Adamski et al., 2009; Du et al., 2014), indicating that the maternal integuments are crucial for 20 determining seed size in Arabidopsis. Consistent with this notion, mature eod7-ko1 ngal3-ko1 ovules were larger than wild-type ovules (Figures 6C and D). The outer integument length of eod7-ko1 ngal3-ko1 ovules and developing seeds was significantly increased, compared with that of wild-type ovules and seeds (Figures 6E and 7C). Considering that the maternal integument or seed coat not only acts as a 25 protective structure but also restricts seed growth, the regulation of maternal integument size is one of important mechanisms for seed size control. The size of the integument is determined by cell proliferation and cell expansion; these two processes are assumed to be coordinated. The number of outer integument cells in sod7-ko1 ngal3-ko1 ovules and seeds was significantly increased, compared with that in wild-30 type ovules and seeds (Figure 6F), indicating that SOD7 controls seed growth by limiting cell proliferation in the maternal integuments. Similarly, several mutants with the increased number of cells in the maternal integuments produced large seeds in Arabidopsis (Schruff et al., 2006; Li et al., 2008; Xia et al., 2013). By contrast, several other mutants with the decreased number of cells in the maternal integuments formed small seeds in Arabidopsis (Adamski et al., 2009; Du et al., 2014). Considering that

35 cells in the integuments mainly undergo expansion after fertilization (Garcia et al.,

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2005), it is possible that the number of cells in the integuments determines the growth potential of the seed coat after fertilization.

The genetic and molecular mechanisms of SOD7 and KLU in seed size control

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The sod7-1D mutant had small seeds and organs (Figure 2), as had been seen in klu mutants (Anastasiou et al., 2007; Adamski et al., 2009). KLU encodes a cytochrome P450 CYP78A5 that has been proposed to generate mobile plant-growth substances (Anastasiou et al., 2007; Adamski et al., 2009). KLU regulates seed size by promoting cell proliferation in the maternal integuments of ovules (Anastasiou et al., 2007; Adamski et al., 2009). By contrast, SOD7 acts maternally to control seed size by limiting cell proliferation in the integuments of ovules and developing seeds (Figure 6). These results suggest that SOD7could function antagonistically in a common pathway with KLU to control seed size. In our growth conditions, klu-4 formed slightly smaller seeds than the wild type due to the decreased cell number and the slightly increased cell length in the integuments of developing seeds (Figures 7A and D), suggesting a possible compensation mechanism between cell proliferation and cell expansion in klu-4 integuments. Importantly, our genetic analyses showed that klu-4 is epistatic to sod7ko1 ngal3-ko1 with respect to seed and organ size (Figures 7A and B). klu-4/s also epistatic to sod7-ko1 ngal3-ko1 for the outer integument length (Figure 7C). Further results revealed that the number of cells in the outer integuments of klu-4 sod7-ko1 ngal3-ko1 ovules and developing seeds was similar to that of klu-4ovules and developing seeds (Figure 7D). Thus, these genetic results demonstrate that SOD7 act in a common pathway with KLU to control seed size by regulating cell proliferation in the maternal integuments.

SOD7 encodes a B3 domain transcriptional repressor NGAL2 that is localized in nuclei of Arabidopsis cells (Figures 4M-R). Thus, it is possible that SOD7 could directly bind to the promoter of *KLU* and repress *KLU* expression. Supporting this idea, the inducible expression of SOD7 resulted in a strong reduction of *KLU* expression (Figure 8A). Our ChIP-qPCR data showed that SOD7 associates with the promoter region of *KLU* in vivo (Figures 8B and C). EMSA experiments revealed that SOD7 directly binds to the CACTTG sequence in the promoter of the KLU gene (Figures 8B and D). Thus, these results illustrate that SOD7 directly targets the promoter region of KLU and represses the expression of *KLU*, thereby determining seed size. Taken together, these findings

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reveal the genetic and molecular mechanisms of SOD7 and KLU in regulating Arabidopsis seed size.

For many plants, the seeds are the main product to be harvested, and an increase in seed size would be beneficial for growers. In this study, we identify SOD7 as a negative regulator of seed size, and demonstrate that SOD7 acts in a common genetic pathway with *KLU* to control seed size. Our current knowledge of SOD7 functions suggests that the SOD7 gene (and its homologs in other plant species) could be used to engineer large seed size in crops. Considering that crop plants have undergone selection for large seed size during domestication (Fan et al., 2006; Song et al., 2007; Gegas et al., 2010), it will be a worthwhile challenge to know whether beneficial alleles of the SOD7 gene have already been utilized by plant breeders.

Knockout experiments in rice using genome editing

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Genome editing experiments to knock out os1 1g01560000 and/or Os12g0157000 in rice are being carried out using the crisper-cas9 system. Four vectors, each with two recognition (CRISPR target) sites, have been constructed, to achieve these knock outs, as described in Fig. 14. In summary, the vectors were obtained as follows:

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1. The target sites were identified. The target site should be (or approximately so) 20 nucleotides before a NGG sequence, N being for any nucleotide. The target sequence was then evaluated using the website: <u>http://cbi.hzau.edu.cn/crispr/help.php</u> (incorporated herein by reference). Of note, the target site should be unique in the genome.

2. Using overlap PCR, the target sequence is linked with the U6 sequence, as shown in Figure 14. U6 is for transcriptional activity.

3. Using infusion technology we connected the U6-guide-gRNA scaffold fragment to the vector pMDC99-cas9 to obtain the pMDC99-cas9- U6-guide-gRNA scaffold constructs. These constructs were named zyy1,zyy2, zyy3 ,zyy4. The full sequences of these constructs are represented in SEQ ID NO: 155, 156, 157 and 158 respectively. Each construct contains two recognition sites, which are highlighted in the sequence information, and are represented separately as SEQ ID Nos 159, 160, 161, 162 and 163.

35 4. We then transformed these constructs into Agrobacteria and used an Agrobacteria mediated method to transform rice and obtain gene-edited rice.

Transformation of plants is a routine technique that is well known to the skilled person. Nonetheless, a brief outline of transformation techniques is provided above.

Knock out lines are being analysed to assess the phenotype.

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	Table 1. Primers used in this study				
	Primer Name Primer Sequences				
	Primers for T-DNA identification				
	SM_3_34191-LP ACCATGACATTCGAGGTTCAC (SEQ ID NO. 8)				
10	SM_3_34191-RP ATCACCACCAAAACGACGTAG (SEQ ID NO. 9)				
	SM_3_36641-RP TACGTCATGCTTCAAATCGTG (SEQ ID NO. 10)				
	SM_3_3664 1-RP AGGACACGAACAATTCATTCG (SEQ ID NO. 11)				
	Spm32 TACGAATAAGAGCGTCCATTTTAGAGTGA (SEQ ID NO. 12)				
	SM_3_39145- LP ACCCAAAGAACAGCAATCATG (SEQ ID NO. 13)				
15	SM_3_39145- RP AAAACACTCCGCCATTAAACC (SEQ ID NO. 14)				
	Primers for TAIL-PCR				
	OJF22 CGAGTATCAATGGAAACTTAACCG (SEQ ID NO. 15)				
	OJF23 AACGGAGAGTGGCTTGAGAT (SEQ ID NO. 16)				
	OJF24 TGGCCCTTATGGTTTCTGCA (SEQ ID NO. 17)				
20	AD1 NTCGA(G/C)T(A/T)T(G/C)G(A/T)GTT (SEQ ID NO. 18)				
	Primers for Constructs				
	SOD7CDS-F ATGTCAGTCAACCATTACCAC (SEQ ID NO. 19)				
	SOD7CDS-F ATGTCAGTCAACCATTACCAC (SEQ ID NO. 19) SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20)				
25	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20)				
25	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21)				
25	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22)				
25	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23)				
25	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24)				
25 30	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25)				
	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25) SOD7-ER-R GCGACTAGTTTATAAAAGAGTTAAAATTA (SEQ ID NO. 25)				
	SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25) SOD7-ER-R GCGACTAGTTTATAAAAGAGTTAAAATTA (SEQ ID NO. 25) MBP-SOD7-FP CGGGATCCTCAGTCAACCATTACC (SEQ ID NO. 27)				
	 SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25) SOD7-ER-R GCGACTAGTTTATAAAAGAGTTAAAATTA (SEQ ID NO. 25) MBP-SOD7-FP CGGGATCCTCAGTCAACCATTACC (SEQ ID NO. 27) MBP-SOD7-RP ACTAGTCGACTCAACCTCGTCCATCTCC (SEQ ID NO. 28) 				
	 SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25) SOD7-ER-R GCGACTAGTTTATAAAAGAGTTAAAATTA (SEQ ID NO. 25) MBP-SOD7-FP CGGGATCCTCAGTCAACCATTACC (SEQ ID NO. 27) MBP-SOD7-RP ACTAGTCGACTCAACCATCCC (SEQ ID NO. 28) Primers for RT-PCR and qRT-PCR 				
	 SOD7CDS-R CAGGTAGGAGATGGACGAGGTTGA (SEQ ID NO. 20) SOD7G-F TGAGAGGAACCATTTCTTAGAGG (SEQ ID NO. 21) SOD7G-R ACCTCGTCCATCTCCTACCTGC (SEQ ID NO. 22) SOD7P-F AAACACGTCAAATATAACGAAT (SEQ ID NO. 23) SOD7P-R CTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (SEQ ID NO. 24) SOD7-ER-F AGTCTGGGCCCATGTCAGTCAACCATTAC (SEQ ID NO. 25) SOD7-ER-R GCGACTAGTTTATAAAAGAGTTAAAATTA (SEQ ID NO. 25) MBP-SOD7-FP CGGGATCCTCAGTCAACCATTACC (SEQ ID NO. 27) MBP-SOD7-RP ACTAGTCGACTCAACCATTACC (SEQ ID NO. 28) Primers for RT-PCR and qRT-PCR ACTIN2-F GAAATCACAGCACTTGCACC (SEQ ID NO. 29) 				

NGAL3-EX-F TTTGAAGACGAGTCAGGCAAGT (SEQ ID NO. 33) NGAL3-EX-R TACGGCGGCTCCATAGTGGG (SEQ ID NO. 34) SOD7-q-FP GTATTGGAGCGGCTTGACTACACC (SEQ ID NO. 35) SOD7-q-RP GACGGCATCACCATGACATTCG (SEQ ID NO. 36) KLU-q-FP TGATTCTGACATGATTGCTGTTCT (SEQ ID NO. 37) 5 KLU-q-RP TCGCAACTGTATCTGTCCCTCTA (SEQ ID NO. 38) Primers for ChIP assay ACTIN7-ChIP-FP CGTTTCGCTTTCCTTAGTGTTAGCT (SEQ ID NO. 29) ACTI N7-Chl P-RP AGCGAACGGATCTAGAGACTCACCTTG (SEQ ID NO. 40) 10 PF1-F CAGGCCTAAGCCTAACAGTAGAC (SEQ ID NO. 41) PF1-R TGTACTAGGATTTATTTACGTAG (SEQ ID NO. 42) PF2-F TATTGTTCATAGAAACCCTGCAAA (SEQ ID NO. 43) PF2-R AGTCAATGGTTTAATGGCGGAGTG (SEQ ID NO. 44) **Probes for EMSA** A-Biotin-FP TTCTACTACACTTGCTCTCTGTA (SEQ ID NO. 45) 15 A-Biotin-RP TACAGAGAGCAAGTGTAGTAGAA (SEQ ID NO. 46)

A-Biotin-m-FP TTCTACTAACACCTCTCTCTGTA (SEQ ID NO. 47)

A-Biotin-m-RP TACAGAGAGAGAGGTGTTAGTAGAA (SEQ ID NO. 48)

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 Plant Cell 16, 3448-3459.

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Sequence information

Identity of homologs to NGAL2 is indicated

AtSOD7 nucleic acid SEQ ID NO. 1 (cDNA) At3g1 1580

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ATGTCAGTCAACCATTACCACAACACTCTCTCGTTGCATCATCACCACCAAAACGA GTCGGAAAGCTAAACCGCTTAGTCATACCAAAACAACACGCCGAGAAATACTTCC CTCTCAATAATAATAATAATGGCGGCAGCGGAGATGACGTGGCGACGACGGA 10 GAAAGGGATGCTTCTTAGCTTCGAGGATGAGTCAGGCAAGTGTTGGAAATTCAGA TACTCTTATTGGAACAGTAGCCAAAGCTACGTGTTGACCAAAGGATGGAGCAGGT TTTTGATCTCCATAGACTCTTCATTGGCTGGCGGAGACGCGGTGAAGCTTCTTCCT CTCCCGCTGTCTCCGTTGTGTCTCAAGAAGCTCTAGTTAATACGACGGCGTATTG 15 GAGCGGCTTGACTACACCTTATCGTCAAGTACACGCGTCAACTACTTACCCTAATA TTCACCAAGAGTATTCACACTATGGCGCCGTCGTTGATCATGCTCAGTCGATACCA CCGGTGGTCGCAGGTAGCTCGAGGACGGTGAGGCTTTTTGGCGTGAACCTCGAA TGTCATGGTGATGCCGTCGAGCCACCACCGCGTCCTGATGTCTATAATGACCAAC ACATTTACTATTACTCAACTCCTCATCCCATGAATATATCATTTGCTGGGGAAGCAT 20 TGGAGCAGGTAGGAGATGGACGAGGTTGA

AtSOD7 nucleic acid SEQ ID NO. 2 (genomic DNA).

ttgtttcggctatttgttatactattgttataacagtcacaagacttgacctcaacgaaaacttttacaaaacgtgaattggaaa 25 tttttacaaaatatgctcttaatcgttaatgcttccccaattaggtgagttaaattgtgagaggaaccatttcttagaggaaatggt tcatgaaaacaaatatgaaatagtatcactagtcttagttttgcgagaaaattaggaaaaatagaaacgtgtaagcacca cttgagaaattcgtcaaataaaatagaagggaccactcacgtaaccatttgcacgtcccattgatttttgtggtagacttgg tatgttatattacttatattcacagaattatatacgaaactcacgacttaagatgcacggtaataactacagatggaaatttac 30 atattagttagaactttttcttctacaattgatcaaatgtttcacactgttctcaatttctcatctagattcatgacttatatgtttggtc aaatatcacagcttgatgagcattaaatagcgtcgaagtataggatggttacgttgttcaatattgtaaaggaaaaaaga agaaaaagagagaataaaatgtagataaagagaaagagaaagagagagagagaacataagggatggtatgaagta 35 gaagtgaagatgcatgcgatggtgtgtgtgggaaaggcaaagcacatgctacacaacttgagcttctcacttgcgtcaggg ataagtatcctctgtaccttcttacttttgcgtaatatgtaccacctcacttctcaaccgtttgatctttaatccttcattatttcttcatt

- atccgttacatgcgcgtgaggagaaccgtccaatccacttagactaacgtgccctttatttcttccttttaattctatgttaaaaa
 aacaatttaactaaaagatgcgcacgtgtcttgacggtggaaaaaaattgtagGCGCCGTCGTTGATCATG
 CTCAGTCGATACCACCGGTGGTCGCAGGTAGCTCGAGGACGGTGAGGCTTTTTG
 GCGTGAACCTCGAATGTCATGGTGATGCCGTCGAGCCACCACCGCGTCCTGATG
 TCTATAATGACCAACACATTTACTATTACTCAACTCCTCATCCCATGgtaaatatttttttttt
 acattttgtcagattcaaatttttgcttacgtatgatataattattaaacagatgtcgtggctgtttctcgagacgagacagatg

AtNGAL2 SEQ ID N0.3 (protein encoded by AtSOD7) ...

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MSVNHYHNTLSLHHHHQNDVAIAQRESLFEKSLTPSDVGKLNRLVIPKQHAEKYFPLN NNNNNGGSGDDVATTEKGMLLSFEDESGKCWKFRYSYWNSSQSYVLTKGWSRYVK DKHLDAGDWFFQRHRFDLHRLFIGWRRRGEASSSPAVSVVSQEALVNTTAYWSGL TTPYRQVHASTTYPNIHQEYSHYGAVVDHAQSIPPWAGSSRTVRLFGVNLECHGDA VEPPPRPDVYNDQHIYYYSTPHPMNISFAGEALEQVGDGRG

AtNGAL3 nucleic acid sequence SEQ ID NO. 4 (cDNA) at5g06250

ATGTCAGTCAACCATTACTCCACAGACCACCACCACACTCTCTTGTGGCAGCAAC 20 AGCAACACCGCCACACCGACACATCGGAGACAACCACCACCGCCACATGGC TCCACGACGACCTAAAAGAGTCACTCTTCGAGAAGTCTCTCACACCAAGCGACGT CGGGAAACTCAACCGCCTCGTCATACCAAAACAACACGCAGAGAAATACTTCCCT CTCAATGCCGTCCTAGTCTCCTCTGCTGCTGCTGACACGTCATCTTCGGAGAAAG GGATGCTTCTAAGCTTTGAAGACGAGTCAGGCAAGTCATGGAGGTTCAGATACTC TTACTGGAACAGCAGTCAAAGCTATGTCTTGACTAAAGGATGGAGCAGATTTGTCA 25 AAGACAAACAGCTCGATCCAGGCGACGTTGTTTTCTTCCAACGACACCGTTCTGA TTCTAGGAGACTCTTCATTGGCTGGCGCAGACGTGGACAAGGCTCCTCATCCTCC CACTATGGAGCCGCCGTAGCAACAGCGGCTGAGACTCACAGCACCGTCGTCT 30 TCCGTCGTCGGGAGCTCAAGGACGGTGAGGCTTTTCGGTGTGAATCTGGAGTGT CAAATGGATGAAAACGACGGAGATGATTCTGTTGCAGTTGCCACCACCGTTGAAT CTCCCGACGGTTACTACGGCCAAAACATGTACTATTATTACTCTCATCCTCATAAC ATGGTAATTTTAACTCTTTTATAA

35

AtNGAL3 amino acid SEQ ID NO.5

MSVNHYSTDHHHTLLWQQQQHRHTTDTSETTTTATWLHDDLKESLFEKSLTPSDVG KLNRLVIPKQHAEKYFPLNAVLVSSAAADTSSSEKGMLLSFEDESGKSWRFRYSYWN SSQSYVLTKGWSRFVKDKQLDPGDWFFQRHRSDSRRLFIGWRRRGQGSSSSVAAT NSAVNTSSMGALSYHQIHATSNYSNPPSHSEYSHYGAAVATAAETHSTPSSSWGSS RTVRLFGVNLECQMDENDGDDSVAVATTVESPDGYYGQNMYYYYSHPHNMVILTLL

Oryza sativa

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10 Osl2g0157000 LOC_Osl2g06080.1 Cover 73% identity 53%

SEQ ID NO: 49

MAMHAGHAWWGVAMYTNHYHHHYRHKTSDVGKNRVKHARYGGGDSGKGSDSGKWRRYSYWTSSSYVTKG 15 WSRYVKKRDAGDVVHRVRGGAADRGCRRRGSAAAVRVTANGGWSMCYSTSGSSYDTSANSYAYHRSVDDHSD HAGSRADAKSSSAASASRRRGVNDCGADATAMYGYMHHSYAAVSTVNYWSV

CDS SEQ ID NO: 50

- 20 ATGGCCATGCACCCTCTCGCCCAGGGGCACCCCCAGGCGTGGCCATGGGGTGTAGCCATG TACACCAACCTGCACTACCACCACCACTACGAGAGGGAGCACCTGTTCGAGAAGCCGCTG ACGCCGAGCGACGTCGGCAAGCTCAACAGGCTGGTGATCCCCAAGCAGCACGCCGAGAGG TACTTCCCGCTCGGCGGCGGCGACTCCGGTGAGAAGGGCCTCCTCCTCCTTCGAGGAC GAGTCCGGCAAGCCATGGCGGTTCCGCTACTCCTACTGGACCAGCAGCCAGAGCTACGTG
- **30** AGCGACATACTACACGCAGGAGAGTCGCAGAGAGAAGCAGACGCCAAGAGCAGCAGCGCG GCGTCGGCGCCGCCGCCGTCGAGGCGGCTCAGGCTGTTCGGCGTTAACCTCGACTGCGGC CCGGAGCCGGAGGCGGATCAGGCGACGGCAATGTACGGCTACATGCACCACCAGAGCCCC TACGCCGCAGTGTCTACAGTGCCAAATTACTGGTCAGTATTTTTTCAGTTTTAA

35

Osllg0156000 LOC_Osllg05740.1 Cover 81% identity 47%

40 SEQ ID NO: 51

MAM NHPLFSQEQPQSWPWGVAMYAN FHYHH HYEKEHMFEKPLTPSDVGKLNRLVIPKQHA ERYFPLGAGDAADKGLILSFEDEAGAPWRFRYSYWTSSQSYVLTKGWSRYVKEKRLDAGD VVHFERVRGSFGVGDRLFIGCRRRGDAAAAQTPAPPPAVRVAPAAQNAGEQQPWSPMCYS TSGGGSYPTSPANSYAYRRAADHDHGDMHADESPRDTDSPSFSAGSAPSRRLRLFGVNL

45 DCGPEPEADTTAAATMYGYMHQQSSYAAMSAVPSYWGNS

CDS SEQ ID NO: 52 ATGGCCATGAACCACCCTCTTCTCCCAGGAGCAACCCCAGTCCTGGCCATGGGGTGTG

GCCATGTACGCCAACTTCCACTACCACCACCACCACCACGAGAAGGAGCACATGTTTGAGAAG

CCCCTGACGCCCAGTGACGTGGGGAAGCTGAACCGGCTGGTGATCCCCAAGCAGCACGCC GAGAGGTACTTCCCCCTCGGCGCCGGCGACGCCGCCGACAAGGGCCTGATCCTGTCGTTC GAGGACGAGGCCGGCGCGCGCGGCGGCGGCTCAGGTACTCCTACTGGACGAGCAGCCAGAGC TACGTGCTCACCAAGGGCTGGAGCCGCTACGTCAAGGAGAAGCGCCTCGACGCCGGCGAC

- 15

os02g0683500	LOC_Os02g45850	
Cover 47%	identity 62%	

20 SEQ ID NO: 53

MEFTTSSRFSKEEEDEEQDEAGRREIPFMTATAEAAPAPTSSSSSPAHHAASASASASAS GSSTPFRSDDGAGASGSGGGGGGGGGAEVVEKEHM FDKVVTPSDVGKLNRLVIPKQYAEK YFPLDAAAN EKGLLLNFEDRAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVS FSRGIGDEAARHRLFIDWKRRADTRDPLRLPRGLPLPMPLTSHYAPWGIGGGGGGFFVQPS PPATLYEHRLRQGLDFRAFNPAAAMGRQVLLFGSARIPPQAPLLARAPSPLHHHYTLQPS

25 PPATLYEHRLRQGLDFRAFNPAAAMGRQVLLFGSARI PPQAPLLARAPSPLHHHYTLQPS GDGVRAAGSPVVLDSVPVI ESPTTAAKRVRLFGVNLDNPHAGGGGGAAAGESSN HGNALS LQTPAWM RRDPTLRLLELPPH HHHGAESSAASSPSSSSSKRDAHSALDLDL

CDS SEQ ID NO: 54

- 35 ACGCCGAGCGACGTTGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGTACGCCGAGAAG TACTTCCCGCTGGACGCGGCGGCGAACGAGAAGGGCCTCCTGCTCAACTTCGAGGACCGC GCGGGGAAGCCATGGCGGTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTGATG ACCAAGGGGTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGGGACACCGTCTCC TTCTCCCGCGGCATCGGCGACGAGGCGGCGCGCGCACCGCCTCTTCATCGACTGGAAGCGC

- 50 AAGAGGGACGCGCATTCGGCCTTGGATCTCGATCTGTAG

os04g0581400 LOC_Os04g49230 Cover 46% identity 64%

- 10 GTGGAGGTGATCGAGAAGGAGCACATGTTCGACAAGGTGGTGACGCCGAGCGACGTGGGG AAGCTGAACCGGCTGGTGATCCCGAAGCAGCACGCCGAGAAGTACTTCCCGCTGGACTCG GCGGCGAACGAGAAGGGCCTTCTCCTCAGCTTCGAGGACCGAACCGGCAAGCTATGGCGC TTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTCATGACCAAGGGTTGGAGCCGC TTCGTCAAGGAGAAGCGCCTCGACGCCGGGGACACCGTCTCCTTCTGCCGCGGCGCCGCC
- 15 GAGGCCACCCGCGACCGCCTCTTCATCGACTGGAAGCGCCGCGCCGACGTCCGCGACCCG CACCGCTTCCAGCGCCTACCGCTCCCCATGACCTCGCCCTACGGCCCGTGGGGGGGCGGC GCGGGCGCTTCTTCATGCCGCCGCCGCCGCCCGCCACGCTCTACGAGCATCACCGCTTTC GCCAGGGCTTCGACTTCCGCAACATCAACCCCGCTGTGCCGGCGAGGCAGCTCGTCTTCT TCGGCTCCCCAGGGACGGGGATTCATCAGCACCCGCCCTTGCCACCGCCGCCGTCGCCAC
 20 CTCCGCCTCCTCACCAACTCCACATTACGGTGCACCACCCGAGCCCCGTAG

SEQ ID NO: 56

M EFATTSSRFSKEEEEEEGEQEMEQEQDEEEEEAEASPREIPFMTSAAAAATASSSSPT SVSPSATASAAASTSASGSPFRSSDGAGASGSGGGGGGDVEVIEKEH MFDKVVTPSDVG KLNRLVIPKQHAEKYFPLDSAANEKGLLLSFEDRTGKLWRFRYSYWNSSQSYVMTKGWSR FVKEKRLDAGDTVSFCRGAAEATRDRLFIDWKRRADVRDPHRFQRLPLPMTSPYGPWGGG AGASSCRPRRPPRSTSITAFARASTSATSTPLCRRGSSSSSAPQGRGFISTRPCHRRRH

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os03g0120900	LOC_Os03g02900
Cover 47%	identity 63%

CDS SEQ ID NO: 57

LRLLTNSTLRCTTRAP

- 35 ATGGAGTTCATCACGCCAATCGTGAGGCCGGCATCGGCGGCGGCGGCGGCGGCGGCGAGGTG CAGGAGAGTGGTGGGAGGAGCTTGGCGGCGGTGGAGAAGGAGCACATGTTCGACAAGGTG GTGACGCCGAGCGACGTGGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGCACGCGGAG AAGTACTTCCCGCTGGACGCGGCGTCCAACGAGAAGGGGGCTCCTGCTCAGCTTCGAGGAC CGCACGGGGAAGCCATGGCGGTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTG

M EFITPIVRPASAAAGGGEVQESGGRSLAAVEKEHM FDKVVTPSDVGKLNRLVI PKQHAE KYFPLDAASNEKGLLLSFEDRTGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTV SFGRGVGEAARGRLFIDWRRRPDVVAALQPPTHRFAHHLPSSI PFAPWAHHHGHGAAAAA AAAAGARFLLPPSSTPIYDH HRRHAHAVGYDAYAAATSRQVLFYRPLPPQQQH HPAVVLE SVPVRMTAGHAEPPSAPSKRVRLFGVNLDCANSEQDHAGVVGKTAPPPLPSPPSSSSSSS **GKARCSLN LDL**

10

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os01g0693400 Cover 47% identity 63%

15 CDS SEQ ID NO: 59

ATGGACAGCTCCAGCTGCCTGGTGGATGATACCAACAGCGGCGGCTCGTCCACGGACAAG CTGAGGGCGTTGGCCGCCGCGGCGGCGGGGGGGGCGCGCCGCTGGAGCGCATGGGGAGCGGG GCGAGCGCGGTGGTGGACGCGGCCGAGCCTGGCGCGGAGGCGGACTCCGGGTCCGGGGGA 20 AAGTTCAAGGGCGTCGTGCCGCAGCCCAACGGGAGGTGGGGCGCGCAGATCTACGAGCGG GCCGACCCGGACGCCGCCGCGAGCTTCGCTTCCTCGCCACGCGCTCCAAGGCCGAGGTC GTCGACATGCTCCGCAAGCACCACCTACTTCGACGAGCTCGCGCAGAGCAAGCGCACCTTC

- 25 ACCCCGAGCGACGTGGGCAAGCTGAACAGGCTCGTCATACCGAAGCAGCACGCCGAGAAG CACTTCCCGCTACAGCTCCCGTCCGCCGGCGGCGAGAGCAAGGGTGTCCTCCTCAACTTC GAGGACGCCGGCCAGGTGTGGCGGTTCCGGTACTCGTACTGGAACAGCAGCCAGAGC
- 30 TACGTGCTAACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGGGTCTCCACGCCGGCGAC GTCGTCGGCTTCTACCGCTCCGCCGCCAGTGCCGGCGACGACGGCAAGCTCTTCATCGAC GAACAGATGGCCGGGTGCAAGAGAGCCAGGGACTTGGCGGCGACGACGCCTCCACAAGCG 35 GCGGCGTTCAAGAAGCAATGCATAGAGCTGGCACTAGTATAG

SEQ ID NO: 49

60M DSSSCLVDDTNSGGSSTDKLRALAAAAAETAPLERMGSGASAVVDAAEPGAEADSGSGG 40 RVCGGGGGGGGGGGGGGKLPSSKFKGVVPQPNGRWGAQIYERHQRVWLGTFAGEDDAARAYD VAAQRFRGRDAVTNFRPLAEADPDAAAELRFLATRSKAEVVDM LRKHTYFDELAQSKRTF AASTPSAATTTASLSNGHLSSPRSPFAPAAARDHLFDKTVTPSDVGKLN RLVIPKQHAEK HFPLQLPSAGGESKGVLLNFEDAAGKVWRFRYSYWNSSQSYVLTKGWSRFVKEKGLHAGD VVGFYRSAASAGDDGKLFI DCKLVRSTGAALASPADQPAPSPVKAVRLFGVDLLTAPAPV 45 EQMAGCKRARDLAATTPPQAAAFKKQCI ELALV

Osl0g0537100 LOC_Osl0g39190 Cover 47% 50 identity 60%

> CDS SEQ ID NO: 61 ATGGAGTTCACCCCAATTTCGCCGCCGACGAGGGTCGCCGGCGGTGAGGAGGATTCCGAG

- 10 TACGGCTACGGCGCGGCGGCGCGCTCCACCCCGGCGTCCAGCCGCCACGTGCTGTTCCTC CGGCCGCAGGTGCCGGCCGCTGTGGTGCTCAAGTCGGTGCCGGTGCACGTCGCGGGCCACC TCGGCGGTGCAGGAGGCGGCGACGACGACAAGGCCGAAGCGTGTCCGGCTGTTCGGGGTG AACCTCGACTGCCCGGCGGCCATGGACGACGACGACGACATCGCCGGAGGCGGCGAGCCGG ACGGCAGCGTCGTCTCTCCTGCAGCTCCCCTCGCCGTCGTCCTCGACGTCGTCGTCGTCGACG
- 15 GCGGGGAAGAAGATGTGCTCCTTGGATCTTGGGTTGTGA

SEQ ID NO: 62

Glycine max

30 L.OC100795470 Cover 75% identity 53%

SEQ ID NO: 63

Msinhysmdlpeptlwwphphhqqqqltlmdpdplrlnlnsddgngndndndenqttttggeqeilddkepmf^ kpltpsdvgklnr
35 Vipkqhaekyfplsgdsggseckglllsfedesgkcwrfrysywnssqsyvltkgwsrykdkrldagdvvlferhrvdaqrlfigwrrrqsd
aalppahvssrksgggdgnsnknegwtrgfysahhpypthhlhhhqp spyqqhdclhagrgsqgqnqrmrpvgnnssssssrvlrl fgvdmecqpehddsgpstpqcsynsnnmlpstqgtdhshhnfyqqqpsnsnpsphhmmvhqpyyy

40 CDS SEQ ID NO: 64

ATGTCCATAAACCACTACTCCATGGACCTTCCCGAACCGACACTCTGGTGGCCACACCCA CACCACCAACAACAACAACTAACCTTAATGGATCCTGACCCTCTCCGTCTCAACCTCAAT AGCGACGATGGCAATGGCAATGACAACGACAACGACGAAAATCAAACAACCACAACAGGA GGAGAACAAGAAATATTAGACGATAAAGAACCGATGTTCGAGAAGCCCTTAACCCCCGAGC

- 45 GACGTGGGGAAGCTGAACCGTCTCGTAATCCCGAAGCAGCACGCGGAGAAGTACTTCCCA CTGAGTGGTGACTCGGGCGGGAGCGAGTGCAAGGGGCTGTTACTGAGTTTCGAGGACGAG TCG GGGAAGTGTTG GCGCTTCCG CTACTCGTACTG GAACAG CAG CCAG A GCTACGTGCTC ACCAAAGGGTGGAGCCGCTACGTCAAGGACAAGCGCCTTGACGCGGGCGACGTCGTTTG TTCGAGCGTCACCGCGTCGACGCGCAGCGCCTCTTCATCGGGTGGAGGCGCAGGCGGCAG
- 50 AGCGATGCCGCCTTGCCGCCTGCGCACGTTAGCAGTAGGAAGAGTGGTGGTGGTGGTGATGGG AATAGTAATAAGAATGAGGGGTGGACCAGAGGGTTCTATTCTGCGCATCATCCTTATCCT ACGCATCATCTTCATCATCATCAGCCCTCGCCATACCAACAACAACATGACTGTCTTCAT

LOC100818164	
Cover 50%	identity 73%

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SEQ ID NO: 65

MSTNHYTM DLPEPTLWWPHPHQQQLTLI DPDPLPLNLN NDDNDNGDDNDNDENQTVTTTT TGGEEEI INNKEPMFEKPLTPSDVGKLNRLVIPKQHAEKYFPLSGGDSGSSECKGLLLSF EDESGKCWRFRYSYWNSSQSYVLTKGWSRYVKDKRLDAGDVVLFQRHRADAQRLFIGWRR RRQSDALPPPAHVSSRKSGGDGNSSKNEGDVGVGWTRGFYPAHHPYPTHHHHPSPYHHQQ DDSLHAVRGSQGQNQRTRPVGNSSSSSSSSRVLRLFGVNMECQPEHDDSGPSTPQCSYN TNNI LPSTQGTDI HSHLNFYQQQQTSNSKPPPHH MMI RHQPYYY

20 SEQ ID NO: 66

ATGTCGACAAACCACTACACCATGGACCTTCCCGAACCAACACTCTGGTGGCCACACCAC CACCAACAACAACTAACCTTAATAGATCCAGACCCTCTCCCTCTGAACCTCAACAACGAC GACAACGACAATGGCGACGACAACGACAACGACGACGAAAACCAAACAGTTACAACAACCACA ACAGGAGGAGAAGAAGAAATAATAAACAATAAAGAACCGATGTTCGAGAAGCCGCTAACC

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LOC100802734 Cover 77% identity 53%

45 SEQ ID NO: 67

MSSINHYSPETTLYWTNDQQQQAAMWLSNSHTPRFN LNDEEEEEDDVIVSDKATNNLTQ EEEKVAMFEKPLTPSDVGKLNRLVPKQHAEKH FPLDSSAAKGLLLSFEDESGKCWRFRY SYWNSSQSYVLTKGWSRYVKDKRLHAGDVVLFHRHRSLPQRFFISCSRRQPNPVPAHVST TRSSASFYSAHPPYPAHHFPFPYQPHSLHAPGGGSQGQNETTPGGNSSSSGSGRVLRLFG VNMECQPDNHN DSQNSTPECSYTHLYHHQTSSYSSSSNPHHHMVPQQP

SEQ ID NO: 68

ATGTCATCGATAAACCACTATTCACCGGAAACAACACTATACTGGACCAACGACCAACAG CAACAAGCCGCCATGTGGCTGAGTAATTCCCACACCCCGCGTTTCAATCTGAACGACGAG GAGGAGGAGGAGGAAGACGACGTTATCGTTTCGGACAAGGCTACTAATAACTTGACGCAA GAGGAGGAGAAGGTAGCCATGTTCGAGAAGCCGTTGACGCCGAGCGACGTCGGGAAGCTG

L oc100781489 Cover 49% identity 64%

20 SEQ ID NO: 69

M ELMQQVKGNYSDSREEEEEEAAAITRESESSRLHQQDTASNFGKKLDLMDLSLGSSKE EEEEGNLQQGGGGVVHHAHQVVEKEHMFEKVATPSDVGKLNRLVIPKQHAEKYFPLDSST NEKGLLLNFEDRNGKVWRFRYSYWNSSQSYVMTKGWSRFVKEKKLDAGDIVSFQRGLGDL YRHRLYIDWKRRPDHAHAHPPH HHDPLFLPSI RLYSLPPTMPPRYHHDHHFH HHLNYNNL

25 FTFQQHQYQQLGAATTTH HNNYGYQNSGSGSLYYLRSSMSMGGGDQN LQGRGSNIVPMI I DSVPVNVAHHNN NRHGNGGITSGGTNCSGKRLRLFGVN MECASSAEDSKELSSGSAAHVT TAASSSSLHHQRLRVPVPVPLEDPLSSSAAAAARFGDHKGASTGTSLLFDLDPSLQYHRH

30 CDS SEQ ID NO: 70

ATGGAGTTGATGCAACAAGTTAAAGGTAATTATTCTGATAGCAGGGAGGAAGAGGAGGAA GAGGAAGCTGCAGCAATCACAAGGGAATCAGAAAGCAGCAGGTTACACCAACAAGATACA GCATCCAATTTTGGAAAGAAGCTAGACTTGATGGACTTGTCACTAGGGAGCAGCAAGGAA GAGGAAGAGGAAGGGAATTTGCAACAAGGAGGAGGAGGAGTGGTTCATCATGCTCACCAA

- 35 GTAGTGGAGAAAGAACACATGTTTGAGAAAGTGGCGACACCGAGCGACGTAGGGAAGCTG AACAGGCTGGTGATACCGAAGCAGCACGCGGAGAAGTACTTCCCCCTTGACTCCTCAACC AACGAGAAGGGTCTGCTCCTGAATTTCGAGGACAGGAATGGGAAGGTGTGGCGATTCAGG TATTCCTATTGGAACAGCAGCCAGAGCTATGTGATGACAAAAGGGTGGAGCCGCTTTGTT AAGGAGAAGAAGCTGGATGCCGGTGACATTGTCTCCTTCCAGCGTGGCCTTGGGGATTTG
- 40 TATAGACATCGGTTGTATATAGATTGGAAGAGAGAGGCCCGATCATGCTCATGCTCATCCA CCTCATCACGATCCTTTGTTTCTTCCCTCTATCAGATTGTACTCTCCCCCCACC ATGCCACCTCGCTACCACCACGATCATCACTTTCACCACCACTCAATTACAACAACCTC TTCACTTTTCAGCAACACCAGTACCAGCAGCTTGGTGCTGCCACTACCACTCATCACAAC AACTATGGTTACCAGAATTCGGGATCTGGTTCACTCTATTACCTAAGGTCCTCTATGTCA
- 45 ATGGGTGGTGGTGATCAAAACTTGCAAGGGAGGAGGGAGCAACATTGTCCCCATGATCATT GATTCTGTGCCGGTTAACGTTGCTCATCACAACAACAATCGCCATGGGAATGGGGGGCATC ACGAGTGGTGGTACTAATTGTAGTGGAAAACGACTAAGGCTATTTGGGGTGAACATGGAA TGCGCTTCTTCGGCAGAAGATTCCAAAGAATTGTCCTCGGGTTCGGCAGCACACGTGACG ACAGCTGCTTCTTCTTCTTCTTCATCATCAGCGCTTGAGGGTGCCAGTGCCAGTGCCA 50 CTTGAAGATCCACTTTCGTCGTCAGCAGCAGCAGCAGCAAGGTTTGGGGATCACAAAGGG
- GCCAGTACTGGGACTTCGCTGCTGCTGTTTGATTTGGATCCCTCTTTGCAGTATCATCGCCAC TGA

LOC100776987

Cover 46% identity 62%

5 SEQ ID NO: 71

MDAISCLDESTTTESLSISQAKPSSTIMSSEKASPSPPPPNRLCRVGSGASAVVDSDGGG GGGSTEVESRKLPSSKYKGVVPQPNGRWGSQIYEKHQRVWLGTFN EEDEAARAYDVAVQR FRGKDAVTNFKPLSGTDDDDGESEFLNSHSKSEIVDMLRKHTYNDELEQSKRSRGFVRRR GSAAGAGNGNSISGACVM KAREQLFQKAVTPSDVGKLNRLVIPKQHAEKHFPLQSAANGV

10 SATATAAKGVLLNFEDVGGKVWRFRYSYWNSSQSYVLTKGWSRFVKEKNLKAGDTVCFQR STGPDRQLYIDWKTRNVVNEVALFGPVVEPIQMVRLFGVNI LKLPGSDSIANNNNASGCC NGKRREM ELFSLECSKKPKI IGAL

15 CDS SEQ ID NO: 72

- 25 GGCTCCGCCGGCGGCGGAAACGGAAACTCAATCTCCGGCGCGTGTGTTATGAAGGCG CGTGAGCAGCTATTCCAGAAGGCCGTTACGCCGAGCGACGTTGGGAAACTGAACCGTTTG GTGATACCGAAGCAGCACGCGGAGAAGCACTTTCCTTTACAGAGCGCTGCTAACGGCGTT AGCGCGACGGCGACGGCGGCGAAGGGCGTTTTGTTGAACTTCGAAGACGTTGGAGGGAAA GTGTGGCGGTTTCGTTACTCGTATTGGAACAGTAGCCAGAGTTACGTCTTGACCAAAGGT

Locl00778733 Cover 44% identity 64%

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SEQ ID NO: 73

MELMQEVKGYSDGREEEEEEEAAEEI ITREESSRLLHQHQEAAGSNFI INNN HHHHQHH HHHTTKQLDFMDLSLGSSKDEGNLQGSSSSVYAHH HHAASASSSANGN NNNSSSSNLQQQ QQQPAEKEHM FDKVVTPSDVGKLNRLVI PKQHAEKYFPLDSSANEKGLLLNFEDRNGKLW 45 RFRYSYWNSSQSYVMTKGWSRFVKEKKLDAGDMVSFQRGVGELYRHRLYI DWWRRPDH HH HHH HGPDHSTTLFTPFLI PNQPHH LMSI RWGATGRLYSLPSPTPPRHHEHLNYNNNAMYH PFHHHGAGSGI NATTH HYNNYHEMSSTTTSGSAGSVFYHRSTPPISMPLADHQTLNTRQQ QQQQQQEGAGNVSLSPMI IDSVPVAHHLHHQQH HGGKSSGPSSTSTSPSTAGKRLRLFG VNMECASSTSEDPKCFSLLSSSSMANSNSQPPLQLLREDTLSSSSARFGDQRGVGEPSM L

50 FDLDPSLQYRQ

SEQ ID NO: 74

ATGGAGTTGATGCAAGAAGTGAAAGGGTATTCTGATGGCAGAGGAGGAGGAGGAGGAGGAGGA GAGGAAGCAGCAGAAGAAATCATCACAAGAGAAGAAAGCAGCAGGTTGTTACACCAGCAC CAG GAGG CAG CAG GTTCCAATTTCATCATCAACAATAATCATCATCATCATCAACATCAC CACCACCACAAAAAAGCAGCTAGACTTCATGGACTTGTCACTTGGTAGCAGCAAGGAT

- 5 GAAGGGAATTTGCAAGGATCATCTTCTTCTGTCTATGCTCATCATCATCATGCAGCAAGT GCTAGTTCTTCTGCCAATGGTAACAACAACAACAGCAGCAGCAGCAACTTGCAGCAACAG CAGCAGCAGCCTGCTGAGAAGGAGCACATGTTTGATAAAGTAGTGACACCAAGTGATGTG GGGAAGCTGAACCGGTTGGTGATACCAAAGCAGCATGCTGAGAAGTATTTCCCTCTTGAT TCCTCAGCCAATGAGAAGGGTCTGTTGCTGAATTTTGAGGACAGGAATGGTAAGTTGTGG
- 10 AGGTTCAGGTACTCCTATTGGAACAGCAGCCAGAGCTATGTGATGACCAAAGGTTGGAGC CGTTTTGTTAAGGAGAAGAAGCTTGATGCTGGTGACATGGTGTCCTTCCAGCGTGGTGTT GGGGAGTTGTATAGGCATAGGTTGTACATAGATTGGTGGAGAAGGCCTGATCATCATCAC CATCACCATCATGGCCCTGACCATTCAACCACACTCTTCACACCTTTCTTAATTCCCAAT
- 15 TCCCCAACCCCACCACCACCATGAACACCTCAATTACAACAATAACGCCATGTATCAT CCCTTTCATCACCATGGTGCTGGAAGTGGAATTAATGCTACTACTACACCAACAACAAC TATCATGAGATGAGTAGTACTACTACTTCAGGATCTGCAGGCTCAGTCTTTTACCACAGG TCAACACCCCCAATATCAATGCCATTGGCTGACCACCAAACCTTGAACACAAGGCAGCAG CAACAACAACAACAACAACAAGAGGGAGCTGGCAATGTTTCTCTTTCCCCTATGATCATT
- 20 GATTCTGTTCCAGTTGCTCACCACCATCCATCAACAACACCATGGTGGCAAGAGTAGT GGTCCTAGTAGTACTAGTACTAGTCCTAGCACTGCAGGGAAAAGACTAAGGCTATTTGGG GTCAACATGGAATGTGCTTCTTCAACATCAGAAGACCCCCAAATGCTTCAGCTTGTTGTCC TCATCTTCAATGGCTAATTCCAATTCACAACCACCACTTCAGCTTTTGAGGGAAGATACA CTTTCGTCATCATCGGCAAGGTTTGGGGATCAGAGAGGAGTAGGGGAACCTTCAATGCTT TTTGATCTGGACCCTTCTTTGCAATACCGGCAGTGA

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LOC732601 identity 62% Cover 44%

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SEQ ID NO: 75

MDGGCVTDETTTSSDSLSVPPPSRVGSVASAVVDPDGCCVSGEAESRKLPSSKYKGVVPQ PNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAALRFRGPDAVTN FKPPAASDDAESEF LNSHSKFEIVDM LRKHTYDDELQQSTRGGRRRLDADTASSGVFDAKAREQLFEKTVTPSD VGKLNRLVIPKQHAEKHFPLSGSGDESSPCVAGASAAKGMLLNFEDVGGKVWRFRYSYWN SSQSYVLTKGWSRFVKEKNLRAGDAVQFFKSTGPDRQLYDCKARSGEVNNNAGGLFVPI GPVVEPVQMVRLFGVNLLKLPVPGSDGVGKRKEMELFAFECCKKLKVIGAL

CDS SEQ ID NO: 76

- 40 ATGGATGGAGGCTGTGTCACAGACGAAACCACCACATCCAGCGACTCTCTTTCCGTTCCG CCGCCCAGCCGCGTCGGCAGCGTTGCAAGCGCCGTCGTCGACCCCGACGGTTGTTGCGTT TCCGGCGAGGCCGAATCCCGGAAACTCCCTTCGTCGAAATACAAAGGCGTGGTGCCGCAA CCGAACGGTCGCTGGGGAGCTCAGATTTACGAGAAGCACCAGCGCGTGTGGCTCGGCACT TTCAACGAGGAAGACGAAGCCGCCAGAGCCTACGACATCGCCGCGCTGCGCTTCCGCGGC 45 CCCGACGCCGTCACCAACTTCAAGCCTCCCGCCGCCTCCGACGACGCCGAGTCCGAGTTC
- CTCAACTCGCATTCCAAGTTCGAGATCGTCGACATGCTCCGCAAGCACACCTACGACGAC GAGCTCCAGCAGAGCACGCGCGGCGGTGGTAGGCGCCGCCTCGACGCTGACACCGCGTCGAGC GGTGTGTTCGACGCGAAAGCGCGTGAGCAGCTGTTCGAGAAAACGGTTACGCCGAGCGAC GTCGGGAAGCTGAATCGATTAGTGATACCGAAGCAGCACGCGGAGAAGCACTTTCCGTTA
- 50 AGCGGATCCGGCGACGAAAGCTCGCCGTGCGTGGCGGGGGCTTCGGCGGCGAAGGGAATG TTGTTGAACTTTGAGGACGTTGGAGGGAAAGTGTGGCGGTTTCGTTACTCTTATTGGAAC AGTAG CCAGAGCTACGTGCTTACCAAAG GATGGAGCCGGTTCGTTAAGGAGAAG AATCTT

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Locl00801107 Cover 44% identity 61%

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SEQ ID NO: 77

MDAISCMDESTTTESLSISLSPTSSSEKAKPSSMITSSEKVSLSPPPSNRLCRVGSGASA VVDPDGGGSGAEVESRKLPSSKYKGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAY DIAAQRFRGKDAVTNFKPLAGADDDDGESEFLNSHSKPEIVDM LRKHTYNDELEQSKRSR GVVRRRGSAAAGTANSISGACFTKAREQLFEKAVTPSDVGKLNRLVIPKQHAEKHFPLQS SNGVSATTIAAVTATPTAAKGVLLN FEDVGGKVWRFRYSYWNSSQSYVLTKGWSRFVKEK NLKAGDTVCFH RSTGPDKQLYIDWKTRNVVNNEVALFGPVGPVVEPIQMVRLFGVNI LKL PGSDTIVGN NNNASGCCNGKRREMELFSLECSKKPKI IGAL

20 CDS SEQ ID NO: 78

ATGGATGCAATTAGTTGCATGGATGAGAGCACCACCACTGAGTCACTCTCTATAAGTCTT TCTCCGACGTCATCGTCGGAGAAAGCGAAGCCTTCTTCGATGATTACATCGTCGGAGAAG GTTTCTCTGTCCCCGCCGCCGTCAAACAGACTATGCCGTGTTGGAAGCGGCGCGAGCGCA GTCGTGGATCCTGATGGCGGCGGCGGCAGCGGCGCTGAGGTAGAGTCGCGGAAACTCCCCTCG

- 30 GGCGTCGTCCGGCGGCGAGGCTCCGCCGCCGCCGGCACCGCAAACTCAATTTCCGGCGCG TGCTTTACTAAGGCACGTGAGCAGCTATTCGAGAAGGCTGTTACGCCGAGCGACGTTGGG AAATTGAACCGTTTGGTGATACCGAAG CAGCACGCGGAGAAG CACTTTCCGTTACAGAGC TCTAACGGCGTTAGCGCGACGACGATAGCGGCGGTGACGGCGACGCCGACGGCGGAGA GGCGTTTTGTTGAACTTCGAAGACGTTGGAGGGAAAGTGTGGCGGTTTCGTTACTCGTAT
- 35 TGGAACAGTAGCCAGAGTTACGTCTTAACCAAAGGTTGGAGCCGGTTCGTTAAGGAGAAG AATCTGAAAGCTGGTGACACGGTTTGTTTTCACCGGTCCACTGGACCGGACAAGCAGCTT TACATCGATTGGAAGACGAGGAATGTTGTTAACAACGAGGTCGCGTTGTTCGGACCGGTC GGACCGGTTGTCGAACCGATCCAGATGGTTCGGCTCTTTGGGGTTAACATTTTGAAACTA CCCGGTTCAGATACTATTGTTGGCAATAACAATAATGCAAGTGGGTGCTGCAATGGCAAG
- 40 AGAAGAGAAATGGAACTGTTCTCGTTAGAGTGTAGCAAGAAACCTAAGATTATTGGTGCT TTGTAA

LOC100789009				
Cover 44%	identity	62%		

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SEQ ID NO: 79

M DGGSVTDETTTTSNSLSVPANLSPPPLSLVGSGATAVVYPDGCCVSGEAESRKLPSSKY KGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAAH RFRGRDAVTNFKPLAGAD DAEAEFLSTHSKSEIVDM LRKHTYDNELQQSTRGGRRRRDAETASSGAFDAKAREQLFEK TVTQSDVGKLNRLVIPKQHAEKHFPLSGSGGGALPCMAAAAGAKGMLLNFEDVGGKVWRF RYSYWNSSQSYVLTKGWSRFVKEKNLRAGDAVQFFKSTGLDRQLYDCKARSGKVN NNAA GLFI PVGPVVEPVQMVRLFGVDLLKLPVPGSDGIGVGCDGKRKEMELFAFECSKKLKVIG AL

SEQ ID NO: 80

- 5 ATGGATGGAGGCAGTGTCACAGACGAAACCACCACAACCAGCAACTCTCTTTCGGTTCCG GCGAATCTATCTCCGCCGCCTCTCAGCCTTGTCGGCAGCGGCGCAACCGCCGTCGTCTAC CCCGACGGTTGTTGCGTCTCCGGCGAAGCCGAATCCCCGGAAACTCCCGTCCTCGAAATAC AAAGGCGTGGTGCCGCAACCGAACGGTCGTTGGGGAGCTCAGATTTACGAGAAGCACCAG CGCGTGTGGCTCGGCACCTTCAACGAGGAAGACGAAGCCGCCAGAGCCTACGACATCGCC
- 10 GCGCATCGCTTCCGCGGCCGCGACGCCGTCACTAACTTCAAGCCTCTCGCCGGCGCCGAC GACGCCGAAGCCGAGTTCCTCAGCACGCATTCCAAGTCCGAGATCGTCGACATGCTCCGC AAGCACACCTACGACAACGAGCTCCAGCAGAGCACCCGCGGCGGCGGCGCGCGGGGAC GCCGAAACCGCGTCGAGCGGCGCGTTCGACGCGAAGGCGCGTGAGCAGCTGTTCGAGAAA ACCGTTACGCAGAGCGACGTCGGGAAGCTGAACCGATTAGTGATACCAAAGCAGCACGCG
- 15 GAGAAGCACTTTCCGTTAAGCGGATCCGGCGGCGGAGCCTTGCCGTGCATGGCGGCGGCGGC GCGGGGGCGAAGGGAATGTTGCTGAACTTTGAGGACGTTGGAGGGAAAGTGTGGCGGTTC CGTTACTCGTATTGGAACAGTAGCCAGAGCTACGTGCTTACCAAAGGATGGAGCCGGTTC GTTAAGGAGAAGAATCTTCGAGCTGGTGACGCGGGTTCAGTTCTTCAAGTCGACCGGACTG GACCGGCAACTATATATAGACTGCAAGGCGAGGAGTGGTAAGGTTAACAATAATGCTGCC
- 20 GGTTTGTTTATTCCCGTTGGACCGGTTGTTGAGCCGGTTCAGATGGTACGGCTTTTCGGG GTCGACCTTTTGAAACTACCCGTACCCGGTTCGGATGGTATTGGGGTTGGCTGTGACGGG AAGAGAAAAGAGATGGAGCTGTTTGCATTTGAATGTAGCAAGAAGTTAAAAGTAATTGGA GCTTTGTAA
- 25 Locl02660503 Cover 36% identity 57%

SEQ ID NO: 81

30 migvekvticmrievntekgrralmdcwqisgvhessdcseikfafdavvkrarheennaaaqkfkgvvsqqngnwgaqi yahqqriwl gtfksereaamaydsasiklrsgechrnfpwndqtvqepqfqshysaetvlnmirdgtypskfatflktrqtqkgvakhiglkgddeeqfcct qlfqkeltpsdvgklnrlvipkkhavsyfpyvggsadesgsvdveavfydklmrlwkfrycywkssqsyvftrgwnrfvkdkklkakdviafft wgksggegeafalidviynnnaeedskgdtkqvlgnqlqlagseegededanigkdfnaqkglrlfgvcit

35

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CDS SEQ ID NO: 82

atgattggagttgagaaagtgacaatttgtatgagaatagagtgaatactgaaaagggaagaagggctttaatggactgttggcaaatatcag gagttcatgaaagttcagattgtagcgaaatcaaatttgcattcgacgcagtagtaaaacgcgcgaggcatgaagagaataatgcagcagcac agaagttcaaaggcgttgtgtctcaacaaaatgggaactggggtgcacagatatatgcacaccagcagagaatctggttggggaccttcaaat ctgaaagagggctgcaatggcttatgacagcgccagcataaaacttagaagcggagggtgcacagaaactttccatggaacgaccaaaca gttcaagggcctcagttccaaagccattacagcgcagaaacagtgctaaacatgattaggggggaccacaaaatttgctacatttcc aaaactcgtcaaacccaaaaaggcgttgcgaaacacataggtctgaagggtgatgacgagggaacagttttgttgcacccaactttttcagaagg

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Hordeum vulgare

MLOC_66387

Cover 47% identity 64%

SEQ ID NO: 83

M EFTATSSRFSKGEEEVEEEQEEASMREIPFMTPAAATCAAAPPSASASASTPASASGSS PPFRSGDDAGASGSGAGDGSRSNVAEAVEKEHM FDKVVTPSDVGKLNRLVI PKQYAEKYF PLDSAANEKGLLLNFEDSAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVSFS RGAGEAARHRLFI DWKRRADTRDPLRLPRLPLPM PLTSHYSPWGLGAGARGFFM PPSPPA TLYEHRLRQGFDFRGMNPSYPTMGRQVI LFGSAARM PPHGPAPLLVPRPPPPLHFTVQQQ GSDAGGSVTAGSPVVLDSVPVI ESPTTATKKRVRLFGVNLDNPQHPGDGGGESSNYGSAL PLQMPASAWRPRDHTLRLLEFPSHGAEASSPSSSSSKREAHSGLDLDL

SEQ ID NO: 84

ATGGAGTTTACTGCGACAAGCAGTAGGTTTTCTAAAGGAGAGGAGGAGGAGGAGGAGGAGGAG 15 CAGGAGGAGGCGTCGATGCGCGAGATCCCTTTCATGACGCCCGCGGCCGCCACCTGCGCC GCGGCGCCGCCTTCTGCTTCTGCGTCGGCCTCGACACCCGCGTCAGCGTCTGGAAGTAGC CCTCCCTTTCGATCTGGGGATGACGCCGGAGCGTCGGGGAGCGGGGCCGGCGACGGCAGC CGCAGCAACGTGGCGGAGGCCGTGGAGAAGGAGCACATGTTCGACAAAGTGGTGACGCCG AGCGACGTGGGGAAGCTTAACCGGCTGGTCATCCCCAAGCAGTACGCCGAGAAGTACTTC 20 CCGCTGGACTCGGCGGCCAACGAGAAGGGCCTTCTGCTCAACTTCGAGGACAGCGCCGGG AAG CCATGGCG CTTCCG CTATTCCTACTGGAAC A GCAG CCAG A GCTACGTCATGACCAAA GGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCTGGGGACACCGTCTCCTTCTCC CGCGGCGCCGGTGAGGCCGCGCGCCACCGCCTCTTCATCGACTGGAAGCGCCGAGCCGAC ACCAGAGACCCGCTCCGCTTGCCCGCCCCCGCTCCCGATGCCGCTGACGTCGCACTAC 25 ACGCTCTACGAGCACCGTCTCCGTCAAGGCTTCGACTTCCGCGGCATGAACCCCAGTTAC

- 30 GTAATCGAAAGCCCCACGACGGCAACGAAGAAGCGCGTGCGCTTGTTCGGCGTGAACTTG GACAACCCGCAGCATCCCGGTGATGGCGGGGGGCGAATCGAGCAATTATGGCAGTGCACTG CCATTGCAGATGCCCGCATCAGCATGGCGGCGCAAGGGACCATACGCTGAGGCTGCTCGAA TTCCCCTCGCACGGTGCCGAGGCGTCGTCTCCATCGTCGTCGTCGTCTTCCAAGAGGGAG GCGCATTCGGGCTTGGATCTCGATCTGTGA

35

M LOC44012

40 Cover 55% identity 63%

SEQ ID NO: 85

 M LRKHTYFDELAQSKRAFAASAALSAPTTSGDAGGSASPPSPAAVREHLFDKTVTPSDVG KLNRLVIPKQNAEKHFPLQLPAGGGESKGLLLNFEDDAGKVWRFRYSYWNSSQSYVLTKG
 WSRFVKEKGLGAGDVVGFYRSAAGRTGEDSKFFI DCRLRPNTNTAAEADPVDQSSAPVQK AVRLFGVDLLAAPEQGM PGGCKRARDLVKPPPPKVAFKKQCI ELALA

SEQ ID NO: 86

50 ATGCTCCGCAAGCACCTACTTCGACGAGCTCGCCCAGAGCAAGCGCGCCTTCGCCGCG TCGGCCGCGCTCTCCGCGCCACCACCTCGGGCGACGCCGGCGGCAGCGCCTCGCCGCCC TCCCCGGCCGCCGTGCGCGAGCACCTCTTCGACAAGACCGTCACGCCAGCGACGTCGGC AAGCTGAACAGGCTGGTGATACCGAAGCAGAACGCCGAGAAGCACTTCCCGCTGCAGCTC

10

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MLOC_57250 Cover 50% identity 57%

15 SEQ ID NO: 87

MYCSRGRI DPAEEGQVMGGLGVRDASWALFKVLEQSDVQVGQNRLLLTKEAVWGGPI PKL FPELEELRGDGLNAENRVAVKILDADGCEGDANFRYLNSSKAYRVMGPQWSRLVKETGMC KGDRLDLYAATATAASSCSGARAAVAPA**P**PGAIVKAAGF

20 CDS SEQ ID NO: 88

ATGTATTGTTCCCGCGGCCGCATCGATCCCGCGGAAGAAGGGCAGGTGATGGGCGGCCTC GGCGTGCGCGACGCCAGCTGGGCGCTGTTCAAGGTGTTGGAGCAGTCCGACGTCCAGGTG GGGCAGAACCGGCTGCTCCTCACCAAGGAGGCGGTGTGGGGCGGCCCTATCCCCAAGCTT TTCCCGGAGCTGGAGGAGCTCCGCGGCGACGGCCTCAACGCCGAGAACAGGGTCGCGGTC

- 25 AAGATCCTCGACGCCGACGGCTGCGAGGGGGGCGCCAACTTCCGCTACCTCAACTCCAGC AAGGCGTACCGGGTCATGGGGCCTCAGTGGAGCCGGCTCGTGAAGGAGACCGGCATGTGC AAGGGAGACCGCCTCGATCTGTACGCGGCAACGGCGACCGCTGCCTCTTCGTGTTCTGGA GCCAGGGCGGCTGTGGCGCCGGCGATACCTCCCGGAGCAATCGTGAAGGCAGCCGGGTTC TAA
- 30

MLOC_38822 Cover 47% identity 56%

SEQ ID NO: 89

35 MLRKH IYPDELAQHKRAFFFAAASSPTSSSSPLASPAPSAAAARREHLFDKTVTPSDVGK LNRLVIPKQHAEKHFPLQLPSASAAVPGECKGVLLNFDDATGKVWRFRYSYWNSSQSYVL TKGWSRFVKEKGLHAGDAVEFYRAASGN NQLFI DCKLRSKSTTTTTSVNSEAAPSPAPVT RTVRLFGVDLLIAPAARHAHEHEDYGMAKTNKRTMEASVAAPTPAHAVWKKRCVDFALTY RLATTPQCPRSRDQLEGVQAAGSTFAL

40

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CDS SEQ ID NO: 90

- ACCGGCAAGGTGTGGAGGTTCCGGTACTCCTACTGGAACAGCAGCCAGAGCTACGTGCTC ACCAAGGGGTGGAGCCGCTTCGTGAAGGAGAAGGGCCTTCACGCCGGCGACGCCGTCGAG TTCTACCGCGCCGCCTCCGGCAACAACCAGCTCTTCATCGACTGCAAGCTCCGGTCCAAG
- 50 AGCACCACGACGACGACCTCCGTCAACTCGGAGGCCGCCCCATCGCCGGCACCCGTGACG AGGACAGTGCGACTCTTCGGGGTCGACCTTCTCATCGCGCCGGCGGCGAGGCACGCGCAT GAGCACGAGGACTACGGCATGGCCAAGACAAACAAGAGAACCATGGAGGCCAGCGTAGCG GCGCCTACTCCGGCGCACGCGGTGTGGAAGAAGCGGTGCGTAGACTTCGCGCTGACCTAC

CGACTTGCCACCACCCCACAGTGCCCGAGGTCAAGAGATCAACTAGAAGGAGTACAAGCA GCTGGGAGTACATTTGCTCTATAG

5 MLOC_7940 Cover 49% identity 52%

SEQ ID NO: 91

MGVEI LSSTGEHSSQYSSGAASTATTESGVGGRPPTAPSLPVSIADESATSRSASAQSTS 10 SRFKGVVPQPNGRWGAQIYERHARVWLGTFPDEDSAARAYDVAALRYRGREAATNFPCAA AEAELAFLAAHSKAEIVDM LRKHTYTDELRQGLRRGRGMGARAQPTPSWAREPLFEKAVT PSDVGKLNRLVVPKQHAEKH FPLKRTPETTTTTGKGVLLNFEDGEGKVWRFRYSYWNSSQ SYVLTKGWSRFVREKGLGAGDSIVFSCSAYGQEKQFFIDCKKNKTMTSCPADDRGAATAS PPVSEPTKGEQVRVVRLFGVDIAGEKRGRAAPVEQELFKRQCVAHSQHSPALGAFVL

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CDS SEQ ID NO: 92

ATGGGGGTGGAGATCCTGAGCTCAACGGGGGGAACACTCCTCCCAGTACTCTTCCGGAGCC 20 GCGTCCACGGCGACGACGGAGTCAGGCCGGGCGGACGGCCGCCGACTGCGCCGAGCCTA CCTGTTTCCATCGCCGACGAGTCGGCGACCTCGCGGTCGGCATCGGCGCAGTCGACGTCG TCGCGGTTCAAGGGCGTGGTGCCGCAGCCCAACGGGCGGTGGGGCGCCCAGATCTACGAG GACGTGGCCGCGCTCCGGTACCGGGGCCGCGAGGCCGCCACCAACTTCCCGTGCGCGGCC 25 GCCGAGGCGGAGCTCGCCTTCCTGGCGGCACACTCCAAGGCCGAGATCGTCGACATGCTC GCGCGCGCGCGCGACGCCGTCGTGGGCGCGCGGGAGCCCCTTTTCGAGAAGGCCGTGACC CCGAGCGACGTGGGCAAGCTCAACCGCCTCGTTGTGCCGAAGCAGCACGCCGAGAAGCAC TTCCCCCTGAAACGCACGCCGGAGACGACGACGACCACCGGCAAGGGGGTGCTTCTCAAC

- 30 TTCGAGGATGGCGAGGGGAAAGTGTGGGGGGTTCCGGTACTCGTATTGGAACAGCAGCCAG AGCTACGTGCTCACCAAGGGATGGAGCCGCTTCGTTCGGGAGAAGGGCCTCGGTGCCGGC GACTCCATCGTGTTCTCCTGCTCGGCGTACGGTCAGGAGAAGCAGTTCTTCATCGACTGC CCGCCAGTGTCAGAGCCAACAAAAGGAGAACAAGTCCGTGTTGTGAGGCTGTTCGGCGTC
- 35
- CAATGCGTGGCACACAGCCAGCACTCTCCAGCCCTAGGTGCCTTCGTCTTATAG

RVVRLFGVDIAGVKRERAATAEQGPQGWFKRQCMAHGQHSPALGDFAL

MLOC_56567

Cover 42% identity 59%

40

45

SEQ ID NO: 93 MGVEI LSSMVEHSFQYSSGASSATAESGAVGTPPRHLSLPVAIADESLTSRSASSRFKGV VPQPNGRWGAQIYERHARVWLGTFPDQDSAARAYDVASLRYRGGDAAFNFPCVVVEAELA FLAAHSKAEIVDM LRKQTYADELRQGLRRGRGMGVRAQPMPSWARVPLFEKAVTPSDVGK LNRLVVPKQHAEKHFPLKRSPETTTTTGNGVLLNFEDGQGKVWRFRYSYWNSSQSYVLTK GWSRFVREKGLGAGDSI MFSCSAYGQEKQFFI DCKKNTTVNGGKSASPLQVMEIAKAEQV

50 SEQ ID NO: 94

ATGGGGGTGGAGATCCTGAGCTCCATGGTGGAGCACTCCTTCCAGTACTCTTCGGGCGCG TCCTCGGCCACCGCGGAGTCAGGCGCCGTCGGAACACCGCCGAGGCATCTGAGCCTACCT GTCGCCATCGCCGACGAGTCCCTGACCTCACGGTCGGCGTCGTCTCGGTTCAAGGGCGTG

MLOC_75135 Cover 43% identity 57%

20 SEQ ID NO: 95

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MGMEILSSTVEHCSQYSSSASTATTESGAAGRSTTALSLPVAITDESVTSRSASAQPASS RFKGVVPQPNGRWGSQIYERHARVWLGTFPDQDSAARAYDVASLRYRGRDAATNFPCAAA EAELAFLTAHSKAEIVDMLRKHTYADELRQGLRRGRGMGARAQPTPSWARVPLFEKAVTP SDVGKLNRLVVPKQHAEKH FPLKCTAETTTTTGNGVLLNFEDGEGKVWRFRYSYWNSSQS YVLTKGWSSFVREKGLGAGDSIVFSSSAYGQEKQLFINCKKNTTMNGGKTALPLPVVETA KGEQDHVVKLFGVDIAGVKRVRAATGELGPPELFKRQSVAHGCGRM NYICYSIGTIGPLM LN

SEQ ID NO: 96

- 40 CCCCTGAAGTGCACCGCAGAGACGACGACCACCACCGGCAACGGCGTGCTGCTAAACTTC GAGGATGGTGAGGGGAAGGTGTGGAGGTTCCGGTACTCGTATTGGAACAGTAGCCAGAGC TACGTGCTCACCAAAGGCTGGAGCAGCTTCGTCCGGGGAGAAGGGCCTCGGCGCAGGCGAC TCCATCGTCTTCTCCTCCTCGGCGTACGGGCAGGAGAAGCAGTTATTCATCAACTGCAAA AAGAACACGACTATGAACGGCGGCAAAACAGCGTTGCCGCTGCCAGTGGTGGAGACTGCC
- 45 AAAGGAGAACAAGACCACGTCGTTAAGTTGTTCGGTGTTGACATCGCCGGTGTGAAGAGG GTGCGAGCGGCGACGGGGGGAGCTAGGCCCGCCGGAGTTGTTCAAGAGACAATCCGTGGCA CACGGATGCGGAAGGATGAACTACATTTGCTACTCCATAGGGACAATAGGACCTCTTATG CTCAACTGA

50

MLOC_63261 Cover 49% identity 51% SEQ ID NO: 97

MASSKPTNPEVDNDMECSSPESGAEDAVESSSPVAAPSSRFKGVVPQPNGRWGAQIYEKH SRVWLGTFGDEEAAACAYDVAALRFRGRDAVTNHQRLPAAEGAGWSSTSELAFLADHSKA EIVDMLRKHTYDDELRQGLRRGHGRAQPTPAWAREFLFEKALTPSDVGKLNRLVVPKQHA EKH FPPTTAAAAGSDGKGLLLNFEDGQGKVWRFRYSYWNSSQSYVLTKGWSRFVQEKGLC AGDTVTFSRSAYVMNDTDEQLFI DYKQSSKNDEAADVATADENEAGHVAVKLFGVDIGWA GMAGSSGG

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SEQ ID NO: 98

- 25 CTCTTCATCGACTACAAGCAGAGTAGCAAGAACGACGAAGCGGCCGACGTAGCCACTGCC GATGAGAATGAGGCCGGCCATGTCGCCGTGAAGCTCTTCGGGGTCGACATTGGCTGGGCT GGGATGGCGGGATCATCAGGTGGGTGA

MLOC_64708

30 Cover 49% identity 51%

SEQ ID NO: 99

MLFDSSVSASLGTM RPLVKKLDMLLAPARGYSTLCKRI KEVM HLLKHDVEEISSYLDELT EVEDPPPMAKCWM NEARDLSYDMEDYI DSLLFVPPGHFI KKKKKKKKKKKKKKKKK WCKQIVFTKQVSDHGI KTSKI IHVNVPRLPNKPKVAKI ILQFRIYVQEAI ERYDKYRLHH CSTLRRRLLSTGSMLSVPIPYEEAAQIVTDGRMNEFISSLAANNAADQQQLKVVSVLGSG CLGKTTLANVLYDRIGMQFECRAFI RVSKKPDMKRLFRDLLSQFHQKQPLPTSCNELGIS DNI IKHLQDKRYLIVI DDLWDLSVWDII KYAFPKGNHGSRII ITTQI EDVALTCCCDHSE

- 40 ETEVSLDLLTDTRDLLRSCLWSNSTSERTKQVLNLSYSNLPDYLKTCLLYLHMYPVGSI I WKDDLVKQLVAEGFIATREGKDQDQEM IEKAAGLCFDALI DRRFIQPIYTKYNNKVLSCT VHEVVHDLIAQKSAEENFIVVADHNRKN IALSHKVRRLSLIFGDTIYAKTPAN ITKSQI R SFRFFGLFECM PCITEFKVLRVLNLQLSGHRGDN DPI DLTGISELFQLRYLKITSDVCIK LPNQMQKLQYLETLDI MDAPRVTAVPWDI IN LPHLLHLTLPVDTYLLDWISSMTDSVISL
- 45 WTLGKLNYLQH LHLTSSSTRPSYHLERSVEALGYLIGGHGKLKTIVVAHVSSAQNTVVRG APEVTISWDRMSPPPLLQRFECPHSCFIFYRI PKWVTELGN LCI LKIAVKELHM ICLGTL RGLHALTDLSLYVETAPIDKII FDKAGFSVLKYCKLRFAAGIAWLKFEADAM PSLWKLM L VFNAIPRMDQNLVFFHHSRPAM HQRGGAVI IVEHMPGLRVISAKFGGAASDLEYASRTVV SNH PSN PTI NMQLVCYSSNGKRSRKRKQQPYDVVKGQPDEYAKRLERPAEKRISTPTKSS
- 50 LRLHVPEITPKPMQITDNNVQRREH MFDTVLTRGDVGMLNRLVVPKKHAEKYFPLDSSST RTSKAIVLSFEDPAGKSWFFHYSYRSSSQNYVMFKGWTGFVKEKFLEAGDTVSFSRGVGE ATRGRLFIDCQNEQRYM FERVLTASDMESDGCSLMVPVNLVWPHPGLRKTI KGRHAVLQF EDGSGNGKVWPFQFEASGQYYLMKGLNYFVNDRDLAAGYTVSFYRAGTRLFVDSGRKDDK

VALGTRSRERIYPKIVRSQ

Brassica rapa

LOC103849927 Cover 99% ident 80%

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CDS SEQ ID NO: 100

- ATGTTGTTTGATAGTTCAGTGAGTGCTTCGTTGGGCACCATGAGACCACTTGTCAAGAAG 10 CTCGACATGCTGCTAGCTCCTGCTCGGGGGATACAGTACCTTGTGCAAGAGGATCAAGGAA GTGATGCACCTTCTCAAACATGATGTTGAAGAGATAAGCTCCTACCTTGATGAACTTACA GAGGTGGAGGACCCTCCACCAATGGCCAAGTGCTGGATGAACGAGGCACGCGACCTGTCT TATGATATGGAGGATTACATTGATAGCTTGTTATTTGTGCCACCTGGCCATTTCATCAAG 15 TGGTGCAAACAGATCGTATTCACAAAGCAAGTGTCAGACCATGGTATCAAGACCAGTAAA ATCATTCATGTTAATGTCCCTCGTCTTCCCAATAAGCCCAAGGTTGCAAAAATAATATTA CAGTTCAGGATCTATGTCCAGGAGGCTATTGAACGGTATGACAAGTATAGGCTTCACCAT TGCAGCACCTTGAGGCGTAGATTGTTGTCCACTGGTAGTATCCTTTCAGTGCCAATACCC 20 GCTGCTAATAATGCAGCAGATCAGCAGCAGCTCAAGGTGGTATCTGTTCTTGGATCTGGG TGTCTAGGTAAAACTACGCTTGCGAATGTGTTGTACGACAGAATTGGGATGCAATTCGAA TGCAGAGCTTTCATTCGAGTGTCCAAAAAGCCTGATATGAAGAGACTTTTCCGTGACTTG CTCTCGCAATTCCACCAGAAGCAGCCACTGCCTACCAGTTGTAATGAGCTTGGCATAAGT GACAATATCATCAAACATCTGCAAGATAAAAGGTATCTAATTGTTATTGATGATTTGTGG 25 GATTTATCAGTATGGGATATTATTAAAATATGCTTTTCCAAAGGGAAACCATGGAAGCAGA ATAATAATAACTACACAGATTGAAGATGTTGCATTAACTTGTTGCTGTGATCACTCGGAG CATGTTTTCGAGATGAAACCTCTCAACATTGGTCACTCAAGAGAGCTATTTTTAATAGA CTTTTTGGTTCTGAAAGTGACTGTCTTGAAGAATTCAAACGAGTTTCAAACGAAATTGTT GATATATGT@TG GTTTACCGCTAGCAACAATCAACATAGCTAGTCATTTGGCAAACCAG 30 GAGACAGAAGTATCATTGGATTTGCTAACAGACACACGTGATTTGTTGAGGTCCTGTTTG TGGTCAAATTCTACTTCAGAAAGAACAAAACAAGTACTGAACCTCAGCTACAGTAATCTT CCTGATTATCTGAAGACATGTTTGCTGTATCTTCATATGTATCCAGTGGGCTCCATAATC TGGAAGGATGATCTGGTGAAGCAATTGGTGGCTGAAGGGTTTATTGCTACAAGAGAAGGG AAAGACCAAGACCAAGAAATGATAGAGAAAGCTGCAGGACTCTGTTTCGATGCACTTATT 35 GATAGAAGATTCATCCAGCCTATATATACCAAGTACAACAATAAGGTGTTGTCCTGCACG GTTCATGAGGTGGTACATGATCTTATTGCCCAAAAGTCTGCTGAAGAGAATTTCATTGTG GTAGCAGACCACAATCGAAAGAATATAGCACTTTCTCATAAGGTTCGTCGACTATCTCTC ATCTTTGGCGACACAATATATGCCAAGACACCAGCAAACATCACAAAGTCACAAATTCGG TCATTCAGATTTTTTG GATTATTCGAGTGTATGCCTTGTATTACAGAGTTCAAG GTTCTC 40 CGTGTTCTAAACCTTCAACTATCTGGTCATCGTGGGGGACAATGACCCTATAGACCTCACT GGGATTTCAGAACTGTTTCAGCTGAGATATTTAAAGATTACAAGTGATGTGTGCATAAAA CTACCAAATCAAATGCAAAAACTGCAATATTTGGAAACGTTGGACATTATGGATGCACCA AGAGTCACTGCTGTTCCATGGGATATTATAAATCTCCCACACCTGTTGCACCTGACTCTT 45 TGGACCCTTGGCAAGCTGAACTACCTGCAGCATCTTCATCTTACTAGTTCTTCTACACGT CCTTCATACCATCTGGAGAGAGAGTGTGGAGGCTCTGGGTTATTTGATCGGAGGACATGGC GCCCCAGAAGTAACCATTTCATGGGATCGTATGTCACCTCCCCCCTTCTCCAGAGATTC GAATGCCCACACAGCTGCTTCATATTTTACCGAATTCCTAAGTGGGTTACAGAACTTGGC
 - 50 AACCTGTGCATTTTGAAGATTGCAGTGAAGGAGCTTCATATGATTTGTCTTGGTACTCTC AGAGGATTGCATGCCCTCACTGATCTGTCGCTGTATGTGGAGACAGCGCCCATTGACAAG ATCATCTTTGACAAGGCCGGGTTCTCAGTTCTCAAGTACTGCAAATTGCGCTTCGCGGCT

- 5 AGTAACCATCCAAG CAATCCTACAATCAACATGCAATTGGTGTGTTATAGTTCCAATGGT AAGAGAAGCAGAAAAAGGAAACAACAACCATGCACGTTGTGAAGGGACAACCAGATGAA TACGCCAAGAGATTGGAGAGACCAGCTGAGAAAAGGATTTCAACGCCGACAAAGTCTTCT TTGCGTCTGCATGTTCCAGAAATTACACCAAAACCTATGCAGATTACAGACAACAATGTT CAGAGGAGGGAGCACATGTTCGATACGGTTCTGACTCGGGGGGACGTGGGGGATGCTGAAC
- 10 CGGCTGGTGGTACCGAAGAAGCACGCGGAGAAGTACTTCCCGCTGGACAGTTCCTCCACC CGCACCAGCAAGGCCATCGTACTCAGCTTTGAGGACCCTGCTGGGAAGTCATGGTTCTTC CACTACTCCTACCGGAGCAGCAGCCAGAACTACGTCATGTTCAAGGGGTGGACTGGCTTC GTCAAGGAGAAGTTTCTCGAAGCCGGCGACACCGTCTCCTTCAGCCGCGGCGTCGGGGAG GCCACGAGGGGGAGGCTCTTCATCGACTGTCAAAATGAGCAGAGGTACATGTTCGAGCGA

LOC103849927

25 SEQ ID NO: 101

msgnhysrdihhntpsvhhqnyavvdreylfeksltpsdv gklnrlvipkqhaekhfplnnagddvaaaettekgmlltfedesgkcwkf rysywnssqsyvltkgwsryvkdkhlhagdvvffqrhrfdlhrvfigwrkrgevssptavsvvsqearvnttaywsglttpyrqvhastssyp nihqeyshygavaeiptvvtgssrtvrlfgvnlechgdvvetppcpdgyngqhfyyystpdpmnisfageameqvgdgrr

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Bra034828 Cover 100% identity 79%

SEQ ID NO: 102

35 MSVNHYSNTLSSHNH HNEH KESLFEKSLTPSDVGKLNRLVIPKQHAERYLPLNNCGGGGD VTAESTEKGVLLSFEDESGKSWKFRYSYWNSSQSYVLTKGWSRYVKDKHLNAGDVVLFQR HRFDI HRLFIGWRRRGEASSSSAVSAVTQDPRANTTAYWNGLTTPYRQVHASTSSYPNNI HQEYSHYGPVAETPTVAAGSSKTVRLFGVNLECHSDVVEPPPCPDAYNGQH IYYYSTPHP M NISFAGEAM EQVG DG RG

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CDS SEQ ID NO: 103

ATGTCAGTCAACCATTACTCAAACACTCTCTCGTCGCACAATCACCACAACGAACATAAA GAGTCTTTGTTCGAGAAGTCACTCACGCCAAGCGATGTTGGAAAGCTAAACCGTTTAGTC ATACCAAAACAACACGCCGAGAGATACCTCCCTCTCAATAATTGCGGCGGCGGCGGCGAC GTGACGGCGGAGTCGACGGAGAAAGGGGTGCTTCTCAGCTTCGAGGACGAGTCGGGAAAA

- TCTTGGAAATTCAGATACTCATATTGGAACAGTAGTCAAAGCTACGTGTTGACCAAAGGA TGGAGCAGGTACGTCAAAGACAAGCACCTCAACGCAGGGGACGTCGTTTTATTTCAACGG CACCGTTTTGATATTCATAGACTCTTCATTGGCTGGAGGAGACGCGGAGAGGCTTCTTCC TCTTCCGCCGTTTCCGCCGTGACTCAAGATCCTCGAGCTAACACGACGGCGTACTGGAAC
- 50 GGTTTGACTACACCTTATCGTCAAGTACACGCGTCAACTAGTTCTTACCCTAACAACATC CACCAAGAGTATTCACATTATGGCCCTGTTGCTGAGACACCGACGGTAGCTGCAGGGAGC TCGAAGACGGTGAGGCTATTTGGAGTTAACCTCGAATGTCACAGTGACGTTGTGGAGCCA CCACCGTGTCCTGACGCCTACAACGGCCAACACATTTACTCATTACTCAACTCCACATCCC

ATGAATATCTCATTTGCTGGAGAAGCAATGGAGCAGGTAGGAGATGGACGAGGTTGA

5 Bra005886 Cover 100% identity 79%

SEQ ID NO: 104

10 MSVN HYSTDH HQVHH HHTLFLQNLHTTDTSEPTTTAATSLREDQKEYLFEKSLTPSDVGK LNRLVIPKQHAEKYFPLNTI ISNNAEEKGMLLSFEDESGKCWRFRYSYWNSSQSYVLTKG WSRYVKDKQLDPADVVFFQRQRSDSRRLFIGWRRRGQGSSSAANTTSYSSSMTAPPYSNY SNRPAHSEYSHYGAAVATATETHFPSSSAVGSSRTVRLFGVNLECQMDEDEGDDSVATA AAAECPRQDSYYDQN MYNYYTPHSSAS

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CDS 105

ATGTCAGTCAACCATTACTCCACGGACCACCACGGGTCCACCACCACCACCACTCTCT TTGCAGAACCTCCACACCGACCACATCGGAGCCAACCACAACCGCCGCCACATCACTC CGCGAAGACCAGAAAGAGTATCTCTTCGAGAAATCTCTCACACCAAGCGACGTTGGCAAA

- 20 CTCAACCGTCTCGTTATACCAAAACAGCACGCGGAGAAGTACTTCCCTCTCAACACCATC ATCTCCAATAATGCTGAGGAGAAAGGGATGCTTCTAAGCTTCGAAGACGAGTCAGGCAAG TGCTGGAGGTTCAGATACTCTTACTGGAACAGCAGTCAAAGCTACGTGTTGACTAAAGGA TGGAGCAGATACGTCAAAGACAAACAGCTCGACCCAGCCGATGTTGTTTTCTTCCAACGT CAACGTTCTGATTCCCGGAGACTCTTTATTGGCTGGCGTAGACGCGGTCAAGGCTCCTCC
- 25 TCCGCCGCGAATACGACGTCGTATTCTAGTTCCATGACTGCTCCACCGTATAGTAATTAC TCTAATCGTCCTGCTCACTCAGAGTATTCCCCACTATGGCGCCGCCGTAGCAACAGCGACG GAGACGCACTTCATACCATCGTCTTCCGCCGTCGGGAGCTCGAGGACGGTGAGGCTTTTT GGTGTGAATTTGGAGTGTCAAATGGATGAAGACGAAGGAGATGATTCGGTTGCCACGGCA GCCGCCGCTGAGTGTCCTCGTCAGGACAGCTACTACGACCAAAACATGTACAATTATTAC 30
- ACTCCTCACTCCTCAGCCTCATAA

Bra005301

Cover 100% identity 58%

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SEQ ID NO: 106

MSI NQYSSDFNYHSLMWQQQQHRHHHHQNDVAEEKEALFEKPLTPSDVGKLNRLVIPKQH AERYFPLAAAAADAMEKGLLLCFEDEEGKPWRFRYSYWNSSQSYVLTKGWSRYVKEKQLD AGDVI LFHRHRVDGGRFFIGWRRRGNSSSSSDSYRH LQSNASLQYYPHAGVQAVESQRGN SKTLRLFGVNMECQLDSDLPDPSTPDGSTICPTSHDQFH LYPQQHYPPPYYMDISFTGDV

HQTRSPQG

CDS SEQ ID NO: 107

- 45 ATGTCAATAAACCAATACTCAAGCGATTTCAACTACCACTCTCATGTGGCAACAACAG CAGCACCGCCACCACCATCAAAACGACGTCGCGGAGGAAAAAGAAGCTCTTTTCGAG AAACCCTTAACCCCAAGTGACGTCGGAAAACTCAACCGCCTCGTCATCCCAAAACAGCAC GCCGAGAGATACTTCCCTCTCGCAGCAGCCGCCGCAGACGCGATGGAGAAGGGATTACTT CTCTGCTTCGAGGACGAGGAAGGTAAGCCATGGAGATTCAGATACTCGTATTGGAACAGT 50 AGCCAGAGTTATGTCTTGACCAAAGGATGGAGCAGATACGTCAAGGAGAAGCAGCTCGAC
- GCCGGTGACGTCATTCTCTTCCACCGCCACCGTGTTGACGGAGGAAGATTCTTCATTGGC TGGAGAAGACGCGGCAACTCTTCCTCCTCTTCCGACTCTTATCGCCATCTTCAGTCCAAT GCCTCGCTCCAATATTATCCTCATGCAGGAGTTCAAGCGGTGGAGAGCCAGAGAGGGAAT

TCGAAGACATTAAGACTGTTCGGAGTGAACATGGAGTGTCAGCTAGACTCCGACTTGCCC GATCCATCTACACCAGACGGTTCCACCATATGTCCGACCAGTCACGACCAGTTTCATCTC TACCCTCAACAACACTATCCTCCTCCGTACTACATGGACATAAGTTTCACAGGAGATGTG CACCAGACGAGAAGCCCACAAGGATAA

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Bra017262 Cover 92% identity 56%

SEQ ID NO: 108
 MSI NQYSSEFYYHSLMWQQQQQHHHQNEVVEEKEALFEKPLTPSDVGKLNRLVIPKQHAE
 RYFPLAAAAVDAVEKGLLLCFEDEEGKPWRFRYSYWNSSQSYVLTKGWSRYVKEKQLDAG
 DVVLFHRH RADGGRFFIGWRRRGDSSSSDSYRNLQSNSSLQYYPHAGAQAVENQRGNSK
 TLRLFGVNM ECQIDSDWSEPSTPDGFTTCPTNHDQFPIYPEH FPPPYYMDVSFTGDVHQT
 SSQQG

CDS SEQ ID NO: 109

 ATGTCAATAAATCAATATTCAAGCGAGTTCTACTACCATTCTCTCATGTGGCAACAACAG
 CAGCAACACCACCATCAAAACGAAGTCGTGGAGGAAAAAGAAGCTCTTTTCGAGAAACCC TTAACCCCAAGTGACGTCGGAAAACTAAACCGCCTAGTCATCCCTAAACAGCACGCCGAG AGATACTTCCCTCTCGCCGCCGCCGCGGTAGACGCCGTGGAGAAGGGATTACTCCTCTGC TTCGAGGACGAGGAAGGTAAGCCATGGAGATTCAGATACTCTTATTGGAATAGTAGCCAG AGTTACGTCTTGACCAAAGGATGGAGCAGATATGTTAAAGAGAAGCAACTTGACGCCGGC
 GACGTTGTTCTCTTCCTCCTCCCGCCGCCGCCGCCGACGACGATCTTCATTGGCTGGAGA AGACGCGGCGACTCTTCCTCCTCCTCCGACTCTTATCGCAATCTTCATTGGCTGGAGA AGACGCGGCGACTCTTCCTCCTCCTCCGACTCTTATCGCAATCTTCAATCTAATTCCTCG CTCCAATATTATCCTCATCCAGGGGCTCAAGCGGTGGAAGACCAG AGAGGTAACTCCAAG

- CICCAATATTATCCICAT@AGGGGCTCAAGCGGTGGAGAACCAGAGAGGTAACTCCAAG ACATTGAGACTTTTTGGAGTGAACATGGAGTGCCAGATAGACTCAGACTGGTCCGAGCCA TCCACACCTGACGGTTTTACCACATGTCCAACCAATCACGACCAGTTTCCTATCTACCCT
- 30 GAACACTTTCCTCCTCCGTACTACATGGACGTAAGTTTCACAGGAGATGTGCACCAGACG AGTAGCCAACAAGGATAG

Bra000434

35 Cover 96% identity 47%

SEQ ID NO: 110 MMTNLSLAREGEEEEEAGAKKPTEEVEREHM FDKVVTPSDVGKLNRLVI PKQHAERYFP LDSSTNEKGLI LNFEDLTGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFLR CVGDTGRDSRLFI DWRRRPKVPDYTTSTSHFPAGAMFPRFYSFQTATTSTSYNPYNHQQP RHHHSGYCYPQI PREFGYGYVVRSVDQRAVVADPLVI ESVPVM MHGGARVNQAAVGTAGK RLRLFGVDMECGESGGTNSTEEESSSSGGSLPRGGASPSSSMFQLRLGNSSEDDH LFKKG KSSLPFN LDQ

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SEQ ID NO: 111

ATGATGACAAATTTGTCTCTTGCAAGAGAAGGAGGAGAAGAAGAAGAAGAAGAAGAGGCAGGAGCA AAGAAGCCCACAGAAGAAGTGGAGGAGAGAGAGCACATGTTCGACAAAGTGGTGACTCCAAGT GACGTCGGGAAACTAAACCGACTCGTGATCCCAAAGCAACACGCGGAGAGATACTTCCCT TTAGATTCATCCACAAACGAGAAGGGTTTGATTCTAAACTTCGAAGATCTCACGGGAAAG TCATGGAGGTTCCGTTACTCTTACTGGAACAGCAGTCAGAGCTATGTCATGACTAAAGGT TGGAGCCGTTTCGTTAAAGACAAGAAGCTAGACGCTGGAGATATTGTCTCTTTCCTGAGA TGTGTCGGAGACACAGGAAGGGACAGCCGCTTGTTTATCGATTGGAGGAGACACGACCACAA

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Bra040478 Cover 96% identity 48%

SEQ ID NO: 112

MMTNLSLAREGEAQVKKPI EEVEREHMFDKVVTPSDVGKLNRLVIPKQHAERYFPLDSSS NEKGLLLNFEDLTGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRCVGDS RLFI DWRRRPKVPDYPTSTAHFAAGAMFPRFYSFPTATTSTCYDLYNHQPPRHHHIGYGY PQI PREFGYGYFVRSVDQRAVVADPLVI ESVPVMMRGGARVSQEVVGTAGKRLRLFGVDM EEESSSSGGSLPRAGGGGASSSSSLFQLRLGSSCEDDH FSKKGKSSLPFDLDQ

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SEQ ID NO: 113

ATGATGACCAACTTGTCTCTTGCAAGGGAAGGAGGAGCACAAGTAAAGAAGCCCATAGAA GAAGTTGAGAGAGAGAGCACATGTTCGACAAAGTGGTGACTCCAAGCGACGTAGGGAAACTA AACAGACTCGTGATCCCAAAGCAACACGCAGAGAGATACTTCCCTCTAGATTCATCCTCA AACGAGAAAGGTTTGCTTCTAAACTTTGAAGATCTAACAGGAAAGTCATGGAGGTTCCGT TACTCTTACTGGAACAGTAGCCAGAGCTATGTCATGACTAAAGGTTGGAGTCGTTTCGTT AAAGACAAGAAGCTTGACGCCGGAGATATTGTCTCTTTCCAGAGATGTGTCGGAGACAGC CGCTTGTTTATCGATTGGAGGAGACGACCTAAAGTCCCTGACTATCCGACATCGACTGCT

- 30 CACTTTG CTG CAG GAG CTATGTTCCCTAGGTTTTACAGTTTTCCG ACAGCAACTACTTCG ACATGTTACGATCTGTACAATCATCAGCCGCCACGTCATCATCACATTGGTTACGGTTAT CCACAGATTCCGAGAGAATTTGGATACGGGTATTTCGTTAGGTCAGTGGACCAGAGAGCG GTGGTGGCTGATCCGTTGGTGATCGAATCTGTGCCGGTGATGATGCGCGGAGGAGCTCGA GTTAGTCAG GAG GTTGTTG GAACGGCCGGGAAGAGG CTGAGG CTTTTTG GAGTCG ATATG
- 40 Bra004501

Cover 74% identity 45%

SEQ ID NO: 114

45 M M MTNLSLSREGEEEEEEQEEAKKPM EEVEREHM FDKVVTPSDVGKLNRLVI PKQYAER 45 YFPLDSSTNEKGLLLNFEDLAGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVS FQRCVGDSGRDSRLFIDWRRRPKVPDHPTSIAHFAAGSM FPRFYSFPTATSYNLYNYQQP RHHHHSGYNYPQI PREFGYGYLVDQRAVVADPLVIESVPVMM HGGAQVSQAVVGTAGKRL RLFGVDMEEESSSSGGSLPRGDASPSSSLFQLRLGSSSEDDH FSKKGKSSLPFDLDQ

TACTTCCCTTTAGATTCATCCACAAACGAGAAAGGTTTGCTTCTAAACTTCGAAGATCTC GCAGGAAAGTCATGGAGGTTCCGTTACTCTTACTGGAACAGTAGTCAGAGCTATGTCATG

ACTAAAGGTTGGAGCCGTTTCGTTAAAGACAAAAAGCTAGACGCCGGAGATATTGTCTCT 5 AGACCTAAAGTTCCTGACCATCCGACATCGATTGCTCACTTTGCTGCCGGATCTATGTTT CCTAGGTTTTACAGTTTTCCGACAGCAACTAGTTACAATCTTTACAACTATCAGCAGCCA CGTCATCATCATCACAGTGGTTATAATTATCCTCAAATTCCGAGAGAATTTGGATACGGG TACTTGGTGGATCAAAGAGCCGTGGTGGCTGATCCGTTGGTGATTGAATCTGTGCCGGTG ATGATGCACGGAGGAGCTCAAGTTAGTCAGGCGGTTGTTGGAACGGCCGGGAAGAGGCTG 10 AGGCTTTTTGGAGTCGATATGGAGGAAGAATCTTCATCTTCCGGTGGGAGTTTGCCACGT GGTGACGCTTCTCCGTCTTCCTCTTTGTTTCAGCTGAGACTTGGAAGCAGCAGCAGTGAAGAT GATCACTTCTCTAAGAAAGGAAAGTCCTCATTGCCTTTTGATTTGGATCAATAA Bra003482 15 Cover 79% identity 44% SEQ ID NO: 115 MNQEEENPVEKASSM EREHM FEKVVTPSDVGKLNRLVIPKQHAERYFPLDNNSDSSKGLL LNFEDRTGNSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRDPGNKDKLFI D 20 WRRRPKI PDHHHQFAGAMFPRFYSFSHPQNLYHRYQQDLGIGYYVSSMERNDPTAVI ESV PLI MQRRAAHVAAI PSSRGEKRLRLFGVDM ECGGGGGGSVNSTEEESSSSGGGGGVSMASV GSLLQLRLVSSDDESLVAMEAASVDEDHHLFTKKGKSSLSFDLDRK 25 SEQ ID NO: 116 TTTGAAAAAGTAGTAACACCAAGCGACGTAGGCAAACTAAACCGACTCGTGATCCCAAAG CAACACGCGGAGAGATACTTCCCTTTAGACAACAATTCTGACAGCAGCAAAGGTTTGCTT CTAAACTTCGAAGACCGAACAGGAAACTCATGGAGATTCCGTTACTCTTACTGGAACAGT 30 AGCCAGAGTTATGTCATGACAAAAGGTTGGAGCCGCTTCGTCAAAGACAAGAAGCTTGAT GCTGGCGACATCGTTTCTTTTCAGAGAGATCCTGGTAATAAAGACAAGCTTTTCATTGAT TGGAGGAGACGACCAAAGATTCCAGATCATCATCATCAATTCGCTGGAGCTATGTTCCCT AGGTTTTACTCTTTCTCTCATCCTCAGAACCTTTATCATCGATATCAACAAGATCTTGGA ATTGGGTATTATGTGAGTTCAATGGAGAGAGAAATGATCCAACGGCTGTAATTGAATCTGTG 35 CCGTTGATAATGCAAAGGAGAGCAGCACACGTGGCTGCTATACCTTCATCAAGAGGAGAG AAGAGGTTAAGGCTGTTTGGAGTGGACATGGAGTGCGGCGGCGGCGGAGGAAGTGTGAAT AGCACGGAGGAAGAGTCGTCGTCTTCCGGTGGTGGCGGCGCGCGTTTCTATGGCTAGTGTT GGTTCTCTCCCAATTGAGGCTAGTGAGCAGTGATGATGAGTCTTTGGTAGCAATGGAA GCTG CAAGTGTCGATGAGGATCATCACTTGTTTACAAAGAAAG GAAAGTCTTCTTTGTCT TTCGATTTGGATAGAAAATGA

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	Bra007646		
45	Cover 74%	identity	45%

SEQ ID NO: 117

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MNQEN KKPLEEASTSMEREN MFDKVVTPSDVGKLNRLVIPKQHAERYFPLDNSSTNNKGL LLDFEDRTGSSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRDPCNKDKLYI DWRRRPKIPDHHQFAGAMFPRFYSFPHPQM PTSFESSHNLYHHRFQRDLGIGYYPTAVIE SVPVI MQRREAQVAN MASSRGEKRLRLFGVDVECGGGGGGSVNSTEEESSSSGGSMSRGG VSMAGVGSLLQLRLVSSDDESLVAMEGATVDEDHH LFTTKKGKSSLSFDLDI

CDS SEQ ID NO: 118

- TATCATCATCGGTTTCAACGAGATCTTGGAATTGGGTATTATCCAACGGCTGTGATTGAA TCTGTGCCGGTGATAATGCAAAGGAGAGAGAGAAGCACAAGTGGCTAATATGGCTTCATCAAGA GGAGAGAAGAGGTTAAGGCTGTTTGGGAGTGGACGTGGAGTGCGGCGGCGGAGGAGGAGGA AGTGTGAATAGCACGGAGGAAGAGTCGTCGTCTTCCGGTGGTAGTATGTCACGTGGCGGC 15
- 15 GTTTCTATGGCTGGTGTTGGTTCTCCCTTCAGTTGAGGTTAGTGAGCAGTGATGATGAG TCTTTAGTAGCGATGGAAGGTGCTACTGTCGATGAGGATCATCACTTGTTTACAACTAAG AAAGGAAAGTCTTCTTTGTCTTTCGATTTGGATATATGA
- 20 Bra014415 Cover 48% identity 60% SEQ ID NO: 119 MERKSNDLERSENI DSQNKKMNLEEERPVQEASSMEREHM FDKVVTPSDVGKLNRLVI PK QHAERYFPLDNNSSDNN KGLLLNFEDRIGI LWSFRYSYWNSSQSYVMTKGWSRFVKDKKL
- 25 DAGDIVSFHRGSCNKDKLFI DWKRRPKI PDHQVVGAM FPRFYSYPYPQIQASYERHNLYH RYQRDIGIGYYVRSM ERYDPTAVIESVPVI MQRRAHVATMASSRGEKRLRLFGVDM ECVR GGRGGGGSVNSTEEESSTSGGSISRGGVSMAGVGSPLQLRLVSSDGDDQSLVARGAARVD EDHH LFTKKGKSSLSFDLDK

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CDS SEQ ID NO: 120

ATGGAGAGGAAGTCCAATGATCTTGAGAGATCTGAGAATATTGATTCTCAAAACAAGAAG TTCGACAAAGTAGTAACACCAAGCGACGTTGGGAAACTAAACCGGCTGGTGATCCCAAAG 35 CAACACGCAGAGCGATACTTCCCTTTAGACAATAATTCCTCAGACAACAACAAAGGTTTG CTTCTAAACTTCGAAGATCGAATAGGAATCTTATGGAGTTTCCGTTACTCCTACTGGAAC AGTAGCCAAAGTTATGTAATGACTAAAGGCTGGAGCCGTTTCGTCAAAGACAAGAAGCTT GATGCTGGCGACATAGTTTCTTTTCATAGAGGTTCTTGTAATAAAGACAAGCTTTTCATT GATTGGAAGAGACGACCAAAGATTCCTGATCACCAAGTCGTCGGAGCTATGTTCCCTAGG 40 TTTTACTCTTACCCTTATCCTCAGATACAGGCTAGTTATGAACGTCACAACCTTTATCAT CGATATCAACGAGATATAGGAATTGGGTATTATGTGAGGTCAATGGAGAGATATGATCCA ACGGCTGTAATTGAATCTGTGCCGGTGATAATGCAAAGGAGAGCACATGTGGCTACTATG GCTTCATCAAGAGGAGAGAGAGAGGTTAAGGCTTTTTGGAGTGGATATGGAGTGCGTCAGA GGCGGCCGAGGAGGAGGAGGAAGTGTGAATAGCACGGAGGAAGAGTCTTCGACTTCCGGT GGTAGTATCTCACGTGGCGGCGTTTCTATGGCTGGTGTTGGCTCTCCACTCCAGTTGAGG

- 50 Bra038346 Cover 51% identity 57% SEQ ID NO: 121 MVFSCI DESSSTSESFSPATATATATATKFSAPPLPPLRLNRMRSGGSNVVLDSKNGVDI

EGYDSPPMADDI HEFTLGDSYAFGEGFSNGYLEEVLRSLPLQEDGQKKLCDAPINADASD CDS SEQ ID NO: 124 ATGGCCGCCTCGCCCTCTTCACCCTTGACAGCGCCGCCAGAGCCGGTGACCCCGCCGTCC 45 CCATGGACCATCACAGACGGAGCCATCTCTGGCACGCTCCCAGCAGCCGAGGCCTTCGCA GTGCACTACCCGGGCTACCCCTCCTCCCGCCCGCGCCGCCGCACCCTCGGCGGTCTC CCCGAGGACCCCTACTGCCATCCAGCCTTTGGCCAGTCCCGCGCCTCCACTGGCCTTCTG CTGCGCCTCTCCAAGCGCAAAGGAGCTGCGGCACCTTGTGCCCATGTGGTCGCTCGTGTC 50 CGGACTGCTTACTACTTCGAAGGTATGGCAGATTTTCAACATGTTGTTCCAGTGCATGCT

GCACAAACAAGAAAAAGAAAACACTCAGATTCTCAAAATGATAATGAGAATTTTGGTAGT GATAAGACAGGACATGATGAAGCAGATGGAGATGTCATGATGTTGGTACCCCCTCTCTTT TCAGTG AAG GATAGGCCAACAAAG ATAGCGCTTGTACCATCGTCCAATGCCATATCTAAA

SEQ ID NO: 123 MAASPSSPLTAPPEPVTPPSPWTITDGAISGTLPAAEAFAVHYPGYPSSPARAARTLGGL PGLAKVRSSDPGARLELRFRPEDPYCHPAFGQSRASTGLLLRLSKRKGAAAPCAHVVARV RTAYYFEGMADFQHVVPVHAAQTRKRKHSDSQNDNENFGSDKTGHDEADGDVM MLVPPI F 35 SVKDRPTKIALVPSSNAISKTMH RGVVQERWEMNVGPTLALPFNTQVVPEKINWEDHI RK NSVEWGWQMAVCKLFDERPVWPRQSLYERFLDDNVHVSQNQFKRLLFRAGYYFSTGPFGK FWI RRGYDPRKDSESQIYQRI DFRM PPELRYLLRLKNSESRKWADMCKLETMPSQSFIYL QLYELKDDFIQAEI RKPSYQSVCSRSTGWFSKPM IKTLRLQVSI RLLSLLHNEEAKNLLR NAHELI ERSKKQEALSRSELSI EYNDADQVSAAHTGTEDQVGPNNSDSEDVDDEEEEEL 40

GTTGTAGACGCGTGCGGTGGAAAGAGATCTCGGGATGTTGATATGTTTGCGCTACGGTGT 25 TCCAAAAAACACGCTATAATCAATGCTTTGTGA Zea mays

identity 47%

- GTCAACTTCAAAACCTTCCTCGCCTCAGAGGACGACAACGGCGAGTTATGTTTCCTTGAA 15 GCTCACTCCAAGGCCGAGATCGTCGACATGTTGAGGAAACACACTTACGCTGACGAGCTT GCGCAGAGCAATAAACGCAGCGGAGCGAATACGAATACGAATACGACTCAAAGCCACACC GTTTCGAGAACACGTGAAGTGCTTTTCGAGAAGGTTGTCACGCCTAGCGACGTTGGTAAG CTAAACCGCCTCGTGATACCTAAACAGCACGCGGAGAAATATTTTCCGTTACCGTCACTG TCGGTGACTAAAGGCGTTCTGATCAACTTCGAAGACGTGACGGGTAAGGTGTGGCGGTTC 20 CGTTACTCATACTGGAACAGTAGTCAAAGTTACGTGTTGACCAAGGGATGGAGTCGGTTC GTTAAGGAGAAGAATCTCCGAGCCGGTGATGTCGTTACTTTCGAGAGATCGACCGGTTCA GACCGGCAGCTTTATATTGATTGGAAAATCCGGTCTGGTCCGAGCAAAAACCCTGTTCAG GTTGTG GTTAGG CTTTTCG GAGTTG ACATCTTCAACGTG ACAAGCG CGAAG CCGAGCAAC
- ATGGTATTCAGTTGCATAGACGAGAGCTCTTCCACTTCAGAATCTTTTTCACCCGCAACC GCAACCGCAACCGCCACCACAAGTTCTCTGCTCCTCCGCTTCCACCGTTACGCCTC 10 AACCGGATGAGAAGCGGTGGAAGCAACGTCGTGTTGGATTCAAAGAATGGCGTAGATATT GATTCACGGAAGCTATCGTCGTCAAAGTACAAAGGCGTGGTTCCTCAGCCCAACGGAAGA TGGGGAGCTCAGATTTACGTGAAGCACCAGCGAGTTTGGCTGGGCACTTTCTGCGATGAA GAGGAAGCTGCTCACTCCTACGACATAGCCGCCCGTAAATTCCGTGGCCGTGACGCCGTT
- DSRKLSSSKYKGVVPQPNGRWGAQIYVKHQRVWLGTFCDEEEAAHSYDIAARKFRGRDAV VNFKTFLASEDDNGELCFLEAHSKAEIVDM LRKHTYADELAQSNKRSGANTNTNTTQSHT VSRTREVLFEKVVTPSDVGKLNRLVI PKQHAEKYFPLPSLSVTKGVLI N FEDVTGKVWRF RYSYWNSSQSYVLTKGWSRFVKEKNLRAGDVVTFERSTGSDRQLYIDWKIRSGPSKNPVQ 5 VVVRLFGVDI FNVTSAKPSNVVDACGGKRSRDVDM FALRCSKKHAIINAL

CDS SEQ ID NO: 122

GRMZM2G053008 Cover 74%

ACCATGCACAGGGGAGTTGTACAAGAACGGTGGGAGATGAATGTTGGACCAACTCTGGCG CTTCCGTTCAACACTCAAGTTGTCCCGGAGAAGATTAATTGGGAAGACCACATTAGAAAG AATTCTGTAGAATGGGGTTGGCAAATGGCTGTTTGCAAATTGTTTGATGAGCGCCCTGTG TGGCCAAGGCAATCACTTTATGAGCGGTTCCTTGATGATAATGTGCATGTCTCTCAAAAC CAATTCAAAAGGCTTCTGTTTAGAGCTGGATACTACTTCTCTACTGGACCCTTTGGAAAA

- TTTTGGATCAGAAGAGGATATGACCCTCGTAAAGACTCTGAGTCACAAATATATCAGAGA ATTGATTTTCGCATGCCTCCCGAGCTACGATATCTTCTAAGGCTGAAGAATTCTGAGTCT CGAAAGTGGGCAGATATGTGCAAGCTTGAAACAATGCCATCACAGAGTTTCATCTACCTG CAATTATATGAACTGAAGGATGATTTTATTCAAGCAGAAATTCGAAAACCTTCTTATCAA
- 10 TCAGTTTGTTCACGTTCTACAGGATGGTTTTCTAAGCCAATGATCAAAACCCTGAGGTTG CAAGTGAGCATAAGGCTCCTCTCTTTATTGCATAATGAAGAGGCTAAAAACTTGTTGAGG AATGCCCATGAGCTTATTGAAAGGTCCAAGAAGCAGGAAGCCCTTTCGAGGATCTGAGCTG TCAATAGAATATAATGATGCTGATCAAGTTTCTGCCGCACATACTGGAACTGAGGATCAA GTCGGCCCTAACAACTCTGATAGTGAAGAAGATGTGGATGATGAAGAAGAGGAAGAGGAAGAGGAATTG
- 15 GAGGGTTATGATTCTCCACCTATGGCAGATGATATTCATGAGTTCACCTTAGGTGATTCC TATGCATTTGGTGAAGGCTTCTCGAATGGATACCTCGAAGAAGTACTGCGCAGCTTGCCA TTGCAGGAAGACGGCCAAAAGAAATTATGTGATGCTCCTATCAACGCTGATGCAAGTGAT GGAGAGTTTGAAATTTACGAACAGCCCAGTGATGATGAAGATTCTGATGGCTAG

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GRMZM2G102059_T01 Cover 47% identity 62% SEQ ID NO: 125

M EFASSSSRFSREEDEEEEQEEEEEEASPREI PFMTAAATADTGAAASSSSPSAAASS 25 GPAAAPRSSDGAGASGSGGGGSDDVQVI EKEHM FDKVVTPSDVGKLNRLVIPKQHAEKYF PLDAAAN EKGQLLSFEDRAGKLWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVSFC RGAGDTARDRLFI DWKRRADSRDPH RM PRLPLPMAPVASPYGPWGGGGGGGGGGGGGGFFM PPA PPATLYEHHRFRQALDFRNI NAAAAPARQLLFFGSAGM PPRASMPQQQQPPPPPHPPLHS IM LVQPSPAPPTASVPM LLDSVPLVNSPTAASKRVRLFGVNLDNPQPGTSAESSQDANAL 30 SLRTPGWQRPGPLRFFESPQRGAESSAASSPSSSSSSKREAHSSLDLDL

CDS SEQ ID NO: 126

- 50 TCGGTACCGCTCGTCAACAGCCCAACGGCAGCGTCGAAGCGCGTCCGCCTGTTTGGGGTC AACCTCGACAACCCGCAACCAGGCACAAGTGCGGAGTCAAGCCAAGATGCCAACGCATTG TCGCTGAGGACACCGGGATGGCAAAGGCCGGGGGCCGTTGAGGTTCTTCGAATCGCCTCAA CGCGGCGCCGAGTCATCTGCAGCCTCCTCGCCGTCGTCATCGTCCTCCAAGAGAGAA

SEQ ID NO: 127

Cover 47% identity 63%

QSLWCRSCOPOPRRTADVP

CDS SEQ ID NO: 128

GRMZM2G098443 T01

GCGCACTCGTCCTTGGATCTCGATCTGTGA

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GACAAGGTGGTGACGCCGAGCGACGTCGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAG CACGCGGAGAAGTACTTCCCGCTGGACGCGGCGGCGAACGAGAAGGGCCTCCTGCTCAGC TTCGAGGACCGCACGGGGAAGCCCTGGCGCTTCCGCTACTCCTACTGGAACAGTAGCCAG AGCTACGTGATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGG 20 CCCTCCGCCGTCGTCCCCTACGCGCCGTGGGCGCGCACGCGCACCACCACCACTACCCA

M EFTTPPPATRSGGGEERAAAEHNQHHQQQHATVEKEHM FDKVVTPSDVGKLNRLVIPKQ HAEKYFPLDAAANEKGLLLSFEDRTGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAG DTVSFGRGISEAARDRLFI DWRCRPDPPVVHHQYHH RLPLPSAVVPYAPWAAHAHHHHYP ADGHTEPVTPCLCATLVATEM RASSSQLSLTRSNLSRPPQPRIARVDGAQPRPSSSPRQP

ATGGAGTTCACCACTCCCCCGCCCGCGACCCGGTCGGGCGGCGGAGAGGGAGAGGGCGGCT

GCTGAGCACCAGCACCACCAGCAGCAGCAGCAGCGGGGGGAGAAGGAGCACATGTTC

GCAGATGGGCACACGGAACCAGTAACACCTTGCCTGTGCGCCACACTCGTTGCCACTGAA ATGAGAGCATCATCTTCGCAACTGTCACTCACACGCTCCAACCTCTCCAGGCCGCCACAA

25 CAGTCGTTGTGGTGCCGGTCGTGCCAACCGCAACCGCCGAACGGCCGACGTTCCTTGA

- GRMZM2G082227_T01

Cover 45% identity 64%

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SEQ ID NO: 129

M EFTAPPPATRSGGGEERAAAEHHQQQQQATVEKEHM FDKVVTPSDVGKLNRLVIPKQHA ERYFPLDAAANDKGLLLSFEDRAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDT VSFGRGVGEAARGRLFIDWRRRPDPPVVHHQYHH HRLPLPSAVVPYAPWAAAAHAHHHHY PAAGVGAARTTTTTTTVLH HLPPSPSPLYLDTRRRHVGYDAYGAGTRQLLFYRPHQQPS TTVM LDSVPVRLPPTPGQHAEPPPPAVASSASKRVRLFGVNLDCAAAAGSEEENVGGWRT SAPPTQQASSSSSYSSGKARCSLNLDL

CDS SEQ ID NO: 130

- 40 ATGGAGTTCACCGCTCCCCCGCCCGCGACCCGGTCGGGCGGCGGCGAGGAGAGGGCGGCT GCTGAGCACCACCAGCAGCAGCAGCAGCAGGCGACGAGAAGGAGCACATGTTCGACAAG GTGGTGACGCCGAGCGACGTCGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGCACGCG GAGAGGTACTTCCCGCTGGACGCGGCGGCGAACGACAAGGGCCTGCTGCTCAGCTTCGAG GACCGCGCGGGGAAGCCCTGGCGCTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTAC GTGATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAGCGCCTCGACGCCGGGGACACC
- 45 CCAGCAGCTGGGGTCGGTGCCGCCAGGACGACGACGACGACGACGACGACGGTGCTCCAC
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AACCTCGACTGCGCCGCCGCCGGCTCAGAGGAGGAGAACGTCGGCGGGTGGAGGACT AGTGCGCCGCCGACGCAGCAGGCGTCCTCCTCCTCATCCTACTCTTCCGGGAAAGCGAGG TGCTCCTTGAACCTTGACTTGTGA

GRMZM2G024948_T01 Cover 46% identity 63% SEQ ID NO: 131 M DQFAASG RFSREEEADEEQEDASNSMREISFMP PAAASSSSAAASASASASASASASASASA GSSSAPFRSASASGDAAGASGSGGPADADAEAEAVEKEHMFDKVVTPSDVGKLNRLVI PK QYAEKYFPLDAAANEKGLLLSFEDSAGKHWRFRYSYWNSSQSYVMTKGWSRFVKEKRLVA GDTVSFSRAAAEDARHRLFI DWKRRVDTRGPLRFSGLALPM PLPSSHYGGPHHYSPWGFG GGGGGGGGFFMPPSPPATLYEHRLRQGLDFRSMTTTYPAPTVGRQLLFFGSARM PPHHAP PPQPRPFSLPLH HYTVQPSAAGVTAASRPVLLDSVPVI ESPTTAAKRVRLFGVNLDNN PD GGGEASHQGDALSLQMPGWQQRTPTLRLLELPRHGGESSAASSPSSSSSKREARSALDL

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DL

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50

GATCTGTGA

Cover 55%

YQHDEEI

CDS 135

SEQ ID NO: 134

GRMZM2G328742 T01

CDS SEQ ID NO: 132

20 GAGGATGCGTCCAATTCCATGCGCGAGATCTCCTTCATGCCGCCGGCTGCGGCCTCGTCA TCTTCGGCGGCTGCTTCCGCGTCCGCGTCCGCCTCCACCAGCGCATCCGCGTGTGCATCG GGAAGCAGCAGCGCCCCCTTCCGCTCCGCCTCCGCGTCGGGGGATGCCGCCGGAGCGTCG GGGAGCGGCCCAGCGGACGCGGACGCGGAGGCGGAGGCGGTGGAGAAGGAGCACATG TTCGACAAGGTGGTCACGCCGAGCGACGTGGGGAAGCTCAACCGGCTGGTGATCCCGAAG 25 CAGTACGCGGAGAAGTACTTCCCGCTGGACGCGGCGGCCAACGAGAAGGGCCTCCTCCTC AGCTTCGAGGACAGCGCCGGCAAGCACTGGCGCTTCCGCTACTCCTACTGGAACAGCAGC CAGAGCTACGTCATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGTCGCC

- GGGGACACCGTCTCCTCCCCGCGCCGCCGAGGACGCGCCACCGCCTCTTCATC GACTGGAAGCGCCGGGTCGACACCCGCGGCCCGCTTCGTTTCTCCGGCCTCGCGCTGCCG 30 ATGCCGCTGCCGTCGTCGCACTACGGCGGGCCCCACCACTACAGCCCGTGGGGCTTCGGC GGCGGCGGCGGCGGCGGCGGCGGCGGATTCTTCATGCCGCCCTCGCCGCCGCCACGCTCTAC
- GAGCACCGCCTCAGACAGGGCCTCGACTTCCGCAGCATGACGACGACCTACCCCGCGCCG ACCGTGGGGAGGCAGCTCCTGTTTTTCGGCTCGGCCAGGATGCCTCCTCATCACGCGCCG
- CCGCCCCAGCCGCCCGTTCTCGCTGCCGCTGCATCACTACACGGTGCAACCGAGCGCC 35 GCCGGCGTCACCGCCGCGTCACCGGCCGGTCCTTCTTGACTCGGTGCCGGTCATCGAGAGC
- GGCGGCGGCGAGGCTAGCCATCAGGGCGATGCATTGTCATTGCAGATGCCCGGGTGGCAG CAAAGGACTCCAACTCTAAGGCTACTAGAATTGCCTCGCCATGGCGGGGGGGCCTCCCGCG

identity 64%

- CCGACGACCGCCGCGAAGCGCGTGCGGCTGTTCGGCGTCAACCTGGACAACAACCCAGAT

MATN HLSQGQHQHPQAWPWGVAMYTNLHYH HQQH HHYEKEH LFEKPLTPSDVGKLNRLVI PKQHAERYFPLSSSGAGDKGLILCFEDDDDDEAAAANKPWRFRYSYWTSSQSYVLTKGWS RYVKEKQLDAGDVVRFQRM RGFGM PDRLFISHSRRGETTATAATTVPPAAAAVRVVVAPA QSAGADHQQQQQPSPWSPMCYSTSGSYSYPTSSPANSQHAYHRHSADH DHSNNMQHAGES

QSDRDNRSCSAASAPPPPSRRLRLFGVN LDCGPGPEPETPTAMYGYMHQSPYAYNNWGSP

ATGGCCACGAACCATCTCTCCCAAGGGCAGCACCAGCACCCGCAGGCCTGGCCCTGGGGC GTGGCCATGTACACCAACCTACACTACCACCACCAGCAGCACCACCACTACGAGAAGGAG CACCTGTTCGAGAAGCCGCTGACGCCGAGCGACGAGGCAAGCTCAACAGGCTGGTGATC CCCAAGCAGCACGCCGAGAGGTACTTCCCTCTCAGCAGCAGCGGCGCCGGCGACAAAGGC

- 5 CTCATCCTGTGCTTCGAGGACGACGACGACGACGAGGCTGCCGCCGCCAACAAGCCGTGG CGGTTCCGCTACTCGTACTGGACCAGCAGCCAGAGCTACGTGCTCACCAAGGGCTGGAGC CGCTACGTCAAGGAGAAGCAGCTTGACGCCGGCGACGTCGTGCGCTTCCAGAGGATGCGT GGTTTCGGCATGCCCGACCGCCTGTTCATCAGCCACAGCCGCCGCGGCGAGACTACTGCT ACTGCTGCAACAACAGTGCCCCCCGCTGCTGCTGCCGTGCGCGTAGTAGTGGCACCTGCA
- 15 ACGGCGATGTACGGCTACATGCACCAAAGCCCCTACGCTTACAACAACTGGGGCAGTCCA TACCAGCATGACGAGGAGATTTAA
- 20 GRMZM2G142999_T01 Cover 44% identity 64%

SEQ ID NO: 136

25 MEFTPAHAHARVVEDSERPRGGVAWVEKEHM FEKVVTPSDVGKLNRLVIPKQHAERYFPA 26 LDASSAAAAAAAAAGGGKGLVLSFEDRAGKAWRFRYSYWNSSQSYVMTKGWSRFVKEKR 26 LGAGDTVLFARGAGGARGRFFI DFRRRRQDLAFLQPTLASAQRLLPLPSVPICPWQDYGA 28 SAPAPNRHVLFLRPQVPAAVVLKSVPVHVAASAVEATMSKRVRLFGVNLDCPPDAEDSAT 29 VPRGRAASTTLLQLPSPSSSTSSSTAGKDVCCLDLGL

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45

GRMZM2G125095_T01 Cover 85% identity 40% SEQ ID NO: 138

50 M EFRPAHARVFEDSERPRGGVAWLEKEHMFEKVVTPSDVGKLNRLVI PKQHAERYFPALD ASAAAASASASAGGGKAGLVLSFEDRAGKAWRFRYSYWNSSQSYVMTKGWSRFVKEKRLG AGDTVLFARGAGATRGRFFI DFRRRRH ELAFLQPPLASAQRLLPLPSVPICPWQGYGASA PAPSRHVLFLRPQVPAAVVLTSVPVRVAASAVEEATRSKRVRLFGVNLDCPPDAEDGATA TRTPSTLLQLPSPSSSTSSSTGGKDVRSLDLGL

CDS SEQ ID NO: 139

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20 Tricum aeseirum

TRAES3BF098300010CFD_tl Cover: 42% ident 60%

25 SEQ ID NO: 140

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30 RVVRLFGVDISGVKRGRAATATAEQGLQELFKRQCVAPGQHSPALGAFAL

CDS SEQ ID NO: 141

- 45 AAGGTGTGGAGGTTCCGGTACTCGTACTGGAACAGCAGCCAGAGCTACGTGCTCACCAAA GGCTGGAGCCGCTTCGTCCGGGAGAAGGGCCTAGGTGCCGGCGACTCCATCCTATTCTCG TGCTCGCTGTACGAACAGGAGAAGCAGTTCTTCATCGACTGCAAGAAGAACACTAGCATG AACGGAGGCAAATCGGCGTCGCCGCTGCCAGTGGGGGTGACTACCAAAGGAGAACAAGTT CGCGTCGTTAGGCTATTCGGTGTCGACATCTCGGGAGTGAAGAGGGGGCGAGCGGCGACG
- 50 GCAACGGCGGAGCAAGGCCTGCAGGAGTTGTTCAAGAGGCAATGCGTGGCACCCGGCCAG CACTCTCCTGCCCTAGGTGCCTTCGCCTTATAG

5 TRAES3BF062700040CFD_tl Cover 47% ident 55%

SEQ ID NO: 142

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CDS SEQ ID NO: 143

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- 25 CCGACGCCGGCGTGGGCACGAGAGCTCCTCTTCGAGAAGGCCGTGACCCCGAGCGACGTC GGCAAGCTCAACCGCCTCGTGGTGCCGAAGCAGCAGGCCGAGAAGCACTTCCCTCCGACC ACTGCGGCGGCCACCGGCAGCAACGGCAAGGGCGTGCTGCTCAACTTCGAGGACGGCGAA GGGAAGGTGTGGCGCTTCCGTACTCGTACTGGAACAGCAGCCAGAGCTACGTGCTCACC AAGGGCTGGAGCCGCTTCGTCAAGGAGACGGGCCTCCGCGCCGGCGACACCGTGGCGTTC
- 30 TACCGGTCGGCGTACGGGAATGACACGGAGGATCAGCTCTTCATCGACTACAAGAAGATG AACAAGAATGACGATGCTGCGGACGCGGCGGATTTCCCGATGAGAATGAGACAGGCCATGTC GCCGTCAAGCTCTTCGGCGTTGACATTGCCGGTGGAGGGATGGCGGGGATCATCAGGTGGC TGA

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TRAES3BF062600010CFD_tl Cover 43% ident 58%

- 40 SEQ ID NO: 144
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 DPELGFLADHSKAEIVDMLRKHTYDDELRQGLRRGRGRAQPTPAWARELLFEKAVTPSDV
 GKLNRLVVPKQQAEKHFPPTTAAATGSNGKGVLLNFEDGEGKVWRFRYSYWNSSQSYVLT
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 - CDS SEQ ID NO: 145

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- 5 GGCAAGCTCAACCGCCTCGTGGTGCCGAAGCAGCAGGCCGAGAAGCACTTCCCTCCGACC ACTGCGGCGGCCACCGGCAGCAACGGCAAGGGCGTGCTGCTCAACTTCGAGGACGGCGAA GGGAAGGTGTGGCGCTTCCGGTACTCGTACTGGAACAGCAGCCAGAGCTACGTGCTCACC AAGGGCTGGAGCCGCTTCGTCAAGGAGACGGGCCTCCGCGCCGGCGACACCGTGGCGTTC TACCGGTCGGCGTACGGGAATGACACGGAGGATCAGCTCTTCATCGACTACAAGAAGATG
- 10 AACAAGAATGACGATGCTGCGGACGCGGCGATTTCCGATGAGAATGAGACAGGCCATGTC GCCGTCAAGCTCTTCGGCGTTGACATTGCCGGTGGAGGGATGGCGGGATCATCAGGTGGC TGA

CLAIMS:

- 1. A plant wherein said plant does not produce a functional NGAL2 polypeptide or does not produce functional NGAL2 and NGAL3 polypeptides.
- A plant according to claim 1 wherein the expression of a nucleic acid sequence encoding a NGAL2 polypeptide or the activity of a NGAL2 polypeptide is reduced or abolished.
 - 3. A plant according to claim 1 or 2 wherein the expression of a nucleic acid sequence encoding a NGAL3 polypeptide or the activity of a NGAL3 polypeptide is reduced or abolished.
 - 4. A plant according to a preceding claim wherein the NGAL2 polypeptide comprises a sequence comprising or consisting of SEQ ID NO: 3, a functional variant or homologue thereof.
- 5. A plant according to a preceding claim wherein the *SOD7* nucleic acid sequence encoding a NGAL2 polypeptide comprises a nucleic acid sequence comprising or consisting of SEQ ID NO: 1 or 2, a functional variant or homologue thereof.
 - 6. A plant according to claim 5 wherein the functional variant or homologue comprises a sequence comprising or consisting of SEQ ID No 6 or 7.
- 7. A plant according to a preceding claim wherein the NGAL3 polypeptide comprises a sequence comprising or consisting of SEQ ID NO: 5, a functional variant or homologue thereof.
 - 8. A plant according to a preceding claim wherein the *NGAL3* nucleic acid sequence encoding a NGAL3 polypeptide comprises a nucleic acid sequence comprising or consisting of SEQ ID NO: 4, a functional variant or homologue thereof.
 - 9. A plant according to claim 8 wherein the functional variant or homologue thereof comprises a sequence comprising or consisting of SEQ ID NO: 6 or 7.
 - 10. A plant according to a preceding claim wherein the endogenous *SOD7* nucleic acid sequence or its promoter carries a functional mutation.
 - 11. A plant according to any of claims 1-9 wherein said plant comprises an RNA interference construct that reduces the expression of *SOD7*, a functional variant or homolog thereof.
 - 12. A plant according to a preceding claim wherein the endogenous *NGAL3* nucleic acid sequence or its promoter carries a functional mutation.

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- 13. A plant according to any of claims 2 to 11 wherein said plant comprises an RNA interference construct that reduces the expression of *NGAL3*, a functional variant or homolog thereof.
- 14. A plant according to a preceding claim wherein said plant is a crop plant.
- 15. A plant according to a preceding claim wherein said plant is a monocotyledonous plant or dicotyledonous plant.
- 16. A plant according to a preceding claim wherein said plant is selected from maize, rice, wheat, oilseed rape/canola, sorghum, soybean, sunflower, alfalfa, potato, tomato, tobacco, grape, barley, pea, bean, field bean, lettuce, cotton, sugar cane, sugar beet, broccoli or other vegetable brassicas or poplar.
- 17. A method for altering a plant phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 polypeptide or reducing or abolishing the activity of a NGAL2 or reducing or abolishing the expression of a nucleic acid sequences encoding NGAL2 and NGAL3 polypeptides or reducing or abolishing the activity of a NGAL2 and NGAL3 polypeptide relative to a control plant.
- 18. A method for making a plant with an altered phenotype comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 polypeptide or reducing or abolishing the activity of a NGAL2 or reducing or abolishing the expression of a nucleic acid sequences encoding NGAL2 and NGAL3 polypeptides or reducing or abolishing the activity of a NGAL2 and NGAL3 polypeptide relative to a control plant..
- 19. A method according to claim 17 or 18 comprising reducing or abolishing the expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reducing or abolishing expression of a nucleic acid sequence encoding a NGAL3 polypeptide.
- 20. A method according to claim 17 or 18 comprising reducing or abolishing activity of a NGAL2 polypeptide and reducing or abolishing activity of a NGAL3 polypeptide.
- 30
- 21. A method according to claim 17 or 18 comprising reducing or abolishing expression of a nucleic acid sequence encoding a NGAL2 polypeptide and reducing or abolishing activity of a NGAL3 polypeptide.
- 22. A method according to claim 17 or 18 comprising reducing or abolishing expression of a nucleic acid sequence encoding a NGAL3 polypeptide and reduced or abolished activity of a NGAL2 polypeptide.

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- 23. A method according to any of claims 17 to 22 wherein the NGAL2 polypeptide comprises a sequence comprising or consisting of SEQ D NO: 1 or 2, a functional variant or homologue thereof.
- 24. A method according to any of claims 17 to 22 wherein the *SOD7* nucleic acid sequence encoding a NGAL2 polypeptide comprises a nucleic acid sequence comprising or consisting of SEQ ID NO: 1 or 2, a functional variant or homologue thereof.
- 25. A method according to claim 24 wherein the functional variant or homologue comprises a nucleic acid sequence as shown in SEQ Id NO:49-145.
- 26. A method according to any of claims 17-25 wherein the NGAL3 polypeptide comprises a sequence comprising or consisting of SEQ ID NO: 5, a functional variant or homologue thereof.
 - 27. A method according to claim 17 to 26 wherein the *NGAL3* nucleic acid sequence encoding a NGAL3 polypeptide comprises a nucleic acid sequence comprising or consisting of SEQ ID NO: 4, a functional variant or homologue thereof.
 - 28. A method according to claim 27 wherein the functional variant or homologue comprises a nucleic acid sequence comprising or consisting of SEQ ID NOs:49-145.
- 29. A method according to any of claims 17 to 28 wherein said method comprises introducing a functional mutation in a nucleic acid sequence encoding a NGAL2 and/or NGAL3 protein or peptide in a plant or in its corresponding promoter.
 - 30. A method according to claim 29 wherein said mutation is introduced using T-DNA insertion, chemical mutagenesis or genome editing.
- 31.A method according to claim 30 comprising using TILLING.
 - 32. A method according to any of claims 17 to 28 comprising silencing of the SOD7 and/or NGAL3 nucleic acid sequence.
 - 33. A method according to claim 32 comprising introducing a RNAi, shRNA, snRNA, dsRNA, siRNA, miRNA, ta-siRNA or co-suppression molecule which targets the SOD7 or NGAL3 nucleic acid sequence gene into a plant.
 - 34. A method according to any of claims 17 to 33 wherein said phenotype is characterised by increased seed size relative to a control plant.
 - 35. A plant obtained or obtainable by the method of any of claims 17 to 34.
 - 36. An isolated nucleic acid comprising a sequence comprising or consisting of SEQ ID NO: 1 or 2 or a functional variant or homologue thereof.
 - 37. A vector comprising an isolated nucleic acid according to claim 36.

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38. A silencing nucleic acid construct targeting sequence comprising or consisting of SEQ ID NO: 1, 2 or 3 or a functional variant, part or homologue thereof.

Figure 1

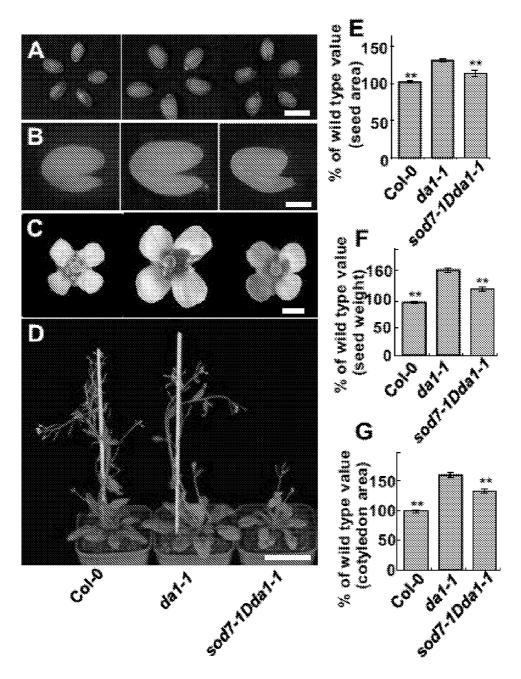
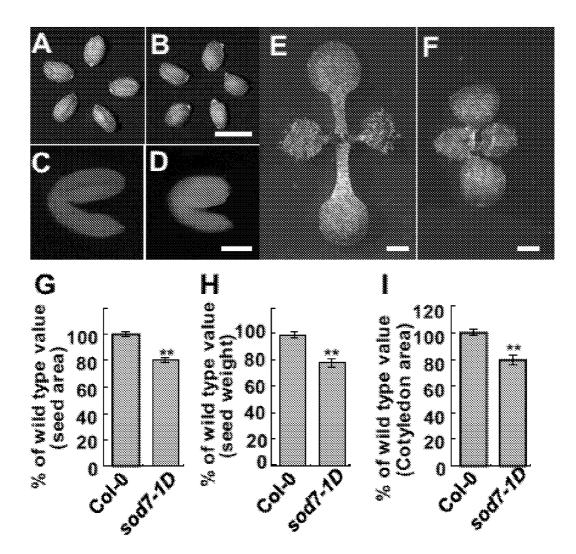


Figure 2



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Figure 3
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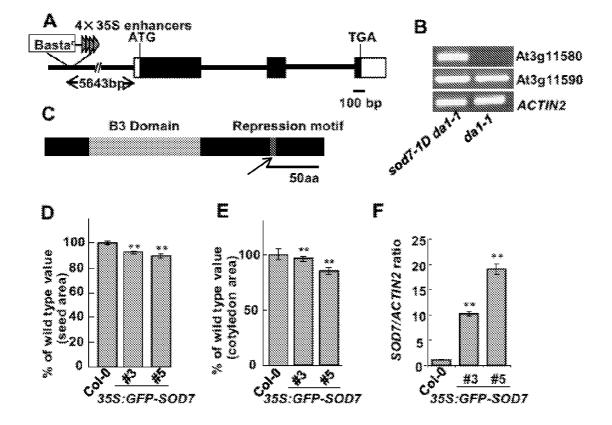


Figure 4

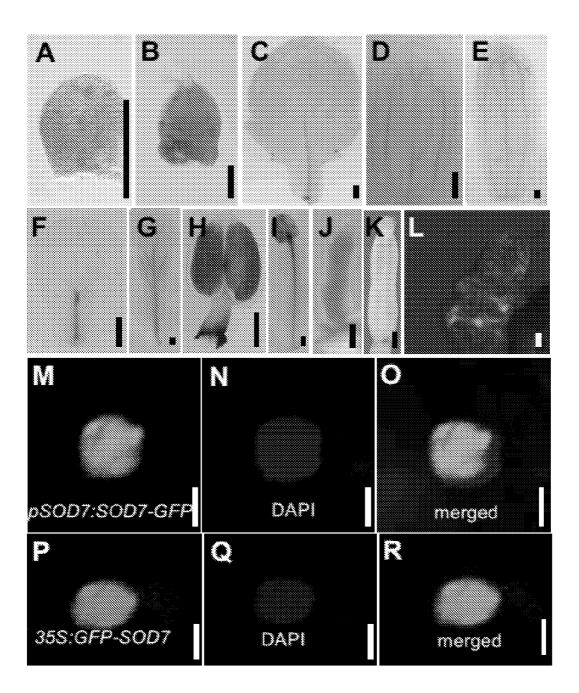
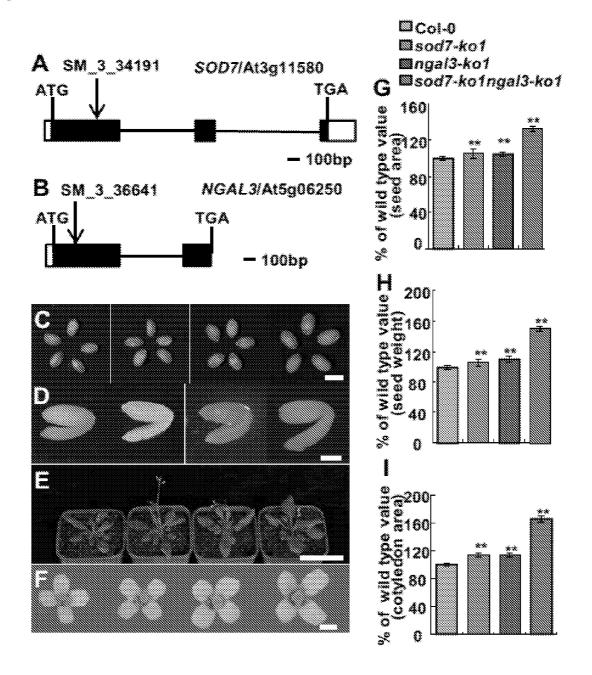
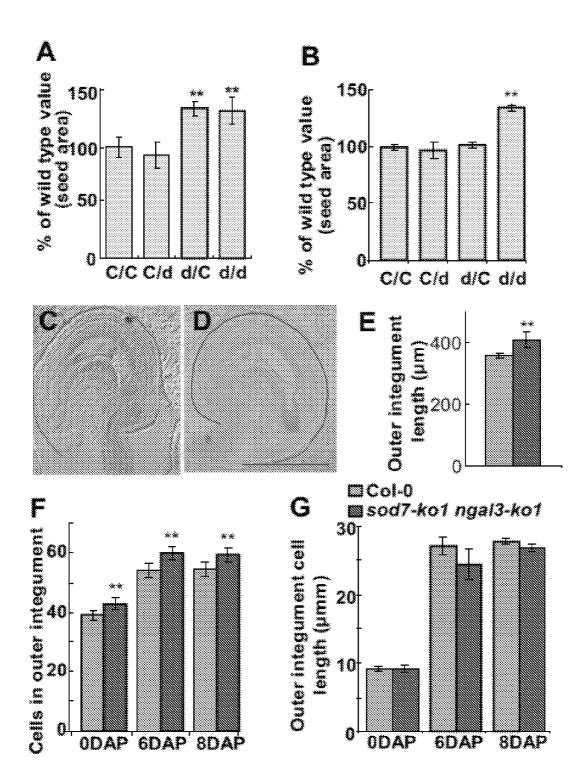


Figure 5









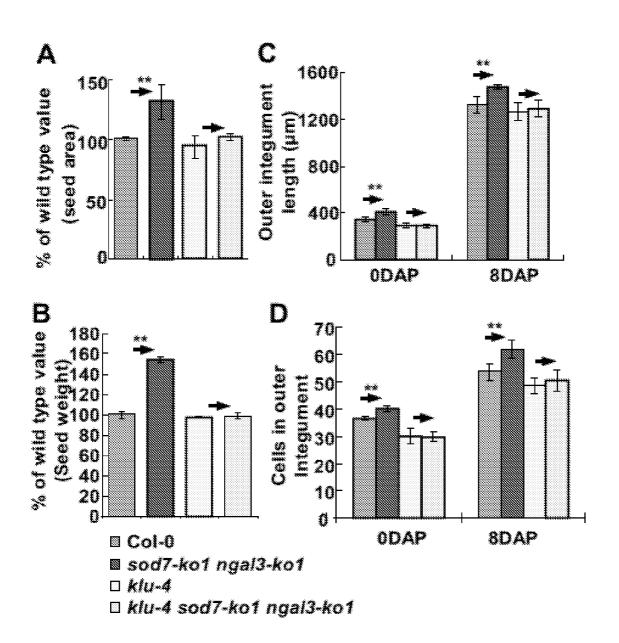


Figure 8

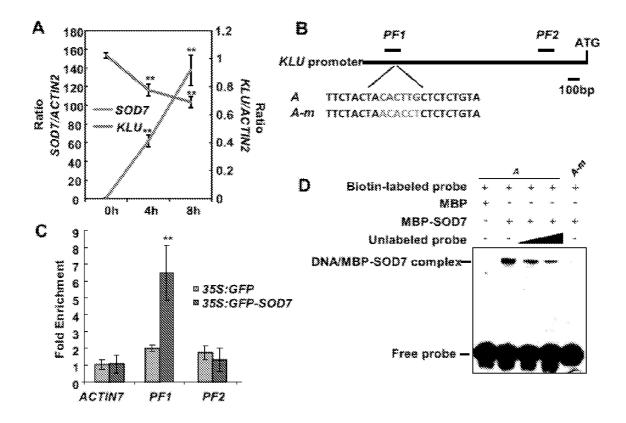
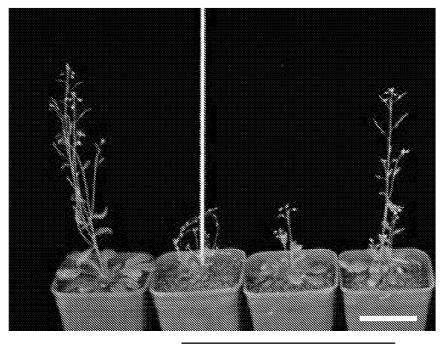


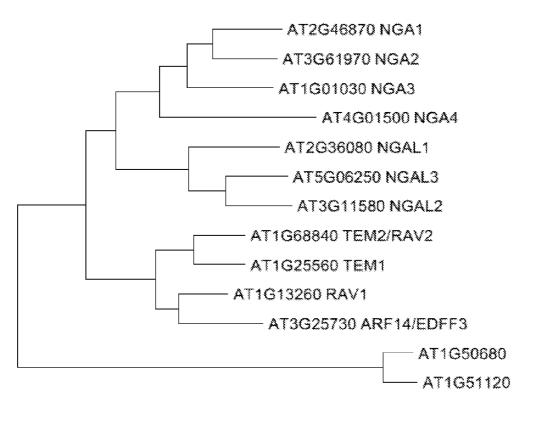
Figure 9



Col-0

35S:GFP-SOD7

Figure 10



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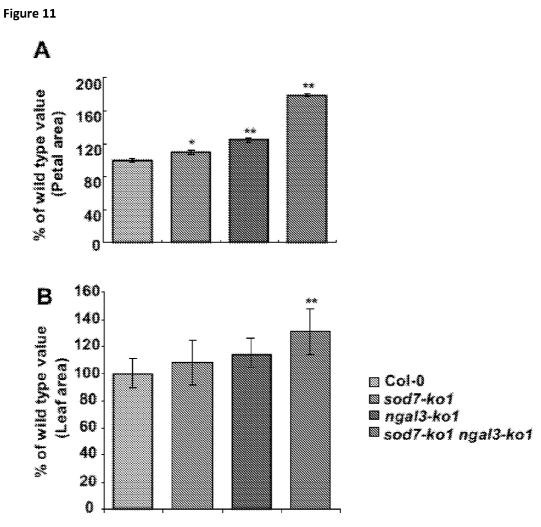


Figure 12

Α

SOD7 1 FEKSLTPSDVGKLNRLVIPKOHAEKYFPLNNNNNGGS	SGDDVA
Brassica rapa2 1 FEKSLTPSDVGKLNRLVIPKQHAEMYLPLNNCGGGG	200000 2000
Glycine max.At3g11580-like1 FEKPLTPSDVGKLNRLVIPKQHAEKYFPLs	200000 0000 0000 0000 0000 0000 0000 0
Glycine max At5q06250-like1 FEKPLTPSDVGKLNRLVIPKOHAEKYFPLS®G	
Glycine max At2g36080-like1 FEKPLTPSDVGKLNRLVIPKQHAEKYFPLDSS	00000
	GD [®] GE- <mark>KGLLLSFEDES</mark>
At5g06250/NAGL3 1 FEKSLTPSDVGKLNRLVIPKQHAEKYFPLNWVLVS-SA	200
Hordeum vulgare1 1 F KV TPSDVGKLNRLVIPKQHAEKYFPL	AANEKGLLLSFEDRG
Zea mays Os02g0683500 1 F	aan <mark>ekglllsfed</mark> ra
Zea mays Os02g0683500-like1 <mark>F</mark> KV TPSDVGKLNRLVIPKQHAEKYFPLD	AANEKGQLLSFEDRA
Hordeum vulgare2 1 <mark>F</mark> K <mark>V TPSDVGKLNRLVIPKQHAEKYFPL</mark> D	<mark>KGLLLSFED</mark> RA
Gossypium hirsutum RAV 1 FEKA TPSDVGKLNRLVIPKQHAEKYFPLQS	GAASKGALLNFEDV
Triticum aestivum 1 <mark>FEK</mark> A <mark>TPSDVGKLNRLV PKQHAEK</mark> H <mark>FPL</mark> KRTP	ETP
58 GKCWFRYSYWNSSQSYVLTKGWSRWVKWKHLDAGDVVFFQRHRFDLHRLFIGW-RRRGE	—
56 GK <mark>SW FRYSYWNSSQSYVLTKGWSR VK KHLNAGDVV</mark> LFORHRFDIHRLFIGWRRRGE 1	
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	107 SEQ ID NO 263
	107 SEQ ID NO 264
	108 SEQ ID NO 265
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	107 SEQ ID NO 267
	105 SEQ ID NO 268
	107 SEQ ID NO 269
	107 SEQ ID NO 270
	104 SEQ ID NO 271
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В

SEQ ID NO 164	LOC_Os04g49230	1 D <mark>RLF</mark> IDWKRR
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SEQ ID No 166	sGmLoc100795470	1 RLFGV D
SEQ ID No 167	Bra000434	1 RLFGV D
SEQ ID No 168	Bra040478	1 RLFGV D
SEQ ID No 169	Bra004501	1 RLFGV D
SEQ ID No 170	Bra003482	1 RLFGV D
SEQ ID No 171	Bra014415	1 <mark>RLFGV</mark> D –
SEQ ID No 172	GmLoc100818164	1 RLFGVN –
SEQ ID No 173	GmLoc100802734	1 RLFGVN
SEQ ID No 174	GmLoc100781489	1 RLFGVN –
SEQ ID No 175	GmLoc100778733	1 RLFGVN –
SEQ ID No 176	Bra005301	1 RLFGVN –
SEQ ID No 177	Bra017262	1 RLFGVN
SEQ ID No 178	GmLoc102660503	1 RLFGV C

SEQ ID No 179	HvMLOC_7940	1 <mark>vrlfgv</mark> d\\A-
SEQ ID No 180	HvMLOC_56567	1 VRLFGV <mark>D</mark> @A-
SEQ ID No 181	Bra038346	1 VRLFGV <mark>D</mark> ∰F-
SEQ ID No 182	TRAES3BF098300010CFD_t1	1 VRLFGV <mark>D</mark> S-
SEQ ID No 183	GmLoc100776987	1 VRLFGVN
SEQ ID No 184	GmLoc100801107	1 VRLFGVN
SEQ ID No 185	os01g0693400	1 VRLFGV <mark>D</mark> L-
SEQ ID No 186	GmLoc100789009	1 VRLFGV <mark>D</mark> L-
SEQ ID No 187	HvMLOC44012	1 VRLFGV <mark>D</mark> L-
SEQ ID No 188	HvMLOC 38822	1 VRLFGV <mark>D</mark> L-
SEQ ID No 189	GmLoc732601	1 VRLFGVNLL-
SEQ ID No 190	BrLOC103849927	1 VRLFGVNL
SEQ ID No 191	Bra034828	1 VRLFGVNL
SEQ ID No 192	Bra005886	1 VRLFGVNL
SEQ ID No 193	SOD7	1 VRLFGVNL
SEQ ID No 194	At5g06250/NGAL3	1 VRLFGVNL
SEQ ID No 195	LOC Os11g05740.1	1 RLFGVNL -
SEQ ID No 196		1 RLFGVNL -
SEQ ID No 197		1 VRLFGVNL
SEQ ID No 198	LOC Os03g02900	1 VRLFGVNL
SEQ ID No 199	Os10g0537100	1 VRLFGVNL
SEQ ID No 200	HvMLOC 66387	1 VRLFGVNL
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Figure 13

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Os12g0157000	SEQ	ID	NO	209	1	
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Bra000434	SEQ	ID	NO	212	1	
Bra040478	SEQ	ID	NO	213	1	
Bra014415	SEQ	ID	NO	214	1	
Bra003482	SEQ	ID	NO	215	1	
Bra007646	SEQ	ID	NO	216	1	
GlycinemaxLoc100781489	9 SEQ	ID	NO	217	1	
GRMZM2G024948_T01	SEQ	ID	NO	218	1	
os02g0683500	SEQ	ID	NO	219	1	
HvMLOC_66387	SEQ	ID	NO	220	1	
os04g0581400	SEQ	ID	NO	221	1	
GRMZM2G102059_T01	SEQ	ID	NO	222	1	
Os10g0537100	SEQ	ID	NO	223	1	
GRMZM2G142999_T01	SEQ	ID	NO	224	1	
GRMZM2G125095 T01	SEQ	ID	NO	225	1	
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GRMZM2G328742 T01	SEQ	ID	NO	230	1	
GmLoc100802734	SEQ	ID	NO	231	1	
GmLoc100795470	SEQ	ID	NO	232	1	
GmLoc100818164	SEQ	ID	NO	233	1	
Bra017262	SEQ	ID	NO	234	1	
At2g36080	SEQ	ID	NO	235	1	
Bra005301	SEQ	ID	NO	236	1	
At3g11580	SEQ	ID	NO	237	1	
BraLOC103849927	SEQ	ID	NO	238	1	
BrassicarapaBra034828				239	1	
At5g06250		ID	NO	240	1	
Bra005886				241	1	
GmLoc102660503				242	1	MIGVEKVTICMRIEVNTA
H∨MLOC 38822	-			243	1	
os01q0693400				244	1	TDKLRALAAAAAET
HvMLOC44012				245	1	
H∨MLOC 7940	-			246		MGVEIL-SSTGEHSSQYSSGAASTATTESGVGGRPPTAP
HvMLOC 75135				247	1	
TRAECDM81004				248	1	MGVEIL-SSMVEDSSQYSSGA-STATTESGTTGRALTAL
HVMLOC 56567				249		MGVEIL-SSMVEHSFQYSSGA-SSATAESGAVGTPPRHL
TRAES3BF098300010CFD t						MGVEIL-SSMVEHSFQYSSGV-STATTESGTAGTPPRPL
HvMLOC 63261				251		MASSKPTNPEVD-NDMECSSPESGAEDAV-ESS
_						MASSKEINF EVD ND M ECSS F ESGAEDAV ESS MASGKPTNHGMEDD-NDMEYSSAESGAEDAA-EPS
_						MASGKPINHGMEDD-NDMEISSAESGAEDAA-EPS MASGKPINHGMEDD-NDMEYSSAESGAEDAA-EPS
Bra038346				254	1	
GmLoc732601	-			255		MDGG-CVTDETT-TSSDSLSVLSVLSV
GmL0C/32801 GmLoc100789009				255		LSVPP- MDGG-SVTDETT-TTSNSLSVPANLSPPP-
GmL02100789009 GmLoc100776987	~			256	1	
GmLoc100776987 GmLoc100801107					1	QAKPSSTIMSSEKASPSPPPP
GULOCIUU0UIIU/	зву	тυ	NU	258	T	MDAI-SCMDESTTTESLSISLSPTS-SSEKAKPSSMITSSEKVSLSPPPS

GRMZM2G053008 HvMLOC_57250 Os12g0157000 GmLoc100778733 Bra004501 Bra000434 Bra040478 Bra014415 Bra003482 Bra007646 GlycinemaxLoc100781489 GRMZM2G024948 T01 os02g0683500 HvMLOC 66387 os04g0581400 GRMZM2G102059 T01 Os10g0537100 GRMZM2G142999 T01 GRMZM2G125095 T01 os03q0120900 GRMZM2G098443_T01 GRMZM2G082227_T01 Os11g0156000 GRMZM2G328742 T01 GmLoc100802734 GmLoc100795470 GmLoc100818164 Bra017262 At2g36080 Bra005301 At3g11580 BraLOC103849927 BrassicarapaBra034828 At5g06250 Bra005886 GmLoc102660503 HvMLOC 38822 os01g0693400 HvMLOC44012 HvMLOC_7940 HVMLOC 75135 TRAECDM81004 HvMLOC 56567 TRAES3BF098300010CFD_t1 HvMLOC_63261 TRAES3BF062700040CFD t1 TRAES3BF062600010CFD_t1 Bra038346 GmLoc732601 GmLoc100789009 GmLoc100776987 GmLoc100801107

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					FAFDAVVKRAR	-HEEN
	APLE-RMGS-	-GA	SAVVDAAE	PGAEADSGS	GGRVCGGGGGGAG	GAGG
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9	SLP		VSIAD-	ES	ATSR	SASA
8	SLP		VAITD	ES	VTSR	SASA
8	SLP		VAIAD	ES	VTS	A
	SLP		VAIAD	ES	LTS	
8	SLP		VAIAD	ES	VTS	
						SPV
8 2					VL	
8 2	 SSP					WT TIC
8 2 4	SSP				VL	
8 2 4 4	SSP				VL VD	APPR
8 2 4 4 8	SSP SSP LRLN-RMRS-		snvvldski	NG		APPR
8 2 4 4 8 2	SSP SSP LRLN-RMRS- PS-RVGS-	-GG	SNVVLDSKI SAVVDPDG	NG CCVS	VD	APPR IDSR AESR
8 2 4 8 2 8	SSP SSP LRLN-RMRS- PS-RVGS- LS-LVGS-	-GG -VA -GA	SNVVLDSKI SAVVDPDG(TAVVYPDG(NG CCVS CCVS	VD GE	APPR IDSR AESR AESR

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HvMLOC 57250		~
Os12g0157000	1	
GmLoc100778733	1	MELMQ-EVKG-YSDGREEEEEEEAAEEII
Bra004501		
Bra000434	1	
Bra040478	1	
Bra014415	1	
Bra003482	1	
Bra007646	1	
GlycinemaxLoc100781489	1	MELMQ-QVKGNYSDSREEEE
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os02q0683500	1	
HvMLOC 66387	1	MEFTATSSRFSKGEEEVE
os04g0581400		
GRMZM2G102059 T01		
Os10g0537100		
GRMZM2G142999 T01	1	
GRMZM2G125095 T01	1	
os03q0120900	1	
GRMZM2G098443 T01	1	
GRMZM2G082227 T01	-	
Os11g0156000	1	
GRMZM2G328742 T01	1	
GRH2H2G520742_101 GmLoc100802734	1	
GmLoc100795470	1	
GmLoc100818164		
Bra017262	-	
	-	
At2g36080	-	
Bra005301	-	
At3g11580	1	
BraLOC103849927	-	
BrassicarapaBra034828	-	
At5g06250	-	
Bra005886	-	
GmLoc102660503		AAAQKFKGVVSQQNGNWGAQIYAHQQRIWLGTFKSEREAAMAYDSASIKLRSGECHRNFP
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HvMLOC44012	-	
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HVMLOC_75135		PASSRFKGVVPQPNGRWGSQIYERHARVWLGTFPDQDSAARAYDVASLRYRGRDAATNFP
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TRAES3BF062700040CFD_t1		APSSRFKGVVPQPNGRWGAQIYEKHSRVWLGTFPDEDAAVRAYDVAALRFRGPDAVINHQ
TRAES3BF062600010CFD_t1		APSSRFKGVVPQPNGRWGAQIYEKHSRVWLGTFPDEDAAARAYDVAALRFRGPDAVINHQ
Bra038346		LSSSKYKGVVPQPNGRWGAQIYVKHQRVWLGTFCDEEEAAHSYDIAARKFRGRDAVVNFK
GmLoc732601		LPSSKYKGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAALRFRGPDAVTNFK
GmLoc100789009		LPSSKYKGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAAHRFRGRDAVTNFK
GmLoc100776987		LPSSKYKGVVPQPNGRWGSQIYEKHQRVWLGTFNEEDEAARAYDVAVQRFRGKDAVTNFK
GmLoc100801107	78	LPSSKYKGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAAQRFRGKDAVTNFK

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HvMLOC_57250	1	
Os12g0157000	1	
GmLoc100778733	29	TREESSRLLHQHQEAAGSNFI MNNHHHHQHHHH
Bra004501	1	
Bra000434	1	
Bra040478	1	
Bra014415	1	
Bra003482	1	
Bra007646	1	
GlycinemaxLoc100781489	20	ЕААЕЕЕААЕАА
GRMZM2G024948_T01	19	DASDAS
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GRMZM2G082227 T01	1	
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GRMZM2G328742 T01	1	
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GmLoc100795470	1	
GmLoc100818164	1	STNHYTMDLPEPTLWWPHPH
Bra017262	1	SIMWQQQQ
At2q36080	1	
Bra005301	- 1	SINQYSSDFNYHSLMWQQQQ
At3q11580	1	
BraLOC103849927	1	
BrassicarapaBra034828	1	STLSH
At5q06250	1	ŴQQQQ
Bra005886	1	SVNHYSTDHHQVHHHHTLFLQ
GmLoc102660503	120	WNDQTVQEPQFQSHYSAETVLN-M RDGTYPSKFAT
HvMLOC 38822	1	MRKHIYPDELAQ
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		CAAAEAELAFLAAHSKAEIVD-MRKHTYTDELRQ
HvMLOC_7940		CAAALAELAF LAANSNAEIVD-MERKHTYADELRQCAAALAELAFLTAHSKAEIVD-MERKHTYADELRQ
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TRAECDM81004		
HvMLOC_56567		
TRAES3BF098300010CFD_t1		
HvMLOC_63261		
TRAES3BF062700040CFD_t1		
TRAES3BF062600010CFD_t1		RPTAAEEAGSSSSRSELDPELGFLADHSKAEIVD-MRKHTYDDELRQ
Bra038346		TFLASEDDNGELCFLEAHSKAEIVD-MRKHTADELAQ
GmLoc732601		PPAASDDAESEFLNSHSKFEIVD-MRKHTYDDELQQ
GmLoc100789009		PLAGADDAEAEFLSTHSKSEIVD-MRKHTYDNELQQ
GmLoc100776987		PLSGTDDDDGESEFLNSHSKSEIVD-MORKHTYNDELEQ
GmLoc100801107	138	PLAGADDDDGESEFLNSHSKPEIVD-M RKHT NDELEQ

GRMZM2G053008		AQT KR		KHSDSQN	
HvMLOC_57250					
Os12g0157000	1				
GmLoc100778733		HTT OLDFMDLSLGSSKDEGNLQGSS			
Bra004501		MMMTNLS			
Bra000434	1			L-	
Bra040478	1	MMTNLS			
Bra014415	1	MERKSNDL		ERSENI-	DSÇ
Bra003482	1				
Bra007646	1				
GlycinemaxLoc100781489	25	AIT ESESSRLHQQDI	ASNFGKKLDL	MDLSLGS-SKEE-	EEE
GRMZM2G024948_T01	25	NSM EISFMPPAAASSSSAAASASASAS	STSASACASGS	SSAPFRSASASG-	DAA
os02g0683500	21	AGRÜEIPFMTATAEAAPAPTSSSSSPAHHAAS	SASASASAS-G	SSTPFRSD	dga
HvMLOC_66387	24	ASMEIPFMTPAAATCAAAPPSASAS	SASTPASAS-G	SSPPFRSG	DDA
os04g0581400	37	ASP EIPFMTSAAAAATASSSSPTSV-SPSAT	ASAAA-STSA	SGSPFRSS	dga
GRMZM2G102059_T01	29	ASPEIPFMTAAATADTGAAASSSSPSA-	A-ASSG	PAAAPRSS	dga
Os10g0537100	1	MEFTPIS		-PP-TRVAGGEE-	D
GRMZM2G142999 T01	1	MEFTPAH		-AH-ARVVE	D
GRMZM2G125095 T01	1	MEFRPA		H-ARVFE	D
os03g0120900	1	MEFITPIVR		-PASAAAGGGEV-	QE-
GRMZM2G098443 T01	1	MEFTTPP			
GRMZM2G082227 T01	1	MEFTAPP			
	21	AM			
GRMZM2G328742 T01	22	AM			
GmLoc100802734	20	000AAMWLSNSH		-TPRFNLNDEEEE	EEDDV
GmLoc100795470		HQQQQLTLMDPD			
GmLoc100818164		QQQLTLIDPD			
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At3q11580		ннн			
BraLOC103849927		-HH			
BrassicarapaBra034828		NHH			
At5q06250		HRHTT			
Bra005886		NLHTT			
GmLoc102660503		-FLÄTROTOKGV			
HvMLOC 38822		-HK [®] AFFFAAAS			
os01q0693400		-SKÄTFAASTPS			
HvMLOC44012		-SK AFAASAAL			
HvMLOC 7940		-GLÄRGRGMGAR			
HvMLOC 75135		-GL [®] RGRGMGAR			
TRAECDM81004		-GL®RGRGMGAR			
		-GLÖRGRGMGAR			
HVMLOC_56567					
TRAES3BF098300010CFD_t1		XX.			
HVMLOC_63261		-GL\\\RGHGR			
TRAES3BF062700040CFD_t1					
TRAES3BF062600010CFD_t1					
Bra038346		-SN\RSGANTN			
GmLoc732601					
GmLoc100789009		-STWGGRRR			
GmLoc100776987		-SK SRGFVRRR			
GmLoc100801107	176	-SK SRGVVRRR		-GSAA	

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GRMZM2G053008			CORPTKIALVPSSNAISKTMHRGVVQ
HvMLOC_57250			VRDASWAL
Os12g0157000	-		MAMHAGHAWWGVA
GmLoc100778733			LQQQQQPAE
Bra004501			EAKKPMEEV E H E H E
Bra000434			GAKKPTEEV
Bra040478			QVKKPIEEV <mark>E</mark> H E
Bra014415			BVQE-ASSME
Bra003482			PVEK-ASSMEBH
Bra007646		-	Pleeastsme
GlycinemaxLoc100781489			VVHHAHQVV E
GRMZM2G024948_T01			DADAEAEAV
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HvMLOC_66387			SRSNVAEAV
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Os10g0537100			RGAAAWAVV
GRMZM2G142999_T01			RPRGGVAWV <mark>E</mark> EH
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os03g0120900			SGGRSLAAV
GRMZM2G098443_T01			HHQQQHATV E H
GRMZM2G082227_T01			QQQQATV <mark>E</mark> EH
Os11g0156000			HHY E H
GRMZM2G328742_T01			ҮННООНННҮВ ВНСЕ
GmLoc100802734			ATNNLTQEE
GmLoc100795470			TGGEQEILD
GmLoc100818164			TGGEEEIINN
Bra017262	25		HQNEVVE <mark>B</mark> & <mark>E</mark> A <mark>LF</mark>
At2g36080	26		HQNDVVE <mark>E</mark> EALF
Bra005301	27		HQNDVAE
At3g11580	17		QNDVAIAQ
BraLOC103849927	20		HQNYAVV
BrassicarapaBra034828			
At5g06250			TTATWLHDDL
Bra005886			TAATSLREDQ
GmLoc102660503			LKGDDEEQFCCTQ
HvMLOC_38822			SPAPSAAAAR
os01g0693400			PRSPFAPAAA
HvMLOC44012			SASPPSPAAV
HVMLOC_7940			AQPTPSWA
HvMLOC_75135			AQPTPSWAÖVPLE
TRAECDM81004			AQPTPSWA
HVMLOC_56567	157		
TRAES3BF098300010CFD_t1	157		AQPTPSWA
HVMLOC_63261	146		AQPTPAWA&PFLF
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TRAES3BF062600010CFD_t1			
Bra038346			TTQSHTVSRT [®] D <mark>VLF</mark> ASSGVFDAKA® <mark>D</mark> QLF
GmLoc732601			ASSGVFDAKA DOLE
GmLoc100789009 GmLoc100776987			ASSGAFDAKA®QL5 SISGACVMKA®BQL5
GmLoc100776987 GmLoc100801107	100		SISGACVMKA&DQLF
GWEOCIOGOTIO/	121	AGIAN	CT2GACITVA®

CD1/// COF 2000	215	MGB LALP-FNIQUEEKINWEDHIRKNSVEWGWQMAVCKLFDERPVWPRQSU
GRMZM2G053008	215	WGE%LALP-FNTQV%EKINWEDHIRKNSVEWGWQMAVCKLFDERPVWPRQSU
HvMLOC_57250	30	-FKV #EQSDVQVGQNRI #EAVWGGPIPKLFPELEELRGDGLNAENR
Os12g0157000	20	NHHYRHKT <mark>SDVGK</mark> -NR- [®] KHA [®] YGGGDSG <mark>KG</mark> -
GmLoc100778733	132	
Bra004501	37	KVVTPSDVGK-LNRLVIPKOYAE YFPLDSSTNEKGL
Bra000434	34	
Bra040478	29	- KVVTPSDVGK-LNRLVIPKQH AE YFPLDSSSNEKGL
Bra014415 Bra003482	42 22	
Bra003482 Bra007646	22	- EKVVTPSDVGK-INRLVIPKOHAEWYFPLDNN-SDSSKGL
GlvcinemaxLoc100781489	23 89	
GRMZM2G024948 T01	102	
os02q0683500	97	
HvMLOC 66387	95	NEW VIEW DAAL
os04q0581400	111	
GRMZM2G102059 T01	95	
Os10q0537100	36	
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GRMZM2G142999_T01	33 31	-EKVVTPSDVGK-INRLVIPKOHAE#YFPALDASSAAA-AAAAAAGGGKGI -EKVVTPSDVGK-INRLVIPKOHAE#YFPALDASAAAA-SASASAGGGKAGI
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2	41	
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GmLoc100802734	74	
GmLoc100795470	74	-EKP#DPSDVGK-LNRLVIPKQHAEKYFPLSG-DSGGSECKGL -EKP#TPSDVGK-INRLVIPKOHAEKYFPLSGGDSGSSECKGL
GmLoc100818164 Bra017262	38	
	\$	
At2g36080	39	- EKP TPSDVGK-LNRLVIPKOH AE YFPLAAAAADAVEKGL
Bra005301	40	- EKPÄTPSDVGK-LNRLVIPKQHAEÄYFPLAAAAADAMEKGL
At3g11580 BroLoc103840027	30 33	- EK S% TPSDVGK-LNRLVIPKQHAEKYFPL NNNNNNGGSGDDVATTE KG - EK S% TPSDVGK-LNRLVIPKQHAEKHFPL NNAGDDVAAAETTE KG
BraLOC103849927	8	
BrassicarapaBra034828 At5q06250	25 47	
Bra005886	50	- ER S% TPSDVGK-LNRLVIPKQHAEKYFPL NAVLVSSA-AADTSSSE KG - ER S% TPSDVGK-LNRLVIPKQHAEKYFPL NTIISNNAEE KG
GmLoc102660503	186	
HvMLOC 38822	50	>−QKE∰TPSDVGK-INRLVIPKKHAVSYFPYVGGSADESGSVD∰ ∭KTVTPSDVGK-INRLVIPKOHAEKHFPIQLPSASAAVPGECKG∭
os01q0693400	217	
HvMLOC44012	51	
HVMLOC 7940	176	SURVISION AND SUBJECT
HVMLOC 75135	175	EKAVTPSDVGA HINKEV FIGH AMARTEL TITTIGKS
TRAECDM81004	171	
HVMLOC 56567	170	
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HvMLOC_63261	159	
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Bra038346	190	
GmLoc732601	173	
GmLoc100789009	179	
GmLoc100776987	206	
GmLoc100801107	211	

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Os12g0157000	49	S-DSGKWR-RYSYWTSSYWTKGWSRWKKRDAGDWKRGG
GmLoc100778733	169	LNFEDRNGKLWRFRYSYWNSSQSY-VWTKGWSRFVKEKWLDAGDWVSFQRGV
Bra004501	74	LNFEDLAGKSWRFRYSYWNSSQSY-VWTKGWSRFVKWKUDAGDWVSFQRCV
Bra000434	71	LNFEDLTGKSWRFRYSYWNSSQSY-VWTKGWSRFVKWKKUDAGDWVSFLRCV
Bra040478	66	LNFEDLTGKSWRFRYSYWNSSQSY-VWTKGWSRFVKWKWLDAGDWVSFQRCV
Bra014415	81	LNFEDRIGILWSFRYSYWNSSQSY-VXTKGWSRFVKKKLDAGDXVSFHRGS
Bra003482	60	LNFEDRTGNSWRFRYSYWNSSQSY <mark>V</mark> WTKGWSRFVK & K&LDAGD&VSFQRDP
Bra007646	61	LDFEDRTGSSWRFRYSYWNSSQSY-VWTKGWSRFVKWKWLDAGDWVSFQRDP
GlycinemaxLoc100781489	126	LINFEDRNGKVWRFRYSYWNSSQSY-VWTKGWSRFVKEK#LDAGDWVSFQRGL
GRMZM2G024948 T01	139	LISFEDSAGKHWRFRYSYWNSSOSY-VÄTKGWSRFVKEKÄLVAGDTVSESRAA
os02q0683500	134	LINFEDRAGKPWRFRYSYWNSSQSY-VÄTKGWSRFVKEKÄLDAGDTVSFSRGI
HvMLOC 66387	132	LINFEDSACKPWRFRYSYWNSSOSY-WITKGWSRFVKEKILDAGDTWSESRGA
os04q0581400	148	LISFEDRTGKLWRFRYSYWNSSOSY-VETKGWSRFVKEKELDAGDTVSFCRGA
GRMZM2G102059 T01	132	LISFEDRAGKLWRFRYSYWNSSQSY-VÄTKGWSRFVKEKÄLDAGDTWSFCRGA
Os10q0537100	86	LSFEDRTGKAWRFRYSYWNSSOSY-VETKGWSRFVKEK
GRMZM2G142999 T01	82	LSFEDRAGKAWRFRYSYWNSSOSY-VWIKGWSRFVKEKWLGAGDTVLFARGA
GRMZM2G125095 T01	80	LSFEDRACKAWRFRYSYWNSSOSY-WITKGWSRFVKEK
os03q0120900	75	LISFEDRTGKPWRFRYSYWNSSOSY-VÄTKGWSRFVKEKÄLDAGDTVSFGRGV
GRMZM2G098443 T01	78	LISFEDRTGKPWRFRYSYWNSSQSY-VÄTKGWSRFVKEKÄLDAGDTVSFGRGI
GRMZM2G082227 T01	76	LISFEDRAGKPWRFRYSYWNSSOSY-VETKGWSRFVKEK
	77	ISFEDEAGAPWRFRYSYWISSOSY-VLTKGWSRWVKEKWLDAGDWVHFERVR
GRMZM2G328742 T01	82	LCFEDDDDDEAAAANKPWRFRYSYWTSSOSY-VLTKGWSRWVKEKOLDAGDWVRFORMR
GmLoc100802734	105	ISEEDESCKCWRFRYSYWNSSOSY-VLTKGWSEWVKWKWLHAGDWVLEHRHR
GmLoc100795470	114	LSFEDESGKCWRFRYSYWNSSOSY-VLTKGWSRWVKWKWLDAGDWVLFERHR
GmLoc100818164	117	LISFEDESGKCWRFRYSYWNSSQSY-VLTKGWSFWVKWKWLDAGDWVLFQRHR
Bra017262	78	LCFEDEECKPWRFRYSYWNSSOSY-VLTKGWSRWVKEKOLDAGDWVLFHRHR
At2g36080	79	LCFEDEECKPWRFRYSYWNSSOSY-VLTKGWSFWVKEKWLDAGDWVLFHRHR
Bra005301	80	LCFEDEECKPWRFRYSYWNSSQSY-VLTKGWSFWVKEKQLDAGDWLFHRHR
At3g11580	78	LSFEDESCKCW#FRYSYWNSSQSY <mark>VLTKGWSF#VK#K#LDAGD#VFFQ</mark> RHR
BraLOC103849927	78	LTFEDESGKCW#FRYSYWNSSQSY-VLTKGWSF#VK#K#LHAGD#VFFQRHR
BrassicarapaBra034828	71	LSFEDESGKSW#FRYSYWNSSQSY-VLTKGWSR#VK#K#LNAGD#VLFQRHR
At5g06250	94	LSFEDESGKSWRFRYSYWNSSQSY <mark>-</mark> VLTKGWSRFVK % K <mark>Q</mark> LDPGD&VFFQRHR
Bra005886	91	LSFEDESGKCWRFRYSYWNSSQSY <mark>-</mark> VLTKGWSFWVKWKQLDFWDWVFFQRQR
GmLoc102660503	227	XAVFYDKLMÜLWÜFRYCYWKSSQSY-VFTÜGWNREVKÜKÜLKAKDÜÜAFFTWG
HvMLOC_38822	94	LNF#DATGKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKGLHAGDAVEFYRAA
os01g0693400	257	LNFEDAAGKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKGLHAGDWVGFYRSA
HvMLOC44012	91	LNFEDDAGKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKGLGAGDWVGFYRSA
HvMLOC_7940	218	LNFEDGE <mark>GK</mark> VWRFRYSYWNSS <u>O</u> SY <mark>-VLTKGWSRFV[®]EK</mark> GLG <mark>AGD</mark> S [®] VFSCSA
HvMLOC_75135	217	LNFEDGECKVWRFRYSYWNSSOSY-VLTKGWSSFVWEKGLGAGDSWVFSSSA
TRAECDM81004	213	LNFEDGE <mark>CK</mark> VWRFRYSYWNSSOSY-VLTKGWSRFV <u>W</u> EK <mark>GLAAGD</mark> SWIFSCSA
H∨MLOC_56567	212	LNFEDGQ <mark>GK</mark> V <mark>WRFRYSYWNSSQSY-VLTKGWSRFV EK</mark> GLGAGDS MFSCSA
TRAES3BF098300010CFD_t1	212	INFEDGE <mark>GK</mark> V <mark>WRFRYSYWNSSQSY</mark> -VLTKGWSRFV <u></u> @EK <mark>GLGAGD</mark> S%LFSCSL
HVMLOC_63261	200	LNFEDGQ GK V <mark>WRFRYSYWNSSQSY</mark> -VLTKGWSRFVQEK <mark>GLCAGD</mark> TVTFSRSA
TRAES3BF062700040CFD_t1	213	LNFEDGE <mark>GK</mark> V <mark>WRFRYSYWNSSOSY</mark> -VLTKGWSRFVKETG <mark>LRAGD</mark> TVAFYRSA
TRAES3BF062600010CFD_t1	213	LNFEDGE <mark>GK</mark> V <mark>WRFRYSYWNSSQSY</mark> -VLTKGWSRFVKE <mark>TGURAGD</mark> TVAFYRSA
Bra038346		MEEDVT <mark>GKVWRFRYSYWNSSQSY</mark> -VLTKGWSREVKEK <mark>NLRAGDWTFER</mark> ST
GmLoc732601		LINFEDVGCKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKNLRAGDAVQEF#ST
GmLoc100789009	227	LINFEDVGGKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKNLRAGDAVQEFSST
GmLoc100776987	251	LINFEDVGGKVWRFRYSYWNSSQSY-VLTKGWSRFVKEKNLKAGDTVCEQRST
GmLoc100801107	263	LINEEDGQGRVWRFRISYWNSSQY-VLIKGWSREVQERGLORDIVIESRSA INFEDGGRVWRFRYSYWNSSQSY-VLIKGWSREVKEIGLRAGDIVAEYRSA INFEDGGRVWRFRYSYWNSSQSY-VLIKGWSREVKERNLRAGDIVAEYRSA INFEDVGRVWRFRYSYWNSSQSY-VLIKGWSREVKERNLRAGDAVQEF SINFEDVGRVWRFRYSYWNSSQSY-VLIKGWSREVKERNLRAGDAVQEF SINFEDVGRVWRFRYSYWNSSQSY-VLIKGWSREVKERNLRAGDAVQEF SINFEDVGRVWRFRYSYWNSSQSY-VLIKGWSREVKERNLRAGDIVCEQRSI INFEDV
		3

GRMZM2G053008	314	§-−−−ESQ _∭ Y(<u>D</u> R ID MPPE	RYLLRLKN	
H√MLOC_57250	132	ATAA		₿VAP-AIPPGAI	VKAAGF
Os12g0157000	90	AA	-DRGC <mark>ÖRR</mark> GS	Saavrvta	
GmLoc100778733	221	GE-LY-RH <mark>RL</mark>	- <mark>@IDW</mark> WRRPD	NHHHHHHGPDHSTTLFT	PFLIPNQPHHLMSIRWGAT-G-
Bra004501	126	GD-SGRDS <mark>RL</mark>	-FIDW <mark></mark> RRPK	PDHPTSIAHFA	AGSMF-P-
Bra000434	123	§d−tgrds <mark>rl</mark>	-FIDW <mark></mark> RRPK	PDYTTSTSHFP	AGAMF-P-
Bra040478	118	GDSRL	-FIDW RRPK	PDYPTSTAHFA	AGAMF-P-
Bra014415	133	CNKD	FIDW	PDHQV	VGAMF-P-
Bra003482	112	§GNKD∰ L	FIDW <mark></mark> RRPK	PDHHHQF	AGAMF-P-
Bra007646	113	cnkd <u></u>	IDWRRPK	₿PDHHQF	AGAMF-P-
GlycinemaxLoc100781489	178	GDLYRH <mark>RL</mark>	- <mark>WIDW</mark> RRPD	🕻ананр	PHHHDPLFLPSI
GRMZM2G024948_T01	191	AEDARH <mark>RL</mark>	-FIDW RRVD	RGPLRFS	GLALPMPLP-S-
os02g0683500	186	GD-EAARH <mark>RL</mark>	FIDW RRAD	§RDPLRLPR	GLPLPMPLT
H∨MLOC 66387	184	GEAARH <mark>RL</mark>	FIDW RRAD	RDPLRLPR	LPLPMPLT
os04g0581400	200	AEATRDRL	FIDW RRAD	RDPHRFQR	T-
GRMZM2G102059_T01	184	GDTARDRL	FIDW RRAD	RDPHRMPR	LPLPMAPV-A-
Os10g0537100	138	GDAARG <mark>RL</mark>	FID	SAGSFMFPPTAA	PPSHSHHHHQRHHPPLPS-
GRMZM2G142999 T01	134	GGARGRF	FID	§laf−lqptla−−−−−	SAQRLLPLPS-
GRMZM2G125095 T01	132	GATRGRF	FID	LAF-LQPPLA	SAQRLLPLPS-
os03g0120900	127	GEAARGRI	FIDW	VAALQ	PPTHRFAHHLPSS-
GRMZM2G098443 T01	130	SEAARDRI	FIDW	§PVVHH	QYH-HRLPLPSAV-
GRMZM2G082227 T01	128	GEAARG <mark>RL</mark>	FIDW	8PVVHH	QYHHHRLPLPSAV-
Os11g0156000	129	GS-FGVGDRL	FIGC [©] RRGD	SAAAQTPAPPPAV	RVAP
GRMZM2G328742 T01	141	GFGMPDRL	FISHSRRGE	TATAATTVPPAAAA	VRVVVAPAQSAGA
GmLoc100802734	157	SLPQRF	-FISCSRRQP	PVPAHVSTTR	
GmLoc100795470	166	VDAQRL	-FIGW [®] RRRQ	DAALPPAHVSSRK	SGGGDGN
GmLoc100818164	169	ADAQRL	FIGWERR	DALPPPAHVSSRK	SGGD-GN
Bra017262	130	ADGGRF	-FIGW [©] RRGD	SSSSDSYRNLQ	
At2q36080	131	SDGGRF		SSSSDSYRHVQ	
Bra005301	132	VDGGRF		SSSSDSYRHLQ	
At3q11580	130	FDLHRL	FIGWÖRRGE	SSSPAVSVVSQEA	L
BraLOC103849927	130	FDLH		SSPTAVSVVSQEA	RR
BrassicarapaBra034828	123	FDIHRL		SSSSAVSAVTQDP	R
At5q06250	146			SSSSVAATNSAVN	T
Bra005886	143	DSRRL		SSSAANTTSY	s
GmLoc102660503	279	8		EEDSKGDTKQVL-	GNOLOLA
HvMLOC 38822	146	бGNNQ <mark>II</mark>		TTTTTSV	NSEA
os01q0693400	309	A-SAGDDG	302	GA-AL	ASPA
HvMLOC44012		AGRTGEDS			ADPV
H∨MLOC 7940				TSCPADDRGAATA	SPPVS
H√MLOC 75135	269	YGQEKQ I	-FINC	NGGKTAL	PLPVV
TRAECDM81004		¥GQEKQ <mark>I</mark>		NSGKSAS	PLPVV
H∨MLOC 56567	264	YGOEKOF	-FIDC.NTT	NGGKSAS	PLOVM
TRAES3BF098300010CFD t1	264	YEQEKQF	-FIDC NTS	NGGKSAS	PLPVG
HvMLOC 63261 —		VVMNDTDEQ 1	FID	DEAAD	VAT
TRAES3BF062700040CFD t1		YG-NDTEDQ L			AI
TRAES3BF062600010CFD t1		YG-NDTEDO		DDAAD	
Bra038346		GSDRQ I		SKN	
GmLoc732601	273	8			FVPI
GmLoc100789009	279	GLDRQ I			FIPV
GmLoc100776987	303	GPDRQ I			F
GmLoc100801107	315	GPDKQ I			FGPV

GRMZM2G053008	338	SESRKWADMCKLETMPSQSFIYLQLYELKDDFIQAEIRKPSYQSVCSRSTGWFS
HvMLOC 57250		
Os12g0157000	109	NGGWSMCYSTSGSSYDTSNGGWSMCYSTSGSSYDTS
GmLoc100778733	275	RLYSLPSPTPPRHHEHLNYNNNAMYHMYH
Bra004501	162	RFYSFPTATSYNLYNYQQP
Bra000434		RFYSFOTATTSTSYNPYNHOOP
Bra040478		RFYSFPTATTSTCYDLYNHQPP
Bra014415		RFYSYPYPOIOASYER
Bra003482		RFYSFSHPQN
Bra007646		RFYSFPHPOMPTSFES
GlycinemaxLoc100781489		RLYSLPPTMPPRYHHDHHFHHHLNYNNLFTT
GRMZM2G024948 T01		SHYGGPHHYSPWGFGGGGGGGGGGFFM
os02a0683500		GGGFFW
2		SHIAPWGIGGGGGFFV
HvMLOC_66387		
os04g0581400		SPYGPWGGGA-GASSCRPRR
GRMZM2G102059_T01		SPYGPWGGGG-GGGAGGFFM
Os10g0537100		VPLCPWRDYTTAYGGGYGY
GRMZM2G142999_T01		VPICPWQDYG
GRMZM2G125095_T01		VPICPWQGYG
os03g0120900		IPFAPWAHHHGHGAAAAA-AAAAGARFLL
GRMZM2G098443_T01	165	VPYAPWAAHAHHHHYPADGHTEPVTPCLCATLVATEM
GRMZM2G082227_T01	164	VPYAPWAAAAHAHHHHYPAAGVGAARTTTTTTTTVLHHL
Os11g0156000	164	-AAQNAGEQQPWSPMCYSTSGGGSY
GRMZM2G328742_T01	186	-DHQQQQQPSPWSPMCYSTSGSYSY
GmLoc100802734	183	SSASFYSAHPPY
GmLoc100795470	202	SNKNEGWTRGFYSAHHPY
GmLoc100818164	204	SSKNEGDVGVGWTRGFYPAHHPY
Bra017262	157	
At2q36080	158	
Bra005301	159	
At3q11580	160	VNTTAYWSGLTTPYY
BraLOC103849927		VNTTAYWSGLTTPYTPY
BrassicarapaBra034828		ANTTAYWNGLTTPY
At5q06250		SSMGALSY
Bra005886		SSMT
GmLoc102660503		55 MI
HvMLOC 38822		
-		
os01g0693400		
HvMLOC44012		
HVMLOC_7940		
HVMLOC_75135		
TRAECDM81004		
HvMLOC_56567		
TRAES3BF098300010CFD_t1		
HvMLOC_63261	280	
TRAES3BF062700040CFD_t1	292	
TRAES3BF062600010CFD_t1	292	
Bra038346	298	
GmLoc732601	301	
GmLoc100789009	307	
GmLoc100776987	325	
GmLoc100801107	341	

GRMZM2G053008	392	KPMIKTL-RLQVSIR		LLSLLHNE
HvMLOC_57250	107			ANSYAYHRSV
Os12g0157000 GmLoc100778733				
				TTSGSAGSVFYHRS-TPPISMPLA FGYGYLV
Bra004501				
Bra000434				FGYGYVVRS-V
Bra040478			-	FGYGYFVRS-V
Bra014415			-	IGIGYYVRS-M
Bra003482				LGIGYYVSS-M
Bra007646			-	LGIGYY
GlycinemaxLoc100781489				QNSGSGSLYYLRSSMSMGG
GRMZM2G024948_T01				YPAPTVGRQLLFFGSARMPPHHAPPP
os02g0683500				-AAMGRQVLLFGSAR-IPPQAP
H√MLOC_66387			-	YPTMGRQVILFGSAARMPPHGPAP
os04g0581400				STPLCRRGSSSSSAPQ
GRMZM2G102059_T01				AAPARQLLFFGSAGMPPRASMPQ
Os10g0537100	204		GYGG	GSTPASSRHVLFL
GRMZM2G142999_T01	180		,	ASAPAPNRHVLFL
GRMZM2G125095_T01	178			ASAPAPSRHVLFL
os03g0120900	191	PPSST-PIYDHHRRH	AHAVGYDAYA	AATSRQVLFY
GRMZM2G098443 T01	202	RASSS-QLSLTRSNLS-	-RPPQPRIARVDGAQPR	PSSSPRQPQSLWC
GRMZM2G082227 T01	203	PPSPS-PLYLDTRRR	HVGYDAY	GAGTRQLLFY
Os11q0156000	188	PT	SPANSY	AY
GRMZM2G328742 T01	210	PT	SSPANSQH	AYH
GmLoc100802734				FF
GmLoc100795470	220	PT	HH	LHH
GmLoc100818164	227	PT	HH	
Bra017262	157			
At2q36080	158			
Bra005301	159			
At3q11580	174	RO	VH	AST
BraLOC103849927		-		AST
BrassicarapaBra034828		-		 AST
At5q06250				ATS
Bra005886		-		PYS
GmLoc102660503				
HvMLOC 38822				
os01g0693400	1,0			
HvML0C44012				
HVMLOC 7940				
-				
HVMLOC_75135	200			
TRAECDM81004				
HvMLOC_56567	200			
TRAES3BF098300010CFD_t1				
HVMLOC_63261	280			
TRAES3BF062700040CFD_t1				
TRAES3BF062600010CFD_t1				
Bra038346				
GmLoc732601	001			
GmLoc100789009				
GmLoc100776987				
GmLoc100801107	341			

GRMZM2G053008	414	EAKNLLRNAHELIERSKKQEALSRSELSIEYNDADQVSAAHTGT
HvMLOC_57250		
Os12g0157000	137	DDHSDHAGSRA
GmLoc100778733	351	DHQTLNTRQQQQQQQQQEGAGNVSLSPMIIDSVPVAHHLHHQQHHGGKSSG
Bra004501	204	DQRAVVADPLVIESVPVMMHGG-A
Bra000434	206	DQRAVVADPLVIESVPVMMHGG-A
Bra040478	197	DQRAVVADPLVIESVPVMMRGG-A
Bra014415	196	ERYDPTAVIESVPVIMQRR-A
Bra003482	169	ERNDPTAVIESVPLIMQRRAA
Bra007646	175	PTAVIESVPVIMQRREA
GlycinemaxLoc100781489	284	GDQNLQGRGSNIVPMIIDSVPVNVAHHNNNRHGNGG
GRMZM2G024948 T01		QPRPFSLPLHHYTVQP-SAAGVTAASRPVLLDSVPVIESP
os02q0683500	283	-LLARAPSPLHHHYTLQP-SGDGVRAAGSPVVLDSVPVIESP
H∨MLOC 66387		LLVPRPPPPLHFTVQQQGSDAGGSVTAGSPVVLDSVPVIESP
os04g0581400		GRGFISTRPCHRRRHLRLLT-NSTLRCTTRAP
GRMZM2G102059 T01		QQQPPPPPPHPPLHSIMLVQ-PSPAPPTASVPMLLDSVPLVNSP
Os10q0537100		RPQVPAAVVLKSVPVHVAATSAVQ
GRMZM2G142999 T01		RPQVPAAVVLKSVPVHVAASAV
GRMZM2G125095 T01		RPQVPAAVVLTSVPVRVAASAV
os03g0120900		RLPPQQQHHPAVVLESVPVRMTAGHAEP
GRMZM2G098443 T01		RSCDV
GRMZM2G098443_101 GRMZM2G082227 T01		RPHQQPSTTVMLDSVPVRLPPTPGQHA-EP
GRM2M2G062227_101 Os11g0156000		RRAADHDHPRD
GRMZM2G328742 T01		RHSADHDHQSD
GRM2M2G328742_101 GmLoc100802734		
		PFPYQPHSLHAPGGGSQGQNE
GmLoc100795470 GmLoc100818164		
		QQDDSLHAVRGSQGQNQ
Bra017262		QYYPHAGAQA
At2g36080		QYYPHAG-AQA
Bra005301		QYYPHAGVQA
At3g11580		QEYSHYG-AVVDHA
BraLOC103849927		SSYPN-IHQEYSHYG-AVA
BrassicarapaBra034828		QEYSHYGPVA
At5g06250		SEYSHYG-AAVATA
Bra005886		SEYSHYG-AAVATA
GmLoc102660503		GSEEGED
HvMLOC_38822		
os01g0693400		
HvMLOC44012		
H√MLOC_7940		
HVMLOC_75135		
TRAECDM81004		
H√MLOC_56567		
TRAES3BF098300010CFD_t1	293	
HvMLOC_63261		
TRAES3BF062700040CFD_t1	292	
TRAES3BF062600010CFD_t1	292	
Bra038346	298	
GmLoc732601		
GmLoc100789009	307	
GmLoc100776987	325	
GmLoc100801107	341	

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GRMZM2G053008	458		edqv <mark>g</mark> p <mark>n</mark> nsds	DVDDEEEEELEGY
HvMLOC_57250				
Os12g0157000	148	DAKSSSAAS	S <mark>R</mark> RR <mark>GVN</mark> -@CG	DATAMYGYMHHS
GmLoc100778733	402	PSSTSTSPSTAG	RLFGVN CA	STSEDPKCFSLLSS
Bra004501	227	QVSQAVVGTAG	RLFGVD	EESSSSGGSLPR-
Bra000434	229	RVNQAAVGTAG	RLFGVD CG	SGGTNSTEEESSSSGGSLPR-
Bra040478	220	RVSQEVVGTAG	RLFGVD	EESSSSGGSLPRA
Bra014415	216	HVATMASSRGE	RLFGVD CV	GGRGGGGGSVNSTEEESSTSGGSISRG
Bra003482	190	HVAAIPSSRGE	RLFGVD.CG	GGG-GSVNSTEEESSSSGGGG
Bra007646	192	QVANMASSRGE	RLFGVD CG	GGGGGGSVNSTEEESSSSGGSMSRG
GlycinemaxLoc100781489	320	ITSGGTNCSG	RLFGVN	SAEDSKELSSGSAAHVTTAASSSSLH
GRMZM2G024948_T01	342	TTAA-	VRLFGVN NN	DGGGEASHQGDALSLQMP
os02g0683500	323	TTAA-	VRLFGVN NP	AGGGGGAAAGESSNHGNALSLQTP
HvMLOC_66387	326	TTATK	VRLFGVN	HPGDGGGESSNYGSALPLQMPAS
os04g0581400				
GRMZM2G102059_T01	329	TAAS-	VRLFGVN	PGTSAESSQDANAL-SLRTP
Os10g0537100	245	EAATTTRP	VRLFGVN	AMDDDDDIAGA
GRMZM2G142999_T01	215	E-ATMS	VRLFGVN	DAEDSATVPP
GRMZM2G125095 T01	213	EEATRS	VRLFGVN	DAEDGATAT
os03g0120900	254	PSAPS	VRLFGVN	SEQDHAGVVGK
GRMZM2G098443 T01	259	P		
GRMZM2G082227 T01	263	PPAVASSAS	VRLFGVN CA	AAGSEEENVGG
Os11g0156000	218	TDSPSFSAGSAPS	RLFGVN	EPEADTTAAATMYGYMHQQ
GRMZM2G328742 T01	244	RDNRSCSAASAPPPPS	RLFGVN	GPEPETPTAMYGYMHQS
GmLoc100802734	221	T-TPGGNSSSSGSG	RLFGVN	DNHNDSQNSTPECSYTHLYHH
GmLoc100795470	251	RMRPVGNNSSSSSSS		EH-DDSGPSTPQCSYNSNNMLPS
GmLoc100818164	256	RTRPVGNSSSSSSSS	RLFGVN	EH-DDSGPSTPQCSYNTNNILPS
Bra017262	172	VENQRGNS	RLFGVN	DS-DWSEPSTPDGFTTCPT
At2g36080	173	VESQRGNS	No	DS-DWSEPSTPDGSNTYTT
Bra005301	174	VESQRGNS	RLFGVN	DS-DLPDPSTPDGSTICPT
At3g11580	200	-QSIPPVVAGSS	VRLFGVN	DA-VEPPP
BraLOC103849927	198	EIPTVVTGSS	VRLFGVN	DV-VETPP
BrassicarapaBra034828	192	etptvaagss	VRLFGVN	DV-VEPPP
At5g06250	212	-AETHSTPSSSVVGSS	VRLFGVN	DE-NDGDDSVAVATTVES
Bra005886	200	-TETHFIPSSSAVGSS	VRLFGVN	DE-DEGDDSVATAAAAECP
GmLoc102660503	325	EDANIGKDFNAQ	RLFGVC T	
HvMLOC_38822	173	APSPAPVT	VRLFGV <mark>D</mark> ®LIA	AARHAHEHEDYGMAKTNKRTMEAS
os01g0693400	336	DQPAPSPV	VRLFGVDÖLTA	APVEQMAGCKRARDL
HvMLOC44012	172	DQSSAPVQ	VRLFGVD	EQ-GMPGGCKRARDL
H∨MLOC 7940	305	EPTKGEQV	VRLFGV <mark>D</mark> &AGE	RGRAAPV
H√MLOC_75135	298	ETAKGEQD	V &LFGV D&AGV	RVRAATGG
TRAECDM81004	294	ETAKGEQV	VRLFGV <mark>D</mark> &AGV	RGRAATAA
H√MLOC 56567	293	EIAKAEQV	VRLFGVDÄAGV	RERAATAA
TRAES3BF098300010CFD t1	293	VTTKGEQV	VRLFGV D <u></u> SGV	RGRAATATAA
HvMLOC 63261	280	ADENEAGHVA	V ELFGV DEGWA	MAGSSGG
TRAES3BF062700040CFD_t1	292	SDENETGHV	V ELFGVD®AGG	MAGSSGG
TRAES3BF062600010CFD t1		SDENETGHVA		
Bra038346	298	PVQVV	VRLFGV <mark>D</mark> @FNV	SAKPSNVVDACGGK
GmLoc732601	301-	GPVVEPVQ-M	RLFGVN LKLP	PGSDGVG
GmLoc100789009	307	GPVVEPVQ-I	VRLFGV <mark>D</mark> ©LKL	VPGSDGIGVGCD-G
GmLoc100776987	325	GPVVEPIQ-N	VRLFGVN	GSDSIA-NN-NNASGCCN-G
GmLoc100801107	341	GPVVEPIQ-N	VRLFGVN	GSDTIVGNN-NNASGCCN-G

GRMZM2G053008	484	PMADDIHEFTLGDSYAFGEGFSNG
HVMLOC 57250	404	
Os12g0157000	181	YAAVSTVNY [©] SV
GmLoc100778733		S-SMANSNSQPPLQLLREDTLSSSSARFGDQRGVGEPSMLFD [®] DPSLQ
Bra004501		G-DASPSSS>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
Bra000434		G-GASPSSS QLRLGNSSEDDHLFKKGKSSLPFN DQ
Bra040478	255	GGG-GASSSSS QLRLGSSCEDDHFSKKGKSSLPFD DQ
Bra014415		GVSMAGVGSPLQLRLVSSDGDDQSLVARGAARVDEDHHLFT-KKGKSSLSFD [®] DK
Bra003482		GVSMASVGS%LQLRLVSSDDESLVAMEAASVDEDHHLFT-KKGKSSLSFD%DRK
Bra007646		GVSMAGVGSÄLQLRLVSSDDESLVAMEGATVDEDHHLFTTKKGKSSLSFDÄDI
GlycinemaxLoc100781489		HQR-LRVPVPVPLEDPLSSSAAAAARFGDHKGASTGTSLLFDÖDPSLQ
GRMZM2G024948 T01		GWQ-QRTPTLR [®] LELPRHGGESSA-ASSPSSSSSSKREARSALDLD [®]
os02q0683500		AWM-RRDPTLRÜLELPPHHHHGAESSAASSPSSSSSSKRDAHSALDLDÜ
HVMLOC 66387		AWR-PRDHTLR [®] LEFPSHGAEASSPSSSSSSKREAHSGLDLD [®]
os04g0581400	000	
GRMZM2G102059 T01	366	GWQ-RPGP-LRFESPQRGAESSAASSPSSSSSSKREAHSSLDLD
Os10g0537100		-AS-RTAA-SSÄLQLPSPSSSTSSSTAGKKMCSLDLGÄ
GRMZM2G142999 T01		-RG-RAAS-TT [®] LQLPSPSSSTSSSTAGKDVCCLDLG [®]
GRMZM2G125095 T01		-RTP-ST [®] LQLPSPSSSTSSSTGGKDVRSLDLG [®]
os03q0120900		TAPPPLPSPP-SSSSSSSGKARCSLNLD
GRMZM2G098443 T01	201	
GRMZM2G082227 T01	298	-WR-TSAPPTQQAS-SSSSYSSGKARCSLNLD
Os11q0156000		SSYAAMSAVPSYWGNSS
GRMZM2G328742 T01		PYAPYQHDEEI
GmLoc100802734		
GmLoc100795470		TOGTDH-SHHNF [®] OO-OPSNSNPSPHHM [®] HHOPY
GmLoc100818164		TQGTDIHSHLNF [®] QQQQTSNSKPPPHHM®RHQPY
Bra017262		NHDQFP:
At2q36080		NHDOFHF [®] POOOHYPPPYYMD [®] SFTGD
Bra005301		
At3q11580		
BraLOC103849927		
BrassicarapaBra034828		
At5q06250		PDGYYGQN
Bra005886		
GmLoc102660503		~ ~ *****
HvMLOC 38822	219	V-A-APTPAHA
os01g0693400		A-A-TTPPQAAA KKQCIELALV
HvMLOC44012		V-K-PP-PPKVAÄKKOCIELALAELALA
H∨MLOC 7940		EQE:::::::::::::::::::::::::::::::
H√MLOC 75135		ELGPPE KRQSVNYI
TRAECDM81004		EQGPPE LKRQCVSPA
HvMLOC 56567	322	EOGPOGWÖKROCMSPA
TRAES3BF098300010CFD t1	324	EQGLQE KRQCVSPA
HvMLOC 63261 -		
TRAES3BF062700040CFD t1		
TRAES3BF062600010CFD t1		
Bra038346	329	RSRDVD ALRCS KKH KKH
GmLoc732601		KRKEME AFECCKKLKKL
GmLoc100789009		KRKEME
GmLoc100776987		KRREME
GmLoc100801107		KRREME

GRMZM2G053008 HvMLOC_57250	511	YLEEVLRSLPLQ	EDGQKKL-CDAPINADASD
Os12g0157000			
GmLoc100778733	489	YRQ	
Bra004501			
Bra000434			
Bra040478			
Bra014415			
Bra003482			
Bra007646			
GlycinemaxLoc100781489	417	YHRH	
GRMZM2G024948_T01			
os02g0683500			
H∨MLOC_66387			
os04g0581400			
GRMZM2G102059_T01			
Os10g0537100			
GRMZM2G142999_T01			
GRMZM2G125095_T01			
os03g0120900			
GRMZM2G098443_T01			
GRMZM2G082227_T01			
Os11g0156000			
GRMZM2G328742_T01			
GmLoc100802734			
GmLoc100795470		YY	
GmLoc100818164			
Bra017262			QG
At2g36080			
Bra005301		-	QG
At3g11580			RG
BraLOC103849927		-	RR
BrassicarapaBra034828	249	AMEQVGDG	RG
At5g06250	283		
Bra005886			
GmLoc102660503			
HvMLOC_38822	248		EGVQAAGSTFAL
os01g0693400			
HvMLOC44012			
HvMLOC_7940			L-GAFVL
HvMLOC_75135			TI-GPLMLN
TRAECDM81004			L-GAFVL
HvMLOC_56567			L-GDFAL
TRAES3BF098300010CFD_t1	345		L-GAFAL
HVMLOC_63261			
TRAES3BF062700040CFD_t1			
TRAES3BF062600010CFD_t1			
Bra038346			II-NAL
GmLoc732601			VI-GAL
GmLoc100789009			VI-GAL
GmLoc100776987			II-GAL
GmLoc100801107	396	K	II-GAL

Figure 14

PCR round 1		20 bp	
Template	[1767]		A scattold TTTTT
	PrimerF1	PrimerR1	
		Product1	
		PrimerF2	PrimerR2
PCR round 2	1	ġ	reduci2
Template	***	Product1 + Product2	

	PrimerF1		PrimerR2
		Final produc	t

gRNA sequence (SEQ ID NO: 146)

os11g01560000

A-R1 GGACTGGGGTTGCTCCTGGGACACAAGCGACAGCGCGCGGG (SEQ ID NO: 147) A-F2 CCCAGGAGCAACCCCAGTCCGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 148) B-R1 TGCTATTTCTAGCTCTAAAACacacaagcgacagcgcgcggg (SEQ ID NO: 149) **B-F2** GCCCCTGACGCCCAGTGACGGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 150) Os12g0157000 C-R1 GGGGGTGCCCCTGGGCGAGAGAACACAAGCGACAGCGCGCGGG (SEQ ID NO: 152) C-F2 TCTCGCCCAGGGGCACCCCCGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 153) D-R1 CTCGTAGTGGTGGTGGTGGTAGTACACAAGCGACAGCGCGCGGG (SEQ ID NO: 154) D-F2 ACTACCACCACCACTACGAGGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 155)

	INTERNATIONAL SEARCH	REPORT	International application No PCT/GB2016/050245	
A. CLASSIF INV. ADD.	CATION OF SUBJECT MATTER A01H5/00 A01H5/10 C07K14/4	415 C12N1	5/82	
B. FIELDS Minimum do	Dinternational Patent Classification (IPC) or to both national classifical SEARCHED cumentation searched (classification system followed by classification C07K C12N			
	on searched other than minimum documentation to the extent that s ata base consulted during the international search (name of data ba			
EPO-Int	ernal , BIOSIS, Sequence Search , EN	IBASE, MEDLIN	IE, WPI Data	
C. DOCUME	NTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the rel	evant passages	Relevant to claim No.	
х	X wo 2004/031349 A2 (MENDEL BIOTECHNOLOGY INC [US] ; JIANG CAI-ZHONG [US] ; HEARD JACQUELINE) 15 Apri I 2004 (2004-04-15) Cl aims 1, 3-9 ; pages 32, 34, 41, 62, 110,			
	115-117, 247; Tables 4-6; Seq. ID Nos 395 and 396			
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		-/		
X Furth	er documents are listed in the continuation of Box C.	X See patent fai	mily annex.	
"A" documer to be c "E" earlier a filing d "L" documen cited to special		date and not in co the principle or th "X" document of partici- considered novel step when the do "Y" document of partici- considered to inv combined with or	blished after the international filing date or priority onflict with the application but cited to understand leavy underlying the invention ular relevance; the claimed invention cannot be or cannot be considered to involve an inventive cument is taken alone ular relevance; the claimed invention cannot be olve an inventive step when the document is ne or more other such documents, such combinatic a person skilled in the art	on
	nt published prior to the international filing date but later than rity date claimed	"&" document member	of the same patent family	
Date of the	actual completion of the international search	Date of mailing of	the international search report	
8	Apri I 2016	26/04/20	016	
Name and r	nailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer	Birgit	
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INTER

Box No. I

2.

3.

1.

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INTERNATIONAL SEARCH REPORT	International application No.
	PCT/GB2016/050245
No. I Nucleotide and/or amino acid sequence(s) (Continuation of item	1.c of the first sheet)
With regard to any nucleotide and/or amino acid sequence disclosed in the international carried out on the basis of a sequence listing:	application, the international search was
a. X forming part of the international application as filed:	
in the form of an Annex C/ST.25 text file.	
on paper or in the form of an image file.	
b. furnished together with the international application under PCT Rule 13fer1 (a only in the form of an Annex C/ST.25 text file.) for the purposes of international search
c. furnished subsequent to the international filing date for the purposes of international	ational search only:
in the form of an Annex C/ST.25 text file (Rule 13fer1 (a)).	
on paper or in the form of an image file (Rule 13fer1 (b) and Administrat	ive Instructions, Section 713).
In addition, in the case that more than one version or copy of a sequence listing statements that the information in the subsequent or additional copies is identicative filed or does not go beyond the application as filed, as appropriate, were furnished.	I to that forming part of the application as
Additional comments:	

INTERNATIONAL SEARCH REPORT

International application No PCT/GB2016/050245

C(Continuat	C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT				
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