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[Continued on nextpage]
(54) Title: PLANTS WITH INCREASED SEED SIZE

## Figure 1


(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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$\mathrm{GN}, \mathrm{GQ}, \mathrm{GW}, \mathrm{KM}, \mathrm{ML}, \mathrm{MR}, \mathrm{NE}, \mathrm{SN}, \mathrm{TD}, \mathrm{TG})$.

## Field of the invention

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

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 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 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 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 Arabidopsis, including HAIKU1(IKU1), IKU2, MINISEED3 (MINI3) and SHORT 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
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).

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

## Summary of the invention

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 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..
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.

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.

## 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 $10-\mathrm{d}$-old wild-type, da $1-1$ and sod7-1D da1-1 seedlings. Values ( $\mathrm{E}-\mathrm{G}$ ) are given as mean $\pm \mathrm{SD}$ relative to the respective wildtype values, set at $100 \%$. **, $\mathrm{P}<0.01$ compared with da $1-1$ (Student's t -test). Bars $=0.5$ mm in (A), 0.2 mm in (B), 1 mm in (C) and 5 cm in (D).

Figure 2. Seed and organ size in the sod7-1D mutant.
(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 $\pm S D$ relative to the respective wild-type values, set at $100 \%$. ${ }^{* *}, P<0.01$ compared with the wild type (Student's t-test). Bars $=0.5 \mathrm{~mm}$ in (A) and (B), 0.2 mm in (C) and (D), and 1 mm in (E) and (F).

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:GFPSOD7\#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:GFPSOD7\#3 and 35S:GFP-SOD7\#5 seedlings. Values (D-F) are given as mean $\pm$ SD relative to the respective wild-type values, set at $100 \%$. ${ }^{* *}, \mathrm{P}<0.01$ compared with the wild type (Student's t-test).

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 ( $\mathrm{D}, \mathrm{E}$ ), the developing petals ( $\mathrm{F}, \mathrm{G}$ ), the developing stamens ( $\mathrm{H}, \mathrm{I}$ ), and the developing carpels (J, K). (L) GFP florescence of SOD7-GFP in a young ovule of $p$ SOD7:SOD7-GFP transgenic plants. (M-O) GFP fluorescence of SOD7-GFP (M),

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 $=100 \mu \mathrm{~m}$ in (A-K), $10 \mu \mathrm{~m}$ in (L), and $2 \mu \mathrm{~m}$ in (M-R).
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-k0 1) 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-ko ${ }_{1}$ ) in the NGAL3 gene was indicated. (C) Seeds from Col-0, sod7-ko 1, ngal3-ko 1 and sod7-ko 1 ngal3-ko 1 plants (from left to right). (D) Mature embryos of Col-0, sod7-ko 1, ngal3-ko 1 and sod7-ko 1 ngal3-ko 1 (from left to right). (E) 25-day-old plants of Col-0, sod7-ko 1, ngal3-ko 1 and sod7-ko 1 ngal3-ko 1 (from left to right). (F) Flowers of Col-0, sod7-ko 1, ngal3-ko 1 and sod7-ko 1 ngal3-ko 1 (from left to right). (G) Projective area of Col-0, sod7-ko 1, ngal3ko 1 and sod7-ko 1 ngal3-ko 1 seeds. (H) Weight of Col-0, sod7-ko 1, ngal3-ko 1 and sod7-ko 1 ngal3-ko 1 seeds. (I) Cotyledon area of Col-0, sod7-ko 1, ngal3-ko 1 and sod7ko 1 ngal3-ko 1 seedlings. Values (G-I) are given as mean $\pm$ SD relative to the respective wild-type values, set at $100 \%$. ${ }^{* *}, \mathrm{P}<0.01$ compared with the wild type (Col0 ) (Student's t-test). Bars $=0.5 \mathrm{~mm}$ in (C), 0.2 mm in (D), 5 cm in (E), and 1 mm in (F).
Figure 6. SOD7 acts maternally to determine seed size.
(A) Projective area of Col-OxCol-0 (C/C) F1, Col-oxsod7-ko 1 ngal3-ko 1 (C/d) F1, sod7ko 1 ngal3-ko $1 \times$ Col-O (d/C) F1 and sod7-ko 1 ngal3-ko Usod7-ko 1 ngal3-ko 1 (d/d) F1 seeds. Values are given as mean $\pm$ SD relative to the respective wild-type values, set at $100 \%$. (B) Projective area of Col-OxCol-0 (C/C) F2, Col-0*sod7-ko 1 ngal3-ko 1 (C/d) F2, sod7-ko 1 ngal3-ko 1*Col-0 (d/C) F2 and sod7-ko 1 ngal3-ko 1*sod7-ko 1 ngal3-ko 1 (d/d) F2 seeds. Values are given as mean $\pm$ SD relative to the respective wild-type values, set at $100 \%$. ( C and D) Mature ovules of Col-0 (C) and sod7-ko 1 ngal3-ko 1 (D). (E) Outer integument length of mature Col-0 (lighter bar to the left) and sod7-ko 1 ngal3-ko 1 (darker bar to the right) ovules. Values are given as mean $\pm$ SD. (F) The number of cells in the outer integuments of Col-0 and sod7-ko 1 ngal3-ko 1 at 0 , 6 and 8 DAP. Values are given as mean $\pm$ SD. (F) The length of cells in the outer integuments of Col-0 and sod7-ko 1 ngal3-ko 1 at 0,6 and 8 DAP. Values are given as
mean $\pm$ SD. ${ }^{* *}, \mathrm{P}<0.01$ compared with the wild type (Col-0) (Student's t-test). Bars $=50$ $\mu \mathrm{m}$ 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 $\pm$ 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 $\pm$ SD relative to the respective wild-type values, set at $100 \%$. (C) The outer integument length of Col-0, klu-4, sod7ko1 ngal3-ko1 and klu-4 sod7-ko1 ngal3-ko1 (from left to right). ngal3-ko1 at 0 and 8 DAP. Values are given as mean $\pm$ 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 (from left to right)at 0 and 8 DAP. Values are given as mean $\pm$ SD. **, $\mathrm{P}<0.01$ 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 $\beta$-estradiol for 0,4 and 8 hours. Means were calculated from three biological samples. Values are given as mean $\pm \mathrm{SD} .{ }^{* *}, \mathrm{P}<0.01$, compared with the expression levelof KLU and SOD7 at 0 hour, respectively (Student's t-test). (B) A 2-kb promoter region of $K L U$ 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 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 $\pm$ SD. ${ }^{* *}, \mathrm{P}<0.01$, compared with 35S:GFP transgenic plants (Student's t-test). (D) Direct interaction 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. 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 $100 \%$. ${ }^{* *}, \mathrm{P}<0.01$ and ${ }^{*}, \mathrm{P}<0.05$ 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 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 , GRMZM2G082227_T01 , Os1 1g01 56000, GRMZM2G328742_T01 , GmLoc100802734 GmLod 00795470, GmLod 00818164 , 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 00801107. Conserved B3 domain and repressor motif are boxed.

Figure 14: Genome editing experiments to knock out rice genes Os1 1 g 01560000 and Os12g0157000 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.

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

As used herein, the words "nucleic acid", "nucleic acid sequence", "nucleotide", "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), 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 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 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.

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
(a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or
(b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or
(c) both (a) and (b)
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
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.

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.

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

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 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 $16,000 \mathrm{~T} 1$ plants (Fig.IA). Seeds of the sod7-1D da1-1 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 OhmeTakagi, 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/DEVELOPMENTRELATED 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.

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 organ size, for example increased seed size, and also increased petal size, increased embryo size, for example. Increased seed size leads to an increase in seed yield and the plants of the invention are thus characterised by increased seed yield.

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.

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 and/or NGAL3 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.

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 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 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 activity of a NGAL2 polypeptide.

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 .

In one embodiment, the domain is: SNNNNNNGGSGDDVACHFQRFDLHRLFIGWRGE
(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 AtNGAL2 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 AtNGAL3 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.

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.

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 $99 \%$.

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 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 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

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 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 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 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 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 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). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing).

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 $30^{\circ} \mathrm{C}$ for short probes (e.g., 10 to 50 nucleotides) and at least about $60^{\circ} \mathrm{C}$ 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.

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 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).

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.

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,
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.

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.

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.

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
a) Knocking out or down SOD7function 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 NGAL3 polypeptide to create a double loss of function mutant.

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 expression of a nucleic acid sequence encoding a NGAL3 polypeptide or reducing or abolishing activity of a NGAL2 polypeptide.

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.

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.

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
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.

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.

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 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. 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. 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 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
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 be fast neutron irradiation or a chemical mutagen, for example selected from the following non-limiting list: ethyl methanesulfonate (EMS), methylmethane sulfonate
(MMS), N -ethyl-N-nitrosurea (ENU), triethylmelamine ( $1^{1} \mathrm{E} \mathrm{M}$ ), 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.

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 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 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 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 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 nonmutagenised 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.

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

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 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 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 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 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 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 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 "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).

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 the mRNA 5' and $3^{\prime}$ 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 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 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.

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 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, 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.

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 amiRNAs have been generalized and integrated into a Web-based 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 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 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 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 $5^{\prime}$ or $3^{\prime}$ 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 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
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 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, 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.

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 hybridises under stringent conditions to the gene transcript.

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.

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 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.

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
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.

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.

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

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 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 double-strand breaks (DSBs) in vivo for genome editing. The use of this technology in genome editing is well described in the art, for example in US 8,440,431, US 8,440, 432 and US $8,450,471$. Reference 30 describes a set of customized plasmids that can be used with the Golden Gate cloning method to assemble multiple DNA fragments. As described therein, the Golden Gate method uses Type IIS restriction endonucleases, which cleave outside their recognition sites to create unique 4 bp overhangs. Cloning is expedited by digesting and ligating in the same reaction mixture because correct assembly eliminates the enzyme recognition site. Assembly of a custom TALEN or TAL effector construct and involves two steps: (i) assembly of repeat modules into intermediary arrays of 1-10 repeats and (ii) joining of the intermediary arrays into a backbone to make the final construct.

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

Cas9 is thus the hallmark protein of the type ॥ 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.

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.

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.

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 at least one CRISPR target sequence, wherein the target sequence is selected from SEQ ID Nos 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 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 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 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.

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.

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 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 be a promoter. The invention also relates to a vector comprising such expression
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 "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The 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 rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility is growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker 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, 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.

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 " $\mathrm{A}, \mathrm{B}$ 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.

## Examples

## 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 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 $/ 8 \mathrm{~h}$ dark) at $22^{\circ} \mathrm{C}$. 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 T 1 plants were selected by using the herbicide Basta. Seeds produced from T1 plants were used to isolate modifiers of da 1-1.

## 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 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 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.

Cloning of the SOD7 gene
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

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 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 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:GFPSOD7, pSOD7:SOD7-GFP and pSOD7:GUS were introduced into Col-0 or sod7-ko1 ngal3ko1 plants using Agrobacterium tumefaciens GV3101, respectively, and transformants were selected on hygromycin ( $30 \mu \mathrm{~g} / \mathrm{ml}$ )-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 into Col-0 plants using Agrobacterium tumefaciens GV3101, and transformants were selected on hygromycin $(30 \mu \mathrm{~g} / \mathrm{ml})$-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 $\mathrm{K} 3 \mathrm{Fe}(\mathrm{CN}) 6 / \mathrm{K} 4 \mathrm{Fe}(\mathrm{CN}) 6$, and $0.1 \%$ ( $\mathrm{v} / \mathrm{v}$ ) Triton X-100) and incubated at $37^{\circ} \mathrm{C}$ for 3 hours. After GUS staining, chlorophyll was removed by $70 \%$ 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 SuperScriptlll 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 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

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. 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 $60 \mu$ l of water. Gene-specific primers (PF1-F, PF1-R, PF-2F, PF2-R, ACTIN7-ChIPF, and ACTIN7-ChIP-R) were used to quantify the enrichment of each fragment (Table 1).

The DNA electrophoretic mobility shift assay (EMSA)
The coding sequence of SOD7 was cloned into the Ndel and BamHI sites of the pMALC 2 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 ( 50 mM Tris- $\mathrm{HCl}, 5 \mathrm{mM}$ EDTA and 250 mM NaCl ) at $95{ }^{\circ} \mathrm{C}$ for 10 min 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

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 ( $\operatorname{sod} 7-1 \mathrm{D})$ 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.
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 $10-\mathrm{d}$ 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.

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
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 At3g1 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 NGATHA like protein (NGAL2) containing a B3 DNA-binding domain and a transcriptional repression motif (Figure 3C) (Alvarez et al., 2009; Ikeda and OhmeTakagi, 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/DEVELOPMENTRELATED 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 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 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 inflorescence in pSOD7:SOD7-GFP transgenic plants. As shown in Figures 4M-0,

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 TDNA 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 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 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 $10-\mathrm{d}$-old seedlings. A synergistic enhancement of cotyledon size of 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.

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
(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 $6 \mathrm{~A})$. 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-ko1l sod7-ko1 ngal3ko1 F2 seeds. As shown in Figure 6B, sod7-ko1 ngal3-ko1l sod7-ko1 ngal3-ko1 F2 seeds were larger than wild-type seeds, while the size of Col-0/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.

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., 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 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 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 wildtype seeds, the number of outer integument cells at 6 DAP was comparable with that at

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 $6 \mathrm{G})$. Thus, these analyses indicate that SOD7 is required for cell proliferation in the maternal integuments of ovules and developing seeds.

## SOD7 acts in a common pathway with KLU to control seed size, but does so independently of $D M$

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 ngal3-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 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 $D M$ to control seed size. We further crossed sod7-ko1 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 da11 was also additive for seed size, compared with their parental lines, further supporting that SOD7 functions to control seed growth separately from $D M$.

SOD7 directly binds to the promoter of $K L U$ and represses the expression of $K L U$ 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 $K L U$. We therefore investigated the expression of $K L U$ in the chemicallyinducible SOD7 \{pER8-SOD7) transgenic plants. After the pER8-SOD7 transgenic plants were treated with the inducer ( $\beta$-estradiol), the expression of SOD7 was strongly induced at 4 and 8 hours (Figure 8A). As expected, the expression of $K L U$ was dramatically repressed at 4 and 8 hours (Figure 8 A ). Thus, these results indicate that SOD7 represses the expression of $K L U$ and also suggest that $K L U$ might be a direct target of SOD7.

To determine whether SOD7 can directly bind to the promoter of the $K L U$ gene, we performed a chromatin immunoprecipitation (ChIP) assay with 35S:GFP and 35.GFPSOD7 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 8 B 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 $K L U$ and represses $K L U$ 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 $K L U$ and represses $K L U$ 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.

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
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 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 7 C ). Considering that the maternal integument or seed coat not only acts as a 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 wildtype 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 cells in the integuments mainly undergo expansion after fertilization (Garcia et al.,
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

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 $K L U$ 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 k/u4 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-4ls 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 $K L U$ and repress $K L U$ expression. Supporting this idea, the inducible expression of SOD7 resulted in a strong reduction of $K L U$ expression (Figure 8A). Our ChIP-qPCR data showed that SOD7 associates with the promoter region of $K L U$ 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 8 B and D). Thus, these results illustrate that SOD7 directly targets the promoter region of KLU and represses the expression of $K L U$, thereby determining seed size. Taken together, these findings
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 $K L U$ 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

Genome editing experiments to knock out os1 1 g 01560000 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:

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: http://cbi.hzau.edu.cn/crispr/help.php (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.
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.

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)
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)
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)
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-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 CTTTTTTTTGGTTTCTTGGAGTGAGAGAGAGAG (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)
Primers for RT-PCR and qRT-PCR
ACTIN2-F GAAATCACAGCACTTGCACC (SEQ ID NO. 29)
ACTIN2-R AAGCCTTTGATCTTGAGAGC (SEQ ID NO. 30)
SOD7-EX-F GCGACGACGGAGAAAGGG (SEQ ID NO. 31)
SOD7-EX-R ACGACGGCGCCATAGTGT (SEQ ID NO. 32)

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) KLU-q-RP TCGCAACTGTATCTGTCCCTCTA (SEQ ID NO. 38)
Primers for ChIP assay
ACTIN7-ChIP-FP CGTTTCGCTTTCCTTAGTGTTAGCT (SEQ ID NO. 29)
ACTIN7-ChIP-RP AGCGAACGGATCTAGAGACTCACCTTG (SEQ ID NO. 40)
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)
A-Biotin-RP tACAGAGAGCAAGTGTAGTAGAA (SEQ ID NO. 46)
A-Biotin-m-FP TTCTACTAACACCTCTCTCTGTA (SEQ ID NO. 47)
A-Biotin-m-RP TACAGAGAGAGGTGTTAGTAGAA (SEQ ID NO. 48)

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Sequence information

Identity of homologs to NGAL2 is indicated

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

ATGTCAGTCAACCATTACCACAACACTCTCTCGTTGCATCATCACCACCAAAACGA CGTAGCTATAGCACAACGAGAGTCTTTGTTCGAGAAATCACTCACACCAAGCGAC GTCGGAAAGCTAAACCGCTTAGTCATACCAAAACAACACGCCGAGAAATACTTCC CTCTCAATAATAATAATAATAATGGCGGCAGCGGAGATGACGTGGCGACGACGGA GAAAGGGATGCTTCTTAGCTTCGAGGATGAGTCAGGCAAGTGTTGGAAATTCAGA TACTCTTATTGGAACAGTAGCCAAAGCTACGTGTTGACCAAAGGATGGAGCAGGT ACGTCAAAGACAAACACCTCGACGCAGGCGACGTTGTTTTCTTTCAACGTCACCG TTTTGATCTCCATAGACTCTTCATTGGCTGGCGGAGACGCGGTGAAGCTTCTTCCT CTCCCGCTGTCTCCGTTGTGTCTCAAGAAGCTCTAGTTAATACGACGGCGTATTG GAGCGGCTTGACTACACCTTATCGTCAAGTACACGCGTCAACTACTTACCCTAATA TTCACCAAGAGTATTCACACTATGGCGCCGTCGTTGATCATGCTCAGTCGATACCA CCGGTGGTCGCAGGTAGCTCGAGGACGGTGAGGCTTTTTGGCGTGAACCTCGAA TGTCATGGTGATGCCGTCGAGCCACCACCGCGTCCTGATGTCTATAATGACCAAC ACATTTACTATTACTCAACTCCTCATCCCATGAATATATCATTTGCTGGGGAAGCAT TGGAGCAGGTAGGAGATGGACGAGGTTGA

AtSOD7 nucleic acid SEQ ID NO. 2 (genomic DNA).
ttgtttcggctatttgttatactattgttataacagtcacaagacttgacctcaacgaaaactttacaaaacgtgaattggaaa ttttacaaaatatgctcttaatcgttaatgcttcccaattaggtgagttaaattgtgagaggaaccatttcttagaggaaatggt tcatgaaaacaaatatgaaatagtatcactagtcttagtttgcgagaaaattaggaaaaatagaaacgtgtaagcacca atgatattcctgaaagcacgtgacagatatttcatgatcctataattaacaagtgataaagatattaaataaaattaacgata cttgagaaattcgtcaaataaaatagaagaggaccactcacgtaaccatttgcacgtcccattgattttgtggtagacttgg tatgttatattacttatattcacagaattatatacgaaactcacgacttaagatgcacggtaataactacagatggaaatttac ccatcaaacaagaaaacaacatttactcaagcatctagctagaccaaaatgtttgttacttgttgacttgcgatccatagat atattagttagaacttttcttctacaattgatcaaatgtttcacactgttctcaatttctcatctagattcatgacttatatgttggtc aaatatcacagcttgatgagcattaaatagcgtcgaagtataggatggttacgttgttcaatattgtaaaggaaaaaaaga gaaagagtgccaaaaggtcaagtcgatttcacaaataaatcttgaagtctttatccctctcgattataaaatgattaggaaa agaaaaagagagaataaaatgtagataaagagaaagagaaagagagagaggaacataagggatggtatgaagta gaagtgaagatgcatgcgatggtgtgtcggaaaggcaaagcacatgctacacaacttgagcttctcacttgcgtcaggg ataagtatcctctgtaccttcttactttgcgtaatatgtaccacctcacttctcaaccgtttgatctttaatccttcattatttcttcatt
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#### Abstract

ctatacacttgactgtaaaaccacatggccaatttggttttatttgattactttgatttgttttgttactctttgtctctgtagcctcct tttgttcattaattaatatcagccgtaagtatatagttcctgtgaaaacagtctctattttggttttactattctaatttgttaggcac cgtcagtttttttgtgaaaccaaattattgactaataagctggaaagcaaaactgactaaaagcattacaaacttatcaatg acataagtttgaatttattaccatgtttgtaatgttcagatataatttgaaatgcttagaattatatatttgtatacttaaattaatg aaataaagtgaatactaaagatagtttattttcatattattctatacaattcggtgtacaatttgttttgatgataataaaaata ataaaattgcgtgttggaattgtgaaacagAATATATCATTTGCTGGGGAAGCATTGGAGCAGGT AGGAGATGGACGAGGT


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

MSVNHYHNTLSLHHHHQNDVAIAQRESLFEKSLTPSDVGKLNRLVIPKQHAEKYFPLN NNNNNGGSGDDVATTEKGMLLSFEDESGKCWKFRYSYWNSSQSYVLTKGWSRYVK DKHLDAGDWFFQRHRFDLHRLFIGWRRRGEASSSPAVSVVSQEALVNTTAYWSGL TTPYRQVHASTTYPNIHQEYSHYGAVVDHAQSIPPWAGSSRTVRLFGVNLECHGDA VEPPPRPDVYNDQHIYYYSTPHPMNISFAGEALEQVGDGRG

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

ATGTCAGTCAACCATTACTCCACAGACCACCACCACACTCTCTTGTGGCAGCAAC AGCAACACCGCCACACCACCGACACATCGGAGACAACCACCACCGCCACATGGC TCCACGACGACCTAAAAGAGTCACTCTTCGAGAAGTCTCTCACACCAAGCGACGT CGGGAAACTCAACCGCCTCGTCATACCAAAACAACACGCAGAGAAATACTTCCCT CTCAATGCCGTCCTAGTCTCCTCTGCTGCTGCTGACACGTCATCTTCGGAGAAAG GGATGCTTCTAAGCTTTGAAGACGAGTCAGGCAAGTCATGGAGGTTCAGATACTC TTACTGGAACAGCAGTCAAAGCTATGTCTTGACTAAAGGATGGAGCAGATTTGTCA AAGACAAACAGCTCGATCCAGGCGACGTTGTTTTCTTCCAACGACACCGTTCTGA TTCTAGGAGACTCTTCATTGGCTGGCGCAGACGTGGACAAGGCTCCTCATCCTCC GTCGCGGCCACTAACTCCGCCGTGAATACGAGTTCTATGGGAGCTCTTTCTTATC ATCAAATCCACGCCACTAGTAATTACTCTAATCCTCССTCTCACTCAGAGTATTCC CACTATGGAGCCGCCGTAGCAACAGCGGCTGAGACTCACAGCACACCGTCGTCT TCCGTCGTCGGGAGCTCAAGGACGGTGAGGCTTTTCGGTGTGAATCTGGAGTGT CAAATGGATGAAAACGACGGAGATGATTCTGTTGCAGTTGCCACCACCGTTGAAT CTCCCGACGGTTACTACGGCCAAAACATGTACTATTATTACTCTCATCCTCATAAC ATGGTAATTTTAACTCTTTTATAA

AtNGAL3 amino acid SEQ ID NO. 5

MSVNHYSTDHHHTLLWQQQQHRHTTDTSETTTTATWLHDDLKESLFEKSLTPSDVG KLNRLVIPKQHAEKYFPLNAVLVSSAAADTSSSEKGMLLSFEDESGKSWRFRYSYWN SSQSYVLTKGWSRFVKDKQLDPGDWFFQRHRSDSRRLFIGWRRRGQGSSSSVAAT NSAVNTSSMGALSYHQIHATSNYSNPPSHSEYSHYGAAVATAAETHSTPSSSWGSS RTVRLFGVNLECQMDENDGDDSVAVATTVESPDGYYGQNMYYYYSHPHNMVILTLL

## Oryza sativa

Osl2g0157000 LOC_Osl2g06080.1
Cover $73 \%$ identity $53 \%$

SEQ ID NO: 49
MAMHAGHAWWGVAMYTNHYHHHYRHKTSDVGKNRVKHARYGGGDSGKGSDSGKWRRYSYWTSSSYVTKG WSRYVKKRDAGDVVHRVRGGAADRGCRRRGSAAAVRVTANGGWSMCYSTSGSSYDTSANSYAYHRSVDDHSD HAGSRADAKSSSAASASRRRGVNDCGADATAMYGYMHHSYAAVSTVNYWSV

CDS SEQ ID NO: 50

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Osllg0156000
LOC_Osllg05740.1
Cover 81\% identity 47\%

SEQ ID NO: 51
MAM NHPLFSQEQPQSWPWGVAMYAN FHYHH HYEKEHMFEKPLTPSDVGKLNRLVIPKQHA ERYFPLGAGDAADKGLILSFEDEAGAPWRFRYSYWTSSQSYVLTKGWSRYVKEKRLDAGD VVHFERVRGSFGVGDRLFIGCRRRGDAAAAQTPAPPPAVRVAPAAQNAGEQQPWSPMCYS TSGGGSYPTSPANSYAYRRAADHDHGDMHHADESPRDTDSPSFSAGSAPSRRLRLFGVNL DCGPEPEADTTAAATMYGYMHQQSSYAAMSAVPSYWGNS

CDS SEQ ID NO: 52
ATGGCCATGAACCACCCTCTCTTCTCCCAGGAGCAACCCCAGTCCTGGCCATGGGGTGTG GCCATGTACGCCAACTTCCACTACCACCACCACTACGAGAAGGAGCACATGTTTGAGAAG

CCCCTGACGCCCAGTGACGTGGGGAAGCTGAACCGGCTGGTGATCCCCAAGCAGCACGCC GAGAGGTACTTCCCCCTCGGCGCCGGCGACGCCGCCGACAAGGGCCTGATCCTGTCGTTC GAGGACGAGGCCGGCGCGCCGTGGCGGTTCAGGTACTCCTACTGGACGAGCAGCCAGAGC TACGTGCTCACCAAGGGCTGGAGCCGCTACGTCAAGGAGAAGCGCCTCGACGCCGGCGAC

SEQ ID NO: 53
MEFTTSSRFSKEEEDEEQDEAGRREIPFMTATAEAAPAPTSSSSSPAHHAASASASASAS GSSTPFRSDDGAGASGSGGGGGGGGEAEVVEKEHM FDKVVTPSDVGKLNRLVIPKQYAEK YFPLDAAAN EKGLLLNFEDRAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVS FSRGIGDEAARHRLFI DWKRRADTRDPLRLPRGLPLPMPLTSHYAPWGIGGGGGFFVQPS PPATLYEHRLRQGLDFRAFNPAAAMGRQVLLFGSARI PPQAPLLARAPSPLHHHYTLQPS GDGVRAAGSPVVLDSVPVI ESPTTAAKRVRLFGVNLDNPHAGGGGGAAAGESSN HGNALS LQTPAWM RRDPTLRLLELPPH HHHGAESSAASSPSSSSSSKRDAHSALDLDL

CDS SEQ ID NO: 54
ATGGAGTTCACTACAAGCAGTAGGTTTTCTAAAGAAGAGGAGGACGAGGAGCAGGATGAG GCGGGAAGGCGAGAGATCCCCTTCATGACGGCCACGGCCGAAGCCGCGCCTGCGCCCACG TCGTCGTCGTCGTCTCCTGCTCATCACGCGGCTTCCGCGTCGGCGTCGGCGTCTGCGTCA GGGAGCAGCACTCCCTTTCGCTCCGACGATGGCGCCGGGGCGTCTGGGAGCGGCGGCGGC GGCGGCGGCGGCGGAGAAGCGGAGGTGGTGGAGAAGGAGCACATGTTCGACAAGGTGGTG ACGCCGAGCGACGTTGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGTACGCCGAGAAG TACTTCCCGCTGGACGCGGCGGCGAACGAGAAGGGCCTCCTGCTCAACTTCGAGGACCGC GCGGGGAAGCCATGGCGGTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTGATG ACCAAGGGGTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGGGACACCGTCTCC TTCTCCCGCGGCATCGGCGACGAGGCGGCGCGGCACCGCCTCTTCATCGACTGGAAGCGC CGCGCCGACACCCGCGACCCGCTCCGGCTGCCCCGCGGGCTGCCGCTCCCGATGCCGCTC ACGTCGCACTACGCCCCGTGGGGGATCGGCGGCGGAGGGGGATTCTTCGTGCAGCCCTCG CCGCCGGCCACGCTCTACGAGCACCGCCTCAGGCAAGGCCTCGACTTCCGCGCCTTCAAC CCCGCCGCCGCGATGGGGAGGCAGGTCCTCCTGTTCGGCTCGGCGAGGATTCCTCCGCAA GCACCACTGCTGGCGCGCGCGCCGTCGCCGCTGCACCACCACTACACGCTGCAGCCGAGC GGCGATGGTGTAAGGGCGGCGGGCTCACCGGTGGTGCTCGACTCGGTTCCGGTCATCGAG AGCCCCACGACGGCCGCGAAGCGCGTGCGGCTGTTCGGCGTGAACCTCGACAACCCGCAT GCCGGCGGCGGCGGCGGCGCCGCCGCCGGCGAGTCGAGCAATCATGGCAATGCACTGTCA TTGCAGACGCCCGCGTGGATGAGGAGGGATCCAACACTGCGGCTGCTGGAATTGCCTCCT CACCACCACCATGGCGCCGAGTCGTCCGCTGCATCGTCTCCGTCGTCGTCGTCTTCCTCC AAGAGGGACGCGCATTCGGCCTTGGATCTCGATCTGTAG

| os04g0581400 | LOC_Os04g49230 |
| :--- | :---: |
| Cover $46 \%$ | identity $64 \%$ |

CDS SEQ ID NO: 55
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SEQ ID NO: 56
M EFATTSSRFSKEEEEEEEGEQEMEQEQDEEEEEAEASPREIPFMTSAAAAATASSSSPT SVSPSATASAAASTSASGSPFRSSDGAGASGSGGGGGGEDVEVIEKEH MFDKVVTPSDVG KLNRLVIPKQHAEKYFPLDSAANEKGLLLSFEDRTGKLWRFRYSYWNSSQSYVMTKGWSR FVKEKRLDAGDTVSFCRGAAEATRDRLFI DWKRRADVRDPHRFQRLPLPMTSPYGPWGGG AGASSCRPRRPPRSTSITAFARASTSATSTPLCRRGSSSSSAPQGRGFISTRPCHRRRRH LRLLTNSTLRCTTRAP
os03g0120900 LOC_Os03g02900 Cover $47 \%$ identity $63 \%$

CDS SEQ ID NO: 57 ATGGAGTTCATCACGCCAATCGTGAGGCCGGCATCGGCGGCGGCGGGCGGCGGCGAGGTG CAGGAGAGTGGTGGGAGGAGCTTGGCGGCGGTGGAGAAGGAGCACATGTTCGACAAGGTG GTGACGCCGAGCGACGTGGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGCACGCGGAG AAGTACTTCCCGCTGGACGCGGCGTCCAACGAGAAGGGGCTCCTGCTCAGCTTCGAGGAC CGCACGGGGAAGCCATGGCGGTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTG ATGACCAAGGGGTGGAGCCGCTTCGTCAAGGAGAAGCGACTCGACGCCGGGGACACCGTC TCCTTCGGCCGCGGCGTCGGCGAGGCCGCGCGCGGGAGGCTCTTCATCGACTGGCGCCGC CGCCCCGACGTCGTCGCCGCGCTCCAGCCGCCCACGCACCGCTTCGCCCACCACCTCCCT TCCTCCATCCCCTTCGCTCCCTGGGCGCACCACCACGGACACGGAGCCGCCGCCGCCGCC GCCGCCGCCGCCGGCGCCAGGTTTCTCCTGCCTCCCTCCTCGACTCCCATCTACGACCAC CACCGCCGACACGCCCACGCCGTCGGGTACGACGCGTACGCCGCGGCCACCAGCAGGCAG GTGCTGTTCTACCGGCCGTTGCCGCCGCAGCAGCAGCATCATCCCGCGGTGGTGCTGGAG TCGGTGCCGGTGCGCATGACGGCGGGGCACGCGGAGCCGCCGTCGGCTCCGTCGAAGCGA GTTCGGCTGTTCGGGGTGAACCTCGACTGCGCGAATTCCGAACAAGACCACGCCGGCGTG GTCGGGAAGACGGCGCCGCCGCCGCTGCCATCGCCGCCGTCATCATCGTCATCTTCCTCC GGGAAAGCGAGGTGCTCCTTGAACCTTGACTTGTGA

## 63

M EFITPIVRPASAAAGGGEVQESGGRSLAAVEKEHM FDKVVTPSDVGKLNRLVI PKQHAE KYFPLDAASNEKGLLLSFEDRTGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTV SFGRGVGEAARGRLFIDWRRRPDVVAALQPPTHRFAHHLPSSI PFAPWAHHHGHGAAAAA AAAAGARFLLPPSSTPIYDH HRRHAHAVGYDAYAAATSRQVLFYRPLPPQQQH HPAVVLE
os01g0693400
Cover 47\% identity 63\%

CDS SEQ ID NO: 59
ATGGACAGCTCCAGCTGCCTGGTGGATGATACCAACAGCGGCGGCTCGTCCACGGACAAG CTGAGGGCGTTGGCCGCCGCGGCGGCGGAGACGGCGCCGCTGGAGCGCATGGGGAGCGGG GCGAGCGCGGTGGTGGACGCGGCCGAGCCTGGCGCGGAGGCGGACTCCGGGTCCGGGGGA CGTGTGTGCGGCGGCGGCGGCGGCGGTGCCGGCGGTGCGGGAGGGAAGCTGCCGTCGTCC AAGTTCAAGGGCGTCGTGCCGCAGCCCAACGGGAGGTGGGGCGCGCAGATCTACGAGCGG CACCAGCGGGTGTGGCTCGGCACGTTCGCCGGGGAGGACGACGCCGCGCGCGCCTACGAC GTCGCCGCGCAGCGCTTCCGCGGCCGCGACGCCGTCACCAACTTCCGCCCGCTCGCCGAG GCCGACCCGGACGCCGCCGCCGAGCTTCGCTTCCTCGCCACGCGCTCCAAGGCCGAGGTC GTCGACATGCTCCGCAAGCACACCTACTTCGACGAGCTCGCGCAGAGCAAGCGCACCTTC GCCGCCTCCACGCCGTCGGCCGCGACCACCACCGCCTCCCTCTCCAACGGCCACCTCTCG TCGCCCCGCTCCCCCTTCGCGCCCGCCGCGGCGCGCGACCACCTGTTCGACAAGACGGTC ACCCCGAGCGACGTGGGCAAGCTGAACAGGCTCGTCATACCGAAGCAGCACGCCGAGAAG CACTTCCCGCTACAGCTCCCGTCCGCCGGCGGCGAGAGCAAGGGTGTCCTCCTCAACTTC GAGGACGCCGCCGGCAAGGTGTGGCGGTTCCGGTACTCGTACTGGAACAGCAGCCAGAGC TACGTGCTAACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGGGTCTCCACGCCGGCGAC GTCGTCGGCTTCTACCGCTCCGCCGCCAGTGCCGGCGACGACGGCAAGCTCTTCATCGAC TGCAAGTTAGTACGGTCGACCGGCGCCGCCCTCGCGTCGCCCGCTGATCAGCCAGCGCCG TCGCCGGTGAAGGCCGTCAGGCTCTTCGGCGTGGACCTGCTCACGGCGCCGGCGCCGGTC GAACAGATGGCCGGGTGCAAGAGAGCCAGGGACTTGGCGGCGACGACGCCTCCACAAGCG GCGGCGTTCAAGAAGCAATGCATAGAGCTGGCACTAGTATAG

SEQ ID NO: 49
60M DSSSCLVDDTNSGGSSTDKLRALAAAAAETAPLERMGSGASAVVDAAEPGAEADSGSGG

| Osl0g0537100 | LOC_Osl0g39190 |
| :--- | :---: |
| Cover $47 \%$ | identity $60 \%$ |

CDS SEQ ID NO: 61 ATGGAGTTCACCCCAATTTCGCCGCCGACGAGGGTCGCCGGCGGTGAGGAGGATTCCGAG

## 64

AGGGGGGCGGCGGCGTGGGCGGTGGTGGAGAAGGAGCACATGTTTGAGAAGGTCGTGACG CCGAGCGACGTGGGGAAGCTGAACCGATTGGTCATCCCCAAGCAGCACGCCGAGAGGTAC TTCCCGCTCGACGCCGCGGCGGGCGCCGGCGGCGGCGGTGGTGGCGGCGGTGGCGGCGGC


SEQ ID NO: 62
MEFTPISPPTRVAGGEEDSERGAAAWAVVEKEH MFEKVVTPSDVGKLNRLVI PKQHAERY FPLDAAAGAGGGGGGGGGGGGGKGLVLSFEDRTGKAWRFRYSYWNSSQSYVMTKGWSRFV KEKRLGAGDTVSFGRGLGDAARGRLFIDFRRRRQDAGSFMFPPTAAPPSHSHHHHQRHHP PLPSVPLCPWRDYTTAYGGGYGYGYGGGSTPASSRHVLFLRPQVPAAVVLKSVPVHVAAT SAVQEAATTTRPKRVRLFGVNLDCPAAMDDDDDIAGAASRTAASSLLQLPSPSSSTSSST AGKKMCSLDLGL

## Glycine max

L.OC100795470

Cover 75\% identity 53\%

SEQ ID NO: 63
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CDS SEQ ID NO: 64
ATGTCCATAAACCACTACTCCATGGACCTTCCCGAACCGACACTCTGGTGGCCACACCCA CACCACCAACAACAACAACTAACCTTAATGGATCCTGACCCTCTCCGTCTCAACCTCAAT AGCGACGATGGCAATGGCAATGACAACGACAACGACGAAAATCAAACAACCACAACAGGA GGAGAACAAGAAATATTAGACGATAAAGAACCGATGTTCGAGAAGCCCTTAACCCCGAGC GACGTGGGGAAGCTGAACCGTCTCGTAATCCCGAAGCAGCACGCGGAGAAGTACTTCCCA CTGAGTGGTGACTCGGGCGGGAGCGAGTGCAAGGGGCTGTTACTGAGTTTCGAGGACGAG TCG GGGAAGTGTTG GCGCTTCCGCTACTCGTACTG GAACAG CAG CCAGAGCTACGTGCTC ACCAAAGGGTGGAGCCGCTACGTCAAGGACAAGCGCCTTGACGCGGGCGACGTCGTTTTG TTCGAGCGTCACCGCGTCGACGCGCAGCGCCTCTTCATCGGGTGGAGGCGCAGGCGGCAG AGCGATGCCGCCTTGCCGCCTGCGCACGTTAGCAGTAGGAAGAGTGGTGGTGGTGATGGG AATAGTAATAAGAATGAGGGGTGGACCAGAGGGTTCTATTCTGCGCATCATCCTTATCCT ACGCATCATCTTCATCATCATCAGCCCTCGCCATACCAACAACAACATGACTGTCTTCAT

GCAGGTAGAGGGTCCCAAGGTCAGAACCAAAGGATGAGACCAGTGGGAAACAACAGTTCT AGCTCTAGTTCGAGTTCAAGGGTACTTAGGCTGTTCGGGGTCGACATGGAATGCCAACCC GAACATGATGATTCTGGTCCCTCCACACCCCAATGCTCCTACAATAGTAACAACATGTTG CCATCAACACAGGGCACAGATCATTCCCATCACAATTTCTACCAACAGCAACCTTCTAAT

SEQ ID NO: 66
ATGTCGACAAACCACTACACCATGGACCTTCCCGAACCAACACTCTGGTGGCCACACCCA CACCAACAACAACTAACCTTAATAGATCCAGACCCTCTCССТСTGAACCTCAACAACGAC GACAACGACAATGGCGACGACAACGACAACGACGAAAACCAAACAGTTACAACAACCACA ACAGGAGGAGAAGAAGAAATAATAAACAATAAAGAACCGATGTTCGAGAAGCCGCTAACC CCGAGCGACGTGGGGAAGCTGAACCGCCTCGTAATCCCGAAGCAGCACGCTGAGAAGTAC TTTCCACTGAGTGGTGGTGACTCGGGCAGTAGCGAGTGCAAGGGGCTGTTACTGAGTTTC GAGGACGAGTCGGGGAAGTGCTGGCGCTTCCGCTACTCGTACTGGAACAGCAGCCAGAGC TACGTGCTCACCAAAGGGTGGAGCCGTTACGTGAAGGACAAGCGCCTCGATGCGGGAGAT GTCGTTTTATTCCAGCGCCACCGCGCCGACGCGCAGCGCCTCTTCATCGGCTGGAGGCGC AGGCGGCAGAGCGACGCCCTGCCGCCGCCTGCGCACGTTAGCAGCAGGAAGAGTGGTGGT GATGGGAATAGTAGTAAGAATGAGGGTGATGTGGGCGTGGGCTGGACCAGAGGGTTCTAT CCTGCGCATCATCCTTATCCTACGCATCATCATCATCCCTCGCCATACCATCACCAACAA GATGACTCTCTTCATGCAGTTAGAGGGTCCCAAGGTCAGAACCAAAGGACGAGACCAGTG GGAAACAG CAGTTCTAGTTCGAGTTCGAGTTCAAGG GTACTTAGGCTATTCGGGGTCAAC ATGGAATGCCAACCCGAACATGATGATTCTGGACCCTCCACACCCCAATGCTCCTACAAT ACTAACAACATATTGCCATCCACACAGGGCACAGATATTCATTCCCATCTCAATTTCTAC CAACAACAACAAACTTCTAATTCCAAGCCTCCCCCTCATCACATGATGATACGTCACCAA CCATACTACTACTAG

Loc100802734
Cover 77\% identity 53\%

SEQ ID NO: 67
MSSINHYSPETTLYWTNDQQQQAAMWLSNSHTPRFN LNDEEEEEEDDVIVSDKATNNLTQ EEEKVAMFEKPLTPSDVGKLNRLVIPKQHAEKH FPLDSSAAKGLLLSFEDESGKCWRFRY SYWNSSQSYVLTKGWSRYVKDKRLHAGDVVLFHRHRSLPQRFFISCSRRQPNPVPAHVST TRSSASFYSAHPPYPAHHFPFPYQPHSLHAPGGGSQGQNETTPGGNSSSSGSGRVLRLFG VNMECQPDNHN DSQNSTPECSYTHLYHHQTSSYSSSSNPHHHMVPQQP

## 66


#### Abstract

ATGTCATCGATAAACCACTATTCACCGGAAACAACACTATACTGGACCAACGACCAACAG CAACAAGCCGCCATGTGGCTGAGTAATTCCCACACCCCGCGTTTCAATCTGAACGACGAG GAGGAGGAGGAGGAAGACGACGTTATCGTTTCGGACAAGGCTACTAATAACTTGACGCAA GAGGAGGAGAAGGTAGCCATGTTCGAGAAGCCGTTGACGCCGAGCGACGTCGGGAAGCTG


CDS SEQ ID NO: 70
ATGGAGTTGATGCAACAAGTTAAAGGTAATTATTCTGATAGCAGGGAGGAAGAGGAGGAA GAGGAAGCTGCAGCAATCACAAGGGAATCAGAAAGCAGCAGGTTACACCAACAAGATACA GCATCCAATTTTGGAAAGAAGCTAGACTTGATGGACTTGTCACTAGGGAGCAGCAAGGAA GAGGAAGAGGAAGGGAATTTGCAACAAGGAGGAGGAGGAGTGGTTCATCATGCTCACCAA GTAGTGGAGAAAGAACACATGTTTGAGAAAGTGGCGACACCGAGCGACGTAGGGAAGCTG AACAGGCTGGTGATACCGAAGCAGCACGCGGAGAAGTACTTCCCCCTTGACTCCTCAACC AACGAGAAGGGTCTGCTCCTGAATTTCGAGGACAGGAATGGGAAGGTGTGGCGATTCAGG TATTCCTATTGGAACAGCAGCCAGAGCTATGTGATGACAAAAGGGTGGAGCCGCTTTGTT AAGGAGAAGAAGCTGGATGCCGGTGACATTGTCTCCTTCCAGCGTGGCCTTGGGGATTTG tatagacatcgattgtatatagattagaigagaiggcccgatcatgctcatgctcatcca ССTCATCATCACGATCCTTTGTTTCTTCССTCTATCAGATTGTACTCTCTCССTCССACC ATGCCACCTCGCTACCACCACGATCATCACTTTCACCACCATCTCAATTACAACAACCTC TTCACTTTTCAGCAACACCAGTACCAGCAGCTTGGTGCTGCCACTACCACTCATCACAAC AACTATGGTTACCAGAATTCGGGATCTGGTTCACTCTATTACCTAAGGTCCTCTATGTCA ATGGGTGGTGGTGATCAAAACTTGCAAGGGAGAGGGAGCAACATTGTCCCCATGATCATT GATTCTGTGCCGGTTAACGTTGCTCATCACAACAACAATCGCCATGGGAATGGGGGCATC ACGAGTGGTGGTACTAATTGTAGTGGAAAACGACTAAGGCTATTTGGGGTGAACATGGAA TGCGCTTCTTCGGCAGAAGATTCCAAAGAATTGTCCTCGGGTTCGGCAGCACACGTGACG ACAGCTGCTTCTTCTTCTTCTCTTCATCATCAGCGCTTGAGGGTGCCAGTGCCAGTGCCA CTTGAAGATCCACTTTCGTCGTCAGCAGCAGCAGCAGCAAGGTTTGGGGATCACAAAGGG GCCAGTACTGGGACTTCGCTGCTGTTTGATTTGGATCCCTCTTTGCAGTATCATCGCCAC TGA

LOC100776987
Cover $46 \%$ identity $62 \%$

SEQ ID NO: 71
MDAISCLDESTTTESLSISQAKPSSTIMSSEKASPSPPPPNRLCRVGSGASAVVDSDGGG GGGSTEVESRKLPSSKYKGVVPQPNGRWGSQIYEKHQRVWLGTFN EEDEAARAYDVAVQR FRGKDAVTNFKPLSGTDDDDGESEFLNSHSKSEIVDMLRKHTYNDELEQSKRSRGFVRRR GSAAGAGNGNSISGACVM KAREQLFQKAVTPSDVGKLNRLVIPKQHAEKHFPLQSAANGV SATATAAKGVLLNFEDVGGKVWRFRYSYWNSSQSYVLTKGWSRFVKEKNLKAGDTVCFQR STGPDRQLYIDWKTRNVVNEVALFGPVVEPIQMVRLFGVNI LKLPGSDSIANNNNASGCC NGKRREM ELFSLECSKKPKI IGAL

CDS SEQ ID NO: 72 ATGGATGCAATTAGTTGCCTGGATGAGAGCACCACCACCGAGTCACTCTCCATAAGTCAG GCGAAGCCTTCTTCGACGATTATGTCGTCCGAGAAGGCTTCTCCTTCCCCGCCGCCGCCG AACAGGCTGTGCCGCGTCGGTAGCGGTGCTAGCGCAGTCGTGGATTCCGACGGCGGCGGC
 GTGCCCCAGCCCAACGGCCGCTGGGGCTCGCAGATTTACGAGAAGCACCAGCGCGTGTGG CTGGGAACGTTCAACGAGGAAGACGAGGCGGCGCGTGCGTACGACGTCGCCGTGCAGCGA TTCCGCGGCAAGGACGCCGTCACAAACTTCAAGCCGCTCTCCGGCACCGACGACGACGAC GGGGAATCGGAGTTTCTCAACTCGCATTCGAAATCCGAGATCGTCGACATGCTGCGTAAG CATACGTACAATGACGAGCTGGAACAAAGCAAGCGCAGCCGCGGCTTCGTACGTCGGCGC GGCTCCGCCGCCGGCGCCGGAAACGGAAACTCAATCTCCGGCGCGTGTGTTATGAAGGCG CGTGAGCAGCTATTCCAGAAGGCCGTTACGCCGAGCGACGTTGGGAAACTGAACCGTTTG GTGATACCGAAGCAGCACGCGGAGAAGCACTTTCCTTTACAGAGCGCTGCTAACGGCGTT AGCGCGACGGCGACGGCGGCGAAGGGCGTTTTGTTGAACTTCGAAGACGTTGGAGGGAAA GTGTGGCGGTTTCGTTACTCGTATTGGAACAGTAGCCAGAGTTACGTCTTGACCAAAGGT TGGAGCCGGTTCGTTAAGGAGAAGAATCTGAAAGCCGGTGACACGGTTTGTTTTCAACGG TCCACTGGACCGGACAGGCAGCTTTACATCGATTGGAAGACGAGGAATGTTGTTAACGAG GTCGCGTTGTTCGGACCGGTTGTCGAACCGATCCAGATGGTTCGGCTCTTTGGTGTTAAC ATTTTGAAACTACCCGGTTCAGATTCTATCGCCAATAACAATAATGCAAGTGGGTGCTGC AATGGCAAGAGAAGAGAAATGGAACTCTTTTCATTAGAGTGTAGCAAGAAACCTAAGATT ATTGGTGCTTTGTAG

## Locl00778733

Cover 44\% identity 64\%

SEQ ID NO: 73
MELMQEVKGYSDGREEEEEEEEAAEEI ITREESSRLLHQHQEAAGSNFI INNN HHHHQHH HHHTTKQLDFMDLSLGSSKDEGNLQGSSSSVYAHH HHAASASSSANGN NNNSSSSNLQQQ QQQPAEKEHM FDKVVTPSDVGKLNRLVI PKQHAEKYFPLDSSANEKGLLLNFEDRNGKLW 5 RFRYSYWNSSQSYVMTKGWSRFVKEKKLDAGDMVSFQRGVGELYRHRLYI DWWRRPDH HH HHH HGPDHSTTLFTPFLI PNQPHH LMSI RWGATGRLYSLPSPTPPRHHEHLNYNNNAMYH PFHHHGAGSGI NATTH HYNNYHEMSSTTTSGSAGSVFYHRSTPPISMPLADHQTLNTRQQ QQQQQQQEGAGNVSLSPMI IDSVPVAHHLHHQQH HGGKSSGPSSTSTSPSTAGKRLRLFG VNMECASSTSEDPKCFSLLSSSSMANSNSQPPLQLLREDTLSSSSARFGDQRGVGEPSM L FDLDPSLQYRQ

SEQ ID NO: 75
MDGGCVTDETTTSSDSLSVPPPSRVGSVASAVVDPDGCCVSGEAESRKLPSSKYKGVVPQ PNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAALRFRGPDAVTN FKPPAASDDAESEF LNSHSKFEIVDM LRKHTYDDELQQSTRGGRRRLDADTASSGVFDAKAREQLFEKTVTPSD VGKLNRLVIPKQHAEKHFPLSGSGDESSPCVAGASAAKGMLLNFEDVGGKVWRFRYSYWN SSQSYVLTKGWSRFVKEKNLRAGDAVQFFKSTGPDRQLYDCKARSGEVNNNAGGLFVPI GPVVEPVQMVRLFGVNLLKLPVPGSDGVGKRKEMELFAFECCKKLKVIGAL

CDS SEQ ID NO: 76
ATGGATGGAGGCTGTGTCACAGACGAAACCACCACATCCAGCGACTCTCTTTCCGTTCCG CCGCCCAGCCGCGTCGGCAGCGTTGCAAGCGCCGTCGTCGACCCCGACGGTTGTTGCGTT TCCGGCGAGGCCGAATCCCGGAAACTCCCTTCGTCGAAATACAAAGGCGTGGTGCCGCAA CCGAACGGTCGCTGGGGAGCTCAGATTTACGAGAAGCACCAGCGCGTGTGGCTCGGCACT TTCAACGAGGAAGACGAAGCCGCCAGAGCCTACGACATCGCCGCGCTGCGCTTCCGCGGC CCCGACGCCGTCACCAACTTCAAGCCTCCCGCCGCCTCCGACGACGCCGAGTCCGAGTTC CTCAACTCGCATTCCAAGTTCGAGATCGTCGACATGCTCCGCAAGCACACCTACGACGAC GAGCTCCAGCAGAGCACGCGCGGTGGTAGGCGCCGCCTCGACGCTGACACCGCGTCGAGC GGTGTGTTCGACGCGAAAGCGCGTGAGCAGCTGTTCGAGAAAACGGTTACGCCGAGCGAC GTCGGGAAGCTGAATCGATTAGTGATACCGAAGCAGCACGCGGAGAAGCACTTTCCGTTA AGCGGATCCGGCGACGAAAGCTCGCCGTGCGTGGCGGGGGCTTCGGCGGCGAAGGGAATG TTGTTGAACTTTGAGGACGTTGGAGGGAAAGTGTGGCGGTTTCGTTACTCTTATTGGAAC AGTAGCCAGAGCTACGTGCTTACCAAAG GATGGAGCCGGTTCGTTAAGGA GAAG AATCTT CGAGCCGGTGACGCGGTTCAGTTCTTCAAGTCGACCGGACCGGACCGGCAGCTATATATA

GACTGCAAGGCGAGGAGTGGTGAGGTTAACAATAATGCTGGCGGTTTGTTTGTTCCGATT GGACCGGTCGTTGAGCCGGTTCAGATGGTTCGGCTTTTCGGGGTCAACCTTTTGAAACTA CCCGTACCCGGTTCGGATGGTGTAGGGAAGAGAAAAGAGATGGAACTGTTTGCATTTGAA tGTtGCAAGAAGTTAAAAGTAATTGGAGCTTTGTAA

SEQ ID NO: 77
MDAISCMDESTTTESLSISLSPTSSSEKAKPSSM ITSSEKVSLSPPPSNRLCRVGSGASA VVDPDGGGSGAEVESRKLPSSKYKGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAY DIAAQRFRGKDAVTNFKPLAGADDDDGESEFLNSHSKPEIVDM LRKHTYNDELEQSKRSR GVVRRRGSAAAGTANSISGACFTKAREQLFEKAVTPSDVGKLNRLVIPKQHAEKHFPLQS SNGVSATTIAAVTATPTAAKGVLLN FEDVGGKVWRFRYSYWNSSQSYVLTKGWSRFVKEK NLKAGDTVCFH RSTGPDKQLYIDWKTRNVVNNEVALFGPVGPVVEPIQMVRLFGVNI LKL PGSDTIVGN NNNASGCCNGKRREMELFSLECSKKPKI IGAL

CDS SEQ ID NO: 78
ATGGATGCAATTAGTTGCATGGATGAGAGCACCACCACTGAGTCACTCTCTATAAGTCTT TCTCCGACGTCATCGTCGGAGAAAGCGAAGCCTTCTTCGATGATTACATCGTCGGAGAAG GTTTCTCTGTCCCCGCCGCCGTCAAACAGACTATGCCGTGTTGGAAGCGGCGCGAGCGCA GTCGTGGATCCTGATGGCGGCGGCAGCGGCGCTGAGGTAGAGTCGCGGAAACTCCCCTCG TCGAAGTACAAGGGCGTGGTGCCCCAGCCCAACGGCCGCTGGGGTGCGCAGATTTACGAG AAGCACCAGCGCGTGTGGCTTGGAACGTTCAACGAGGAAGACGAGGCGGCGCGTGCGTAC GACATCGCCGCGCAGCGGTTCCGCGGCAAGGACGCCGTCACGAACTTCAAGCCGCTCGCC GGCGCCGACGACGACGACGGAGAATCGGAGTTTCTCAACTCGCATTCCAAACCCGAGATC GTCGACATGCTGCGAAAGCACACGTACAATGACGAGCTGGAGCAGAGCAAGCGCAGCCGC GGCGTCGTCCGGCGGCGAGGCTCCGCCGCCGCCGGCACCGCAAACTCAATTTCCGGCGCG TGCTTTACTAAGGCACGTGAGCAGCTATTCGAGAAGGCTGTTACGCCGAGCGACGTTGGG AAATTGAACCGTTTGGTGATACCGAAG CAGCACGCGGAGAAG CACTTTCCGTTACAGAGC TCTAACGGCGTTAGCGCGACGACGATAGCGGCGGTGACGGCGACGCCGACGGCGGCGAAG GGCGTTTTGTTGAACTTCGAAGACGTTGGAGGGAAAGTGTGGCGGTTTCGTTACTCGTAT TGGAACAGTAGCCAGAGTTACGTCTTAACCAAAGGTTGGAGCCGGTTCGTTAAGGAGAAG AATCTGAAAGCTGGTGACACGGTTTGTTTTCACCGGTCCACTGGACCGGACAAGCAGCTT TACATCGATTGGAAGACGAGGAATGTTGTTAACAACGAGGTCGCGTTGTTCGGACCGGTC GGACCGGTTGTCGAACCGATCCAGATGGTTCGGCTCTTTGGGGTTAACATTTTGAAACTA CCCGGTTCAGATACTATTGTTGGCAATAACAATAATGCAAGTGGGTGCTGCAATGGCAAG AGAAGAGAAATGGAACTGTTCTCGTTAGAGTGTAGCAAGAAACCTAAGATTATTGGTGCT tTGTAA

Loc100789009
Cover $44 \%$ identity $62 \%$

SEQ ID NO: 79
M DGGSVTDETTTTSNSLSVPANLSPPPLSLVGSGATAVVYPDGCCVSGEAESRKLPSSKY KGVVPQPNGRWGAQIYEKHQRVWLGTFNEEDEAARAYDIAAH RFRGRDAVTNFKPLAGAD DAEAEFLSTHSKSEIVDM LRKHTYDNELQQSTRGGRRRRDAETASSGAFDAKAREQLFEK TVTQSDVGKLNRLVIPKQHAEKHFPLSGSGGGALPCMAAAAGAKGMLLNFEDVGGKVWRF RYSYWNSSQSYVLTKGWSRFVKEKNLRAGDAVQFFKSTGLDRQLYDCKARSGKVN NNAA GLFIPVGPVVEPVQMVRLFGVDLLKLPVPGSDGIGVGCDGKRKEMELFAFECSKKLKVIG AL

50
SEQ ID NO: 80
ATGGATGGAGGCAGTGTCACAGACGAAACCACCACAACCAGCAACTCTCTTTCGGTTCCG
GCGAATCTATCTCCGCCGCCTCTCAGCCTTGTCGGCAGCGGCGCAACCGCCGTCGTCTAC
CCCGACGGTTGTTGCGTCTCCGGCGAAGCCGAATCCCGGAAACTCCCGTCCTCGAAATAC
AAAGGCGTGGTGCCGCAACCGAACGGTCGTTGGGGAGCTCAGATTTACGAGAAGCACCAG
CGCGTGTGGCTCGGCACCTTCAACGAGGAAGACGAAGCCGCCAGAGCCTACGACATCGCC
GCGCATCGCTTCCGCGGCCGCGACGCCGTCACTAACTTCAAGCCTCTCGCCGGCGCCGAC
GACGCCGAAGCCGAGTTCCTCAGCACGCATTCCAAGTCCGAGATCGTCGACATGCTCCGC
AAGCACACCTACGACAACGAGCTCCAGCAGAGCACCCGCGGCGGCAGGCGCCGCCGGGAC
GCCGAAACCGCGTCGAGCGGCGCGTTCGACGCGAAGGCGCGTGAGCAGCTGTTCGAGAAA
ACCGTTACGCAGAGCGACGTCGGGAAGCTGAACCGATTAGTGATACCAAAGCAGCACGCG
GAGAAGCACTTTCCGTTAAGCGGATCCGGCGGCGGAGCCTTGCCGTGCATGGCGGCGGCT
GCGGGGGCGAAGGGAATGTTGCTGAACTTTGAGGACGTTGGAGGGAAAGTGTGGCGGTTC
CGTTACTCGTATTGGAACAGTAGCCAGAGCTACGTGCTTACCAAAGGATGGAGCCGGTTC
GTTAAGGAGAAGAATCTTCGAGCTGGTGACGCGGTTCAGTTCTTCAAGTCGACCGGACTG
GACCGGCAACTATATATAGACTGCAAGGCGAGGAGTGGTAAGGTTAACAATAATGCTGCC
GGTTTGTTTATTCCGGTTGGACCGGTTGTTGAGCCGGTTCAGATGGTACGGCTTTTCGGG
GTCGACCTTTTGAAACTACCCGTACCCGGTTCGGATGGTATTGGGGTTGGCTGTGACGGG
AAGAGAAAAGAGATGGAGCTGTTTGCATTTGAATGTAGCAAGAAGTTAAAAGTAATTGGA
GCTTTGTAA

660503
Cover $36 \%$ identity $57 \%$

SEQ ID NO: 81
migvekvticmrievntekgrralmdcwqisgvhessdcseikfafdavvkrarheennaaaqkfkgvvsqqngnwgaqi yahqqriwl gtfksereaamaydsasiklrsgechrnfpwndqtvqepqfqshysaetvInmirdgtypskfatflktrqtqkgvakhiglkgddeeqfcct qlfqkeltpsdvgkInrlvipkkhavsyfpyvggsadesgsvdveavfydklmrlwkfrycywkssqsyvftrgwnrfvkdkklkakdviafft wgksggegeafalidviynnnaeedskgdtkqvlgnqlqlagseegededanigkdfnaqkglrlfgvcit

CDS SEQ ID NO: 82
atgattggagttgagaaagtgacaatttgtatgagaatagaggtgaatactgaaaagggaagaagggctttaatggactgttggcaaatatcag gagttcatgaaagttcagattgtagcgaaatcaaattgcattcgacgcagtagtaaaacgcgcgaggcatgaagagaataatgcagcagcac agaagttcaaaggcgttgtgtctcaacaaaatgggaactggggtgcacagatatatgcacaccagcagagaatctggttggggaccttcaaat ctgaaagagaggctgcaatggcttatgacagcgccagcataaaacttagaagcggagagtgccacagaaacttccatggaacgaccaaaca gttcaagagcctcagttccaaagccattacagcgcagaaacagtgctaaacatgattagagatggcacctatccatcaaaatttgctacatttctc aaaactcgtcaaacccaaaaaggcgttgcgaaacacataggtctgaagggtgatgacgaggaacagtttgttgcacccaacttttcagaagg aattaacaccaagtgatgtgggcaagctcaacaggcttgtcatcccaaagaagcatgcagttagctatttccttacgttggtggcagtgctgatg agagtggtagtgttgacgtggaggctgtgtttatgacaaactcatgcgattgtggaagttccgatactgctattggaagagcagccaaagttacg tgttcaccagaggctggaatcggtttgtgaaggataagaagttgaaggctaaagatgtcattgcgtttttacgtggggaaaaagtggaggaga gggagaagctttgcattgatcgatgtaatttataataataatgcagaagaagacagcaagggagacaccaaacaagtttgggaaaccaatta caattagctggcagtgaagaaggtgaagatgaagatgcaaacattggaaaggattcaatgcacaaaagggtctgaggctcttggtgtgtgta tcacctaa

Hordeum vulgare

MLOC_66387

Cover 47\% identity 64\%

SEQ ID NO: 83
M EFTATSSRFSKGEEEVEEEQEEASMREIPFMTPAAATCAAAPPSASASASTPASASGSS

M LOC44012
SEQ ID NO: 84

SEQ ID NO: 85

SEQ ID NO: 86 PPFRSGDDAGASGSGAGDGSRSNVAEAVEKEHM FDKVVTPSDVGKLNRLVI PKQYAEKYF PLDSAANEKGLLLNFEDSAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVSFS RGAGEAARHRLFI DWKRRADTRDPLRLPRLPLPM PLTSHYSPWGLGAGARGFFM PPSPPA TLYEHRLRQGFDFRGMNPSYPTMGRQVI LFGSAARM PPHGPAPLLVPRPPPPLHFTVQQQ GSDAGGSVTAGSPVVLDSVPVI ESPTTATKKRVRLFGVNLDNPQHPGDGGGESSNYGSAL PLQMPASAWRPRDHTLRLLEFPSHGAEASSPSSSSSSKREAHSGLDLDL


#### Abstract

ATGGAGTTTACTGCGACAAGCAGTAGGTTTTCTAAAGGAGAGGAGGAGGTGGAGGAGGAG CAGGAGGAGGCGTCGATGCGCGAGATCCCTTTCATGACGCCCGCGGCCGCCACCTGCGCC GCGGCGCCGCCTTCTGCTTCTGCGTCGGCCTCGACACCCGCGTCAGCGTCTGGAAGTAGC CСТСССТТTCGATCTGGGGATGACGCCGGAGCGTCGGGGAGCGGGGCCGGCGACGGCAGC CGCAGCAACGTGGCGGAGGCCGTGGAGAAGGAGCACATGTTCGACAAAGTGGTGACGCCG AGCGACGTGGGGAAGCTTAACCGGCTGGTCATCCCCAAGCAGTACGCCGAGAAGTACTTC CCGCTGGACTCGGCGGCCAACGAGAAGGGCCTTCTGCTCAACTTCGAGGACAGCGCCGGG AAG CCATGGCG CTTCCG CTATTCCTACTGGAAC A GCAGCCAGAGCTACGTCATGACCAAA GGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCTGGGGACACCGTCTCCTTCTCC CGCGGCGCCGGTGAGGCCGCGCGCCACCGCCTCTTCATCGACTGGAAGCGCCGAGCCGAC ACCAGAGACCCGCTCCGCTTGCCCCGCCTCCCGCTCCCGATGCCGCTGACGTCGCACTAC AGCCCGTGGGGCCTCGGCGCCGGCGCCAGAGGATTCTTCATGCCTCCCTCGCCGCCAGCC ACGCTCTACGAGCACCGTCTCCGTCAAGGCTTCGACTTCCGCGGCATGAACCCCAGTTAC CCCACAATGGGGAGACAGGTCATCCTTTTCGGCTCGGCCGCCAGGATGCCTCCGCACGGA CCAGCACCACTCCTCGTGCCGCGCCCGCCGCCGCCGCTGCACTTCACGGTGCAGCAACAA GGCAGCGACGCCGGCGGAAGTGTAACCGCAGGATCCCCAGTGGTGCTCGACTCAGTGCCG GTAATCGAAAGCCCCACGACGGCAACGAAGAAGCGCGTGCGCTTGTTCGGCGTGAACTTG GACAACCCGCAGCATCCCGGTGATGGCGGGGGCGAATCGAGCAATTATGGCAGTGCACTG CCATTGCAGATGCCCGCATCAGCATGGCGGCCAAGGGACCATACGCTGAGGCTGCTCGAA TTCCCCTCGCACGGTGCCGAGGCGTCGTCTCCATCGTCGTCGTCGTCTTCCAAGAGGGAG GCGCATTCGGGCTTGGATCTCGATCTGTGA


Cover 55\% identity 63\%

M LRKHTYFDELAQSKRAFAASAALSAPTTSGDAGGSASPPSPAAVREHLFDKTVTPSDVG KLNRLVIPKQNAEKHFPLQLPAGGGESKGLLLNFEDDAGKVWRFRYSYWNSSQSYVLTKG WSRFVKEKGLGAGDVVGFYRSAAGRTGEDSKFFI DCRLRPNTNTAAEADPVDQSSAPVQK AVRLFGVDLLAAPEQGM PGGCKRARDLVKPPPPKVAFKKQCIELALA

ATGCTCCGCAAGCACACCTACTTCGACGAGCTCGCCCAGAGCAAGCGCGCCTTCGCCGCG TCGGCCGCGCTCTCCGCGCCCACCACCTCGGGCGACGCCGGCGGCAGCGCCTCGCCGCCC TCCCCGGCCGCCGTGCGCGAGCACCTCTTCGACAAGACCGTCACGCCCAGCGACGTCGGC AAGCTGAACAGGCTGGTGATACCGAAGCAGAACGCCGAGAAGCACTTCCCGCTGCAGCTC

## 72

CCGGCCGGCGGCGGCGAGAGCAAGGGCCTGCTCCTCAACTTCGAGGACGATGCGGGCAAG GTGTGGCGGTTCCGCTACTCGTACTGGAACAGCAGCCAGAGCTACGTCCTCACCAAGGGC TGGAGCCGCTTCGTGAAGGAGAAGGGCCTCGGCGCCGGAGACGTCGTCGGGTTCTACCGC TCCGCCGCCGGGAGGACCGGCGAAGACAGCAAGTTCTTCATTGACTGCAGGCTGCGGCCG

MLOC_57250
Cover $50 \%$ identity $57 \%$

SEQ ID NO: 87
MYCSRGRIDPAEEGQVMGGLGVRDASWALFKVLEQSDVQVGQNRLLLTKEAVWGGPI PKL FPELEELRGDGLNAENRVAVKILDADGCEGDANFRYLNSSKAYRVMGPQWSRLVKETGMC KGDRLDLYAATATAASSCSGARAAVAPAPPGAIVKAAGF

CDS SEQ ID NO: 88
ATGTATTGTTCCCGCGGCCGCATCGATCCCGCGGAAGAAGGGCAGGTGATGGGCGGCCTC GGCGTGCGCGACGCCAGCTGGGCGCTGTTCAAGGTGTTGGAGCAGTCCGACGTCCAGGTG GGGCAGAACCGGCTGCTCCTCACCAAGGAGGCGGTGTGGGGCGGCCCTATCCCCAAGCTT TTCCCGGAGCTGGAGGAGCTCCGCGGCGACGGCCTCAACGCCGAGAACAGGGTCGCGGTC AAGATCCTCGACGCCGACGGCTGCGAGGGGGACGCCAACTTCCGCTACCTCAACTCCAGC AAGGCGTACCGGGTCATGGGGCCTCAGTGGAGCCGGCTCGTGAAGGAGACCGGCATGTGC AAGGGAGACCGCCTCGATCTGTACGCGGCAACGGCGACCGCTGCCTCTTCGTGTTCTGGA GCCAGGGCGGCTGTGGCGCCGGCGATACCTCCCGGAGCAATCGTGAAGGCAGCCGGGTTC TAA

MLOC_38822
Cover 47\% identity 56\%

SEQ ID NO: 89
MLRKH IYPDELAQHKRAFFFAAASSPTSSSSPLASPAPSAAAARREHLFDKTVTPSDVGK LNRLVIPKQHAEKHFPLQLPSASAAVPGECKGVLLNFDDATGKVWRFRYSYWNSSQSYVL TKGWSRFVKEKGLHAGDAVEFYRAASGN NQLFI DCKLRSKSTTTTTSVNSEAAPSPAPVT RTVRLFGVDLLIAPAARHAHEHEDYGMAKTNKRTMEASVAAPTPAHAVWKKRCVDFALTY RLATTPQCPRSRDQLEGVQAAGSTFAL

CDS SEQ ID NO: 90
ATGCTGCGCAAGCACATCTATCCCGACGAGCTCGCGCAGCACAAGCGCGCCTTCTTCTTC GCCGCGGCGTCGTCCCCTACGTCGTCGTCGTCACCTCTCGCCTCGCCGGCTCCTTCAGCC GCGGCGGCGCGGCGCGAGCACCTGTTCGACAAGACGGTCACGCCCAGCGACGTGGGGAAG CTGAACCGGCTGGTGATCCCCAAGCAGCACGCCGAGAAGCACTTCCCGCTGCAGCTCCCT TCTGCCAGCGCCGCCGTGCCAGGCGAGTGCAAGGGCGTGCTGCTCAACTTCGATGACGCG ACCGGCAAGGTGTGGAGGTTCCGGTACTCCTACTGGAACAGCAGCCAGAGCTACGTGCTC ACCAAGGGGTGGAGCCGCTTCGTGAAGGAGAAGGGCCTTCACGCCGGCGACGCCGTCGAG TTCTACCGCGCCGCCTCCGGCAACAACCAGCTCTTCATCGACTGCAAGCTCCGGTCCAAG AGCACCACGACGACGACCTCCGTCAACTCGGAGGCCGCCCCATCGCCGGCACCCGTGACG AGGACAGTGCGACTCTTCGGGGTCGACCTTCTCATCGCGCCGGCGGCGAGGCACGCGCAT GAGCACGAGGACTACGGCATGGCCAAGACAAACAAGAGAACCATGGAGGCCAGCGTAGCG GCGCCTACTCCGGCGCACGCGGTGTGGAAGAAGCGGTGCGTAGACTTCGCGCTGACCTAC

CGACTTGCCACCACCCCACAGTGCCCGAGGTCAAGAGATCAACTAGAAGGAGTACAAGCA GCTGGGAGTACATTTGCTCTATAG

MLOC_7940
Cover $49 \%$ identity $52 \%$

SEQ ID NO: 91
MGVEI LSSTGEHSSQYSSGAASTATTESGVGGRPPTAPSLPVSIADESATSRSASAQSTS SRFKGVVPQPNGRWGAQIYERHARVWLGTFPDEDSAARAYDVAALRYRGREAATNFPCAA AEAELAFLAAHSKAEIVDM LRKHTYTDELRQGLRRGRGMGARAQPTPSWAREPLFEKAVT PSDVGKLNRLVVPKQHAEKH FPLKRTPETTTTTGKGVLLNFEDGEGKVWRFRYSYWNSSQ SYVLTKGWSRFVREKGLGAGDSIVFSCSAYGQEKQFFIDCKKNKTMTSCPADDRGAATAS PPVSEPTKGEQVRVVRLFGVDIAGEKRGRAAPVEQELFKRQCVAHSQHSPALGAFVL

CDS SEQ ID NO: 92
ATGGGGGTGGAGATCCTGAGCTCAACGGGGGAACACTCCTCCCAGTACTCTTCCGGAGCC GCGTCCACGGCGACGACGGAGTCAGGCGTGGGCGGACGGCCGCCGACTGCGCCGAGCCTA CCTGTTTCCATCGCCGACGAGTCGGCGACCTCGCGGTCGGCATCGGCGCAGTCGACGTCG TCGCGGTTCAAGGGCGTGGTGCCGCAGCCCAACGGGCGGTGGGGCGCCCAGATCTACGAG CGCCACGCCCGCGTCTGGCTCGGCACGTTCCCGGACGAAGACTCTGCGGCGCGCGCCTAC GACGTGGCCGCGCTCCGGTACCGGGGCCGCGAGGCCGCCACCAACTTCCCGTGCGCGGCC GCCGAGGCGGAGCTCGCCTTCCTGGCGGCACACTCCAAGGCCGAGATCGTCGACATGCTC CGGAAGCACACCTACACCGACGAGCTCCGCCAGGGCCTGCGGCGCGGCCGCGGCATGGGG GCGCGCGCGCAGCCGACGCCGTCGTGGGCGCGGGAGCCCCTTTTCGAGAAGGCCGTGACC CCGAGCGACGTGGGCAAGCTCAACCGCCTCGTTGTGCCGAAGCAGCACGCCGAGAAGCAC TTCCCCCTGAAACGCACGCCGGAGACGACAACGACCACCGGCAAGGGGGTGCTTCTCAAC TTCGAGGATGGCGAGGGGAAAGTGTGGAGGTTCCGGTACTCGTATTGGAACAGCAGCCAG AGCTACGTGCTCACCAAGGGATGGAGCCGCTTCGTTCGGGAGAAGGGCCTCGGTGCCGGC GACTCCATCGTGTTCTCCTGCTCGGCGTACGGTCAGGAGAAGCAGTTCTTCATCGACTGC AAGAAGAACAAGACGATGACGAGCTGCCCCGCCGATGACCGCGGCGCCGCAACAGCGTCG CCGCCAGTGTCAGAGCCAACAAAAGGAGAACAAGTCCGTGTTGTGAGGCTGTTCGGCGTC GACATCGCCGGAGAGAAGAGGGGGCGAGCGGCGCCGGTGGAGCAGGAGTTGTTCAAGAGG CAATGCGTGGCACACAGCCAGCACTCTCCAGCCCTAGGTGCCTTCGTCTTATAG

MLOC_56567
Cover $42 \%$ identity $59 \%$

SEQ ID NO: 93
MGVEI LSSMVEHSFQYSSGASSATAESGAVGTPPRHLSLPVAIADESLTSRSASSRFKGV VPQPNGRWGAQIYERHARVWLGTFPDQDSAARAYDVASLRYRGGDAAFNFPCVVVEAELA FLAAHSKAEIVDM LRKQTYADELRQGLRRGRGMGVRAQPMPSWARVPLFEKAVTPSDVGK LNRLVVPKQHAEKHFPLKRSPETTTTTGNGVLLNFEDGQGKVWRFRYSYWNSSQSYVLTK GWSRFVREKGLGAGDSI MFSCSAYGQEKQFFIDCKKNTTVNGGKSASPLQVMEIAKAEQV RVVRLFGVDIAGVKRERAATAEQGPQGWFKRQCMAHGQHSPALGDFAL

SEQ ID NO: 94
ATGGGGGTGGAGATCCTGAGCTCCATGGTGGAGCACTCCTTCCAGTACTCTTCGGGGGCG TCCTCGGCCACCGCGGAGTCAGGCGCCGTCGGAACACCGCCGAGGCATCTGAGCCTACCT GTCGCCATCGCCGACGAGTCCCTGACCTCACGGTCGGCGTCGTCTCGGTTCAAGGGCGTG

GTGCCGCAGCCCAACGGGCGGTGGGGCGCCCAGATCTACGAGCGCCACGCTCGCGTCTGG CTCGGCACGTTCCCAGACCAGGACTCGGCGGCGCGCGCCTACGACGTTGCCTCGCTCAGG TACCGCGGCGGCGACGCCGCCTTCAACTTCCCGTGCGTGGTGGTGGAGGCGGAGCTCGCC TTCCTGGCGGCGCACTCCAAGGCTGAGATCGTTGACATGCTCCGGAAGCAGACCTACGCC GATGAACTCCGCCAGGGACTACGGCGCGGCCGTGGCATGGGGGTGCGCGCGCAGCCGATG CCGTCGTGGGCGCGGGTTCCCCTTTTCGAGAAGGCCGTGACCCCTAGCGATGTCGGCAAG CTCAATCGCCTGGTGGTGCCGAAGCAGCACGCCGAGAAGCACTTCCCCCTGAAGCGCAGC CCGGAGACGACGACCACCACCGGCAACGGCGTACTGCTCAACTTTGAGGACGGCCAGGGA AAAGTGTG GAGGTTCCGGTACTCATATTGGAACAGCAGCCAGAGCTACGTGCTCACCAAA GGCTGGAGCCGCTTCGTCCGGGAGAAGGGCCTCGGCGCCGGTGACTCCATCATGTTCTCC TGCTCGGCGTACGGGCAGGAGAAGCAGTTCTTCATCGACTGCAAGAAGAACACGACCGTG AACGGAGGCAAATCGGCGTCGCCGCTGCAGGTGATGGAGATTGCCAAAGCAGAACAAGTC CGCGTCGTTAGACTGTTCGGTGTCGACATCGCCGGGGTGAAGAGGGAGCGAGCGGCGACG GCGGAGCAAGGCCCGCAGGGGTGGTTCAAGAGGCAATGCATGGCACACGGCCAGCACTCT CCTGCCCTAGGTGACTTCGCCTTATAG

MLOC_75135
Cover $43 \%$ identity $57 \%$

SEQ ID NO: 95
MGMEILSSTVEHCSQYSSSASTATTESGAAGRSTTALSLPVAITDESVTSRSASAQPASS RFKGVVPQPNGRWGSQIYERHARVWLGTFPDQDSAARAYDVASLRYRGRDAATNFPCAAA EAELAFLTAHSKAEIVDMLRKHTYADELRQGLRRGRGMGARAQPTPSWARVPLFEKAVTP SDVGKLNRLVVPKQHAEKH FPLKCTAETTTTTGNGVLLNFEDGEGKVWRFRYSYWNSSQS YVLTKGWSSFVREKGLGAGDSIVFSSSAYGQEKQLFINCKKNTTMNGGKTALPLPVVETA KGEQDHVVKLFGVDIAGVKRVRAATGELGPPELFKRQSVAHGCGRM NYICYSIGTIGPLM LN

SEQ ID NO: 96
ATGGGGATGGAAATCCTGAGCTCCACGGTGGAGCACTGCTCCCAGTACTCTTCCAGCGCG TCCACGGCCACAACGGAGTCAGGCGCCGCCGGAAGATCGACGACGGCTCTGAGCCTACCA GTTGCCATCACCGACGAGTCCGTTACCTCGCGGTCGGCATCGGCGCAGCCGGCGTCATCA CGGTTCAAGGGCGTGGTGCCGCAGCCCAACGGGCGGTGGGGCTCCCAGATCTACGAGCGC CACGCTCGCGTCTGGCTCGGCACCTTCCCGGATCAGGACTCGGCGGCGCGTGCCTACGAC GTtGCCTCGCTCAGGTACCGGGGCCGCGATGCCGCCACCAACTTCCCGTGCGCCGCTGCG GAAGCGGAGCTCGCCTTCCTGACCGCGCACTCCAAGGCCGAGATCGTCGACATGCTCCGG AAGCACACCTACGCCGACGAACTCCGCCAGGGCCTGCGGCGCGGCCGCGGCATGGGTGCG CGCGCGCAGCCGACGCCGTCGTGGGCGCGGGTTCCCCTTTTCGAGAAGGCTGTGACCCCT AGCGATGTCGGCAAGCTCAATCGCCTGGTGGTGCCGAAGCAGCACGCCGAGAAGCACTTC CCCCTGAAGTGCACCGCAGAGACGACGACCACCACCGGCAACGGCGTGCTGCTAAACTTC GAGGATGGTGAGGGGAAGGTGTGGAGGTTCCGGTACTCGTATTGGAACAGTAGCCAGAGC TACGTGCTCACCAAAGGCTGGAGCAGCTTCGTCCGGGAGAAGGGCCTCGGCGCAGGCGAC TCCATCGTCTTCTCCTCCTCGGCGTACGGGCAGGAGAAGCAGTTATTCATCAACTGCAAA AAGAACACGACTATGAACGGCGGCAAAACAGCGTTGCCGCTGCCAGTGGTGGAGACTGCC AAAGGAGAACAAGACCACGTCGTTAAGTTGTTCGGTGTTGACATCGCCGGTGTGAAGAGG GTGCGAGCGGCGACGGGGGAGCTAGGCCCGCCGGAGTTGTTCAAGAGACAATCCGTGGCA CACGGATGCGGAAGGATGAACTACATTTGCTACTCCATAGGGACAATAGGACCTCTTATG CTCAACTGA

MLOC_63261
Cover 49\% identity 51\%

SEQ ID NO: 97
MASSKPTNPEVDNDMECSSPESGAEDAVESSSPVAAPSSRFKGVVPQPNGRWGAQIYEKH SRVWLGTFGDEEAAACAYDVAALRFRGRDAVTNHQRLPAAEGAGWSSTSELAFLADHSKA EIVDMLRKHTYDDELRQGLRRGHGRAQPTPAWAREFLFEKALTPSDVGKLNRLVVPKQHA

SEQ ID NO: 98
ATGGCGTCTAGCAAGCCGACAAACCCCGAGGTAGACAATGACATGGAGTGCTCCTCCCCG GAATCGGGTGCCGAGGACGCCGTGGAGTCGTCGTCGCCGGTGGCAGCGCCATCTTCGCGG TTCAAGGGCGTCGTGCCGCAGCCTAACGGGCGCTGGGGCGCGCAGATCTACGAGAAGCAC TCGCGGGTGTGGCTTGGCACGTTCGGGGACGAGGAAGCCGCCGCGTGCGCCTACGACGTG GCCGCGCTCCGCTTCCGCGGCCGCGACGCCGTCACCAACCACCAGCGCCTGCCGGCGGCG GAGGGGGCCGGCTGGTCGTCCACGAGCGAGCTCGCCTTCCTCGCCGACCACTCCAAGGCC GAGATCGTCGACATGCTCCGGAAGCACACCTACGACGACGAGCTCCGGCAGGGCCTGCGC CGCGGCCACGGGCGCGCGCAGCCCACGCCGGCGTGGGCGCGAGAGTTCCTCTTCGAGAAG GCCCTGACCCCGAGCGACGTCGGCAAGCTCAACCGCCTGGTCGTTCCGAAGCAGCACGCC GAGAAGCACTTCCCCCCGACGACGGCGGCGGCCGCCGGAAGCGACGGCAAGGGCTTGCTG CTCAACTTCGAGGACGGCCAAGGGAAGGTGTGGAGGTTCCGGTACTCATACTGGAACAGC AGCCAGAGCTACGTGCTCACCAAGGGCTGGAGCCGCTTCGTCCAAGAAAAGGGCCTCTGC GCCGGCGACACCGTGACGTTCTCCCGGTCGGCGTACGTGATGAATGACACGGATGAGCAG CTCTTCATCGACTACAAGCAGAGTAGCAAGAACGACGAAGCGGCCGACGTAGCCACTGCC GATGAGAATGAGGCCGGCCATGTCGCCGTGAAGCTCTTCGGGGTCGACATTGGCTGGGCT GGGATGGCGGGATCATCAGGTGGGTGA

MLOC_64708
Cover $49 \%$ identity $51 \%$

SEQ ID NO: 99
MLFDSSVSASLGTM RPLVKKLDMLLAPARGYSTLCKRI KEVM HLLKHDVEEISSYLDELT EVEDPPPMAKCWM NEARDLSYDMEDYI DSLLFVPPGHFI KKKKKKKKKGKKKMVI KKRLK WCKQIVFTKQVSDHGI KTSKIIHVNVPRLPNKPKVAKI ILQFRIYVQEAI ERYDKYRLHH CSTLRRRLLSTGSMLSVPIPYEEAAQIVTDGRMNEFISSLAANNAADQQQLKVVSVLGSG CLGKTTLANVLYDRIGMQFECRAFI RVSKKPDMKRLFRDLLSQFHQKQPLPTSCNELGIS DNI IKHLQDKRYLIVI DDLWDLSVWDII KYAFPKGNHGSRIIITTQI EDVALTCCCDHSE HVFEM KPLNIGHSRELFFNRLFGSESDCLEEFKRVSNEIVDICGGLPLATI NIASHLANQ WKDDLVKQLVAEGFIATREGKDQDQEM IEKAAGLCFDALI DRRFIQPIYTKYNNKVLSCT VHEVVHDLIAQKSAEENFIVVADHNRKN IALSHKVRRLSLIFGDTIYAKTPAN ITKSQIR SFRFFGLFECM PCITEFKVLRVLNLQLSGHRGDN DPIDLTGISELFQLRYLKITSDVCIK LPNQMQKLQYLETLDI MDAPRVTAVPWDI INLPHLLHLTLPVDTYLLDWISSMTDSVISL 5 WTLGKLNYLQH LHLTSSSTRPSYHLERSVEALGYLIGGHGKLKTIVVAHVSSAQNTVVRG APEVTISWDRMSPPPLLQRFECPHSCFIFYRI PKWVTELGN LCILKIAVKELHM ICLGTL RGLHALTDLSLYVETAPIDKII FDKAGFSVLKYCKLRFAAGIAWLKFEADAM PSLWKLM L VFNAIPRMDQNLVFFHHSRPAM HQRGGAVI IVEHMPGLRVISAKFGGAASDLEYASRTVV SNH PSN PTI NMQLVCYSSNGKRSRKRKQQPYDVVKGQPDEYAKRLERPAEKRISTPTKSS LRLHVPEITPKPMQITDNNVQRREH MFDTVLTRGDVGMLNRLVVPKKHAEKYFPLDSSST RTSKAIVLSFEDPAGKSWFFHYSYRSSSQNYVMFKGWTGFVKEKFLEAGDTVSFSRGVGE ATRGRLFIDCQNEQRYM FERVLTASDMESDGCSLMVPVNLVWPHPGLRKTI KGRHAVLQF EDGSGNGKVWPFQFEASGQYYLMKGLNYFVNDRDLAAGYTVSFYRAGTRLFVDSGRKDDK

VALGTRSRERIYPKIVRSQ

## Brassica rapa

LOC103849927
Cover $99 \%$ ident $80 \%$

CDS SEQ ID NO: 100
ATGTTGTTTGATAGTTCAGTGAGTGCTTCGTTGGGCACCATGAGACCACTTGTCAAGAAG CTCGACATGCTGCTAGCTCCTGCTCGGGGATACAGTACCTTGTGCAAGAGGATCAAGGAA GTGATGCACCTTCTCAAACATGATGTTGAAGAGATAAGCTCCTACCTTGATGAACTTACA GAGGTGGAGGACCCTCCACCAATGGCCAAGTGCTGGATGAACGAGGCACGCGACCTGTCT TATGATATGGAGGATTACATTGATAGCTTGTTATTTGTGCCACCTGGCCATTTCATCAAG AAGAAGAAGAAGAAGAAGAAGAAGGGAAAGAAGAAGATGGTGATAAAGAAGAGGCTCAAG TGGTGCAAACAGATCGTATTCACAAAGCAAGTGTCAGACCATGGTATCAAGACCAGTAAA ATCATTCATGTTAATGTCCCTCGTCTTCCCAATAAGCCCAAGGTTGCAAAAATAATATTA CAGTTCAGGATCTATGTCCAGGAGGCTATTGAACGGTATGACAAGTATAGGCTTCACCAT TGCAGCACCTTGAGGCGTAGATTGTTGTCCACTGGTAGTATGCTTTCAGTGCCAATACCC TATGAAGAAGCTGCCCAAATTGTAACTGATGGCCGGATGAATGAGTTTATCAGCTCACTG GCTGCTAATAATGCAGCAGATCAGCAGCAGCTCAAGGTGGTATCTGTTCTTGGATCTGGG tGTCTAGGTAAAACTACGCTTGCGAATGTGTTGTACGACAGAATTGGGATGCAATTCGAA TGCAGAGCTTTCATTCGAGTGTCCAAAAAGCCTGATATGAAGAGACTTTTCCGTGACTTG CTCTCGCAATTCCACCAGAAGCAGCCACTGCCTACCAGTTGTAATGAGCTTGGCATAAGT GACAATATCATCAAACATCTGCAAGATAAAAGGTATCTAATTGTTATTGATGATTTGTGG GATTTATCAGTATGGGATATTATTAAATATGCTTTTCCAAAGGGAAACCATGGAAGCAGA ATAATAATAACTACACAGATTGAAGATGTTGCATTAACTTGTTGCTGTGATCACTCGGAG CATGTTTTCGAGATGAAACCTCTCAACATTGGTCACTCAAGAGAGCTATTTTTTTAATAGA CTTTTTGGTTCTGAAAGTGACTGTCTTGAAGAATTCAAACGAGTTTCAAACGAAATTGTT GATATATGTGGTGGTTTACCGCTAGCAACAATCAACATAGCTAGTCATTTGGCAAACCAG GAGACAGAAGTATCATTGGATTTGCTAACAGACACACGTGATTTGTTGAGGTCCTGTTTG TGGTCAAATTCTACTTCAGAAAGAACAAAACAAGTACTGAACCTCAGCTACAGTAATCTT CCTGATTATCTGAAGACATGTTTGCTGTATCTTCATATGTATCCAGTGGGCTCCATAATC TGGAAGGATGATCTGGTGAAGCAATTGGTGGCTGAAGGGTTTATTGCTACAAGAGAAGGG AAAGACCAAGACCAAGAAATGATAGAGAAAGCTGCAGGACTCTGTTTCGATGCACTTATT GATAGAAGATTCATCCAGCCTATATATACCAAGTACAACAATAAGGTGTTGTCCTGCACG GTTCATGAGGTGGTACATGATCTTATTGCCCAAAAGTCTGCTGAAGAGAATTTCATTGTG GTAGCAGACCACAATCGAAAGAATATAGCACTTTCTCATAAGGTTCGTCGACTATCTCTC ATCTTTGGCGACACAATATATGCCAAGACACCAGCAAACATCACAAAGTCACAAATTCGG TCATTCAGATTTTTTG GATTATTCGAGTGTATGCCTTGTATTACAGAGTTCAAG GTTCTC CGTGTTCTAAACCTTCAACTATCTGGTCATCGTGGGGACAATGACCCTATAGACCTCACT GGGATTTCAGAACTGTTTCAGCTGAGATATTTAAAGATTACAAGTGATGTGTGCATAAAA CTACCAAATCAAATGCAAAAACTGCAATATTTGGAAACGTTGGACATTATGGATGCACCA AGAGTCACTGCTGTTCCATGGGATATTATAAATCTCCCACACCTGTTGCACCTGACTCTT CCTGTTGATACATATCTGCTGGATTGGATTAGCAGCATGACTGACTCCGTCATCAGTCTG TGGACCCTTGGCAAGCTGAACTACCTGCAGCATCTTCATCTTACTAGTTCTTCTACACGT CCTTCATACCATCTGGAGAGAAGTGTGGAGGCTCTGGGTTATTTGATCGGAGGACATGGC AAG CTGAAAACTATAGTAGTCGCTCATGTCTCCTCTGCTCAAAATACTGTGGTTCGTGGC GCCCCAGAAGTAACCATTTCATGGGATCGTATGTCACCTCCCCCCCTTCTCCAGAGATTC GAATGCCCACACAGCTGCTTCATATTTTACCGAATTCCTAAGTGGGTTACAGAACTTGGC AACCTGTGCATTTTGAAGATTGCAGTGAAGGAGCTTCATATGATTTGTCTTGGTACTCTC AGAGGATTGCATGCCCTCACTGATCTGTCGCTGTATGTGGAGACAGCGCCCATTGACAAG ATCATCTTTGACAAGGCCGGGTTCTCAGTTCTCAAGTACTGCAAATTGCGCTTCGCGGCT

GGTATAGCTTGGCTGAAATTTGAGGCTGATGCAATGCCTAGTCTATGGAAACTGATGCTA GTTTTCAACGCCATCCCACGAATGGACCAAAATCTTGTTTTCTTTCACCACAGCCGACCG GCGATGCATCAACGTGGTGGTGCAGTAATCATTGTCGAGCATATGCCAGGGCTTAGAGTG ATCTCCGCAAAATTTGGGGGCGCAGCTTCTGATCTAGAGTATGCTTCGAGGACCGTCGTT AGTAACCATCCAAG CAATCCTACAATCAACATGCAATTGGTGTGTTATAGTTCCAATGGT AAGAGAAGCAGAAAAAGGAAACAACAACCTTACGACGTTGTGAAGGGACAACCAGATGAA TACGCCAAGAGATTGGAGAGACCAGCTGAGAAAAGGATTTCAACGCCGACAAAGTCTTCT TTGCGTCTGCATGTTCCAGAAATTACACCAAAACCTATGCAGATTACAGACAACAATGTT CAGAGGAGGGAGCACATGTTCGATACGGTTCTGACTCGGGGGGACGTGGGGATGCTGAAC CGGCTGGTGGTACCGAAGAAGCACGCGGAGAAGTACTTCCCGCTGGACAGTTCCTCCACC CGCACCAGCAAGGCCATCGTACTCAGCTTTGAGGACCCTGCTGGGAAGTCATGGTTCTTC CACTACTCCTACCGGAGCAGCAGCCAGAACTACGTCATGTTCAAGGGGTGGACTGGCTTC GTCAAGGAGAAGTTTCTCGAAGCCGGCGACACCGTCTCCTTCAGCCGCGGCGTCGGGGAG GCCACGAGGGGGAGGCTCTTCATCGACTGTCAAAATGAGCAGAGGTACATGTTCGAGCGA GTGCTGACGGCGAGTGATATGGAGTCGGATGGCTGCTCGCTGATGGTCCCAGTGAACTTG GTGTGGCCGCACCCCGGCCTCCGCAAGACGATCAAGGGGAGGCACGCCGTGCTGCAGTTT GAGGACGGCAGCGGCAACGGGAAGGTGTGGCCATTTCAGTTTGAGGCCTCCGGCCAATAC TATCTCATGAAGGGCTTGAACTACTTTGTTAACGACCGCGACCTTGCGGCTGGCTATACC GTCTCCTTCTACCGCGCCGGCACGCGGTTGTTCGTCGACTCCGGGCGTAAAGATGACAAA GTAGCCTTGGGAACCAG AAG CCGCGAAAG GATCTATCCTAAGATCGTGCGGTCGCAGTAG

LOC103849927

SEQ ID NO: 101
msgnhysrdihhntpsvhhhqnyavvdreylfeksltpsdv gklnrlvipkqhaekhfplnnagddvaaaettekgmlltfedesgkcwkf rysywnssqsyvltkgwsryvkdkhlhagdvvffqrhrfdlhrvfigwrkrgevssptavsvvsqearvnttaywsglttpyrqvhastssyp nihqeyshygavaeiptvvtgssrtvrlfgvnlechgdvvetppcpdgyngqhfyyystpdpmnisfageameqvgdgrr

Bra034828
Cover 100\% identity 79\%

SEQ ID NO: 102
MSVNHYSNTLSSHNH HNEH KESLFEKSLTPSDVGKLNRLVIPKQHAERYLPLNNCGGGGD VTAESTEKGVLLSFEDESGKSWKFRYSYWNSSQSYVLTKGWSRYVKDKHLNAGDVVLFQR HRFDI HRLFIGWRRRGEASSSSAVSAVTQDPRANTTAYWNGLTTPYRQVHASTSSYPNNI HQEYSHYGPVAETPTVAAGSSKTVRLFGVNLECHSDVVEPPPCPDAYNGQH IYYYSTPHP M NISFAGEAM EQVGDGRG

CDS SEQ ID NO: 103
ATGTCAGTCAACCATTACTCAAACACTCTCTCGTCGCACAATCACCACAACGAACATAAA GAGTCTTTGTTCGAGAAGTCACTCACGCCAAGCGATGTTGGAAAGCTAAACCGTTTAGTC ATACCAAAACAACACGCCGAGAGATACCTCCCTCTCAATAATTGCGGCGGCGGCGGCGAC GTGACGGCGGAGTCGACGGAGAAAGGGGTGCTTCTCAGCTTCGAGGACGAGTCGGGAAAA TCTTGGAAATTCAGATACTCATATTGGAACAGTAGTCAAAGCTACGTGTTGACCAAAGGA TGGAGCAGGTACGTCAAAGACAAGCACCTCAACGCAGGGGACGTCGTTTTATTTCAACGG CACCGTTTTGATATTCATAGACTCTTCATTGGCTGGAGGAGACGCGGAGAGGCTTCTTCC TCTTCCGCCGTTTCCGCCGTGACTCAAGATCCTCGAGCTAACACGACGGCGTACTGGAAC GGTTTGACTACACCTTATCGTCAAGTACACGCGTCAACTAGTTCTTACCCTAACAACATC CACCAAGAGTATTCACATTATGGCCCTGTTGCTGAGACACCGACGGTAGCTGCAGGGAGC TCGAAGACGGTGAGGCTATTTGGAGTTAACCTCGAATGTCACAGTGACGTTGTGGAGCCA CCACCGTGTCCTGACGCCTACAACGGCCAACACATTTACTATTACTCAACTCCACATCCC

ATGAATATCTCATTTGCTGGAGAAGCAATGGAGCAGGTAGGAGATGGACGAGGTTGA

Bra005886
Cover 100\% identity 79\%

SEQ ID NO: 104
MSVN HYSTDH HQVHH HHTLFLQNLHTTDTSEPTTTAATSLREDQKEYLFEKSLTPSDVGK LNRLVIPKQHAEKYFPLNTI ISNNAEEKGMLLSFEDESGKCWRFRYSYWNSSQSYVLTKG WSRYVKDKQLDPADVVFFQRQRSDSRRLFIGWRRRGQGSSSAANTTSYSSSMTAPPYSNY SNRPAHSEYSHYGAAVATATETHFPSSSAVGSSRTVRLFGVNLECQMDEDEGDDSVATA AAAECPRQDSYYDQN MYNYYTPHSSAS

CDS 105

ATGTCAGTCAACCATTACTCCACGGACCACCACCAGGTCCACCACCACCACACTCTCTTC TTGCAGAACCTCCACACCACCGACACATCGGAGCCAACCACAACCGCCGCCACATCACTC CGCGAAGACCAGAAAGAGTATCTCTTCGAGAAATCTCTCACACCAAGCGACGTTGGCAAA CTCAACCGTCTCGTTATACCAAAACAGCACGCGGAGAAGTACTTCCCTCTCAACACCATC ATCTCCAATAATGCTGAGGAGAAAGGGATGCTTCTAAGCTTCGAAGACGAGTCAGGCAAG TGCTGGAGGTTCAGATACTCTTACTGGAACAGCAGTCAAAGCTACGTGTTGACTAAAGGA TGGAGCAGATACGTCAAAGACAAACAGCTCGACCCAGCCGATGTTGTTTTCTTCCAACGT CAACGTTCTGATTCCCGGAGACTCTTTATTGGCTGGCGTAGACGCGGTCAAGGCTCCTCC TCCGCCGCGAATACGACGTCGTATTCTAGTTCCATGACTGCTCCACCGTATAGTAATTAC TCTAATCGTCCTGCTCACTCAGAGTATTCCCACTATGGCGCCGCCGTAGCAACAGCGACG GAGACGCACTTCATACCATCGTCTTCCGCCGTCGGGAGCTCGAGGACGGTGAGGCTTTTT GGTGTGAATTTGGAGTGTCAAATGGATGAAGACGAAGGAGATGATTCGGTTGCCACGGCA GCCGCCGCTGAGTGTCCTCGTCAGGACAGCTACTACGACCAAAACATGTACAATTATTAC ACTCCTCACTCCTCAGCCTCATAA

Bra005301
Cover 100\% identity 58\%
SEQ ID NO: 106
MSI NQYSSDFNYHSLMWQQQQHRHHHHQNDVAEEKEALFEKPLTPSDVGKLNRLVIPKQH AERYFPLAAAAADAMEKGLLLCFEDEEGKPWRFRYSYWNSSQSYVLTKGWSRYVKEKQLD AGDVI LFHRHRVDGGRFFIGWRRRGNSSSSSDSYRHLQSNASLQYYPHAGVQAVESQRGN SKTLRLFGVNMECQLDSDLPDPSTPDGSTICPTSHDQFH LYPQQHYPPPYYMDISFTGDV HQTRSPQG

CDS SEQ ID NO: 107
ATGTCAATAAACCAATACTCAAGCGATTTCAACTACCACTCTCTCATGTGGCAACAACAG CAGCACCGCCACCACCACCATCAAAACGACGTCGCGGAGGAAAAAGAAGCTCTTTTCGAG AAACCCTTAACCCCAAGTGACGTCGGAAAACTCAACCGCCTCGTCATCCCAAAACAGCAC GCCGAGAGATACTTCCCTCTCGCAGCAGCCGCCGCAGACGCGATGGAGAAGGGATTACTT CTCTGCTTCGAGGACGAGGAAGGTAAGCCATGGAGATTCAGATACTCGTATTGGAACAGT AGCCAGAGTTATGTCTTGACCAAAGGATGGAGCAGATACGTCAAGGAGAAGCAGCTCGAC GCCGGTGACGTCATTCTCTTCCACCGCCACCGTGTTGACGGAGGAAGATTCTTCATTGGC TGGAGAAGACGCGGCAACTCTTCCTCCTCTTCCGACTCTTATCGCCATCTTCAGTCCAAT GCCTCGCTCCAATATTATCCTCATGCAGGAGTTCAAGCGGTGGAGAGCCAGAGAGGGAAT

TCGAAGACATTAAGACTGTTCGGAGTGAACATGGAGTGTCAGCTAGACTCCGACTTGCCC GATCCATCTACACCAGACGGTTCCACCATATGTCCGACCAGTCACGACCAGTTTCATCTC TACCCTCAACAACACTATCCTCCTCCGTACTACATGGACATAAGTTTCACAGGAGATGTG CACCAGACGAGAAGCCCACAAGGATAA

Bra017262
Cover 92\% identity 56\%

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

CDS SEQ ID NO: 109
ATGTCAATAAATCAATATTCAAGCGAGTTCTACTACCATTCTCTCATGTGGCAACAACAG CAGCAACACCACCATCAAAACGAAGTCGTGGAGGAAAAAGAAGCTCTTTTTCGAGAAACCC TTAACCCCAAGTGACGTCGGAAAACTAAACCGCCTAGTCATCCCTAAACAGCACGCCGAG AGATACTTCCCTCTCGCCGCCGCCGCGGTAGACGCCGTGGAGAAGGGATTACTCCTCTGC tTCGAGGACGAGGAAGGTAAGCCATGGAGATTCAGATACTCTTATTGGAATAGTAGCCAG AGTTACGTCTTGACCAAAGGATGGAGCAGATATGTTAAAGAGAAGCAACTTGACGCCGGC GACGTTGTTCTCTTTCATCGCCACCGTGCTGACGGTGGAAGATTCTTCATTGGCTGGAGA AGACGCGGCGACTCTTCCTCCTCCTCCGACTCTTATCGCAATCTTCAATCTAATTCCTCG CTCCAATATTATCCTCATœAGGGGCTCAAGCGGTGGAGAACCAG AGAGGTAACTCCAAG ACATTGAGACTTTTTGGAGTGAACATGGAGTGCCAGATAGACTCAGACTGGTCCGAGCCA TCCACACCTGACGGTTTTACCACATGTCCAACCAATCACGACCAGTTTCCTATCTACCCT GAACACTTTCCTCCTCCGTACTACATGGACGTAAGTTTCACAGGAGATGTGCACCAGACG AGTAGCCAACAAGGATAG

Bra000434
Cover 96\% identity 47\%

SEQ ID NO: 110
MMTNLSLAREGEEEEEEAGAKKPTEEVEREHM FDKVVTPSDVGKLNRLVI PKQHAERYFP LDSSTNEKGLI LNFEDLTGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFLR CVGDTGRDSRLFIDWRRRPKVPDYTTSTSHFPAGAMFPRFYSFQTATTSTSYNPYNHQQP RHHHSGYCYPQI PREFGYGYVVRSVDQRAVVADPLVI ESVPVM MHGGARVNQAAVGTAGK RLRLFGVDMECGESGGTNSTEEESSSSGGSLPRGGASPSSSMFQLRLGNSSEDDH LFKKG KSSLPFNLDQ

SEQ ID NO: 111
ATGATGACAAATTTGTCTCTTGCAAGAGAAGGAGAAGAAGAAGAAGAAGAGGCAGGAGCA AAGAAGCCCACAGAAGAAGTGGAGAGAGAGCACATGTTCGACAAAGTGGTGACTCCAAGT GACGTCGGGAAACTAAACCGACTCGTGATCCCAAAGCAACACGCGGAGAGATACTTCCCT TTAGATTCATCCACAAACGAGAAGGGTTTGATTCTAAACTTCGAAGATCTCACGGGAAAG TCATGGAGGTTCCGTTACTCTTACTGGAACAGCAGTCAGAGCTATGTCATGACTAAAGGT TGGAGCCGTTTCGTTAAAGACAAGAAGCTAGACGCTGGAGATATTGTCTCTTTCCTGAGA TGTGTCGGAGACACAGGAAGGGACAGCCGCTTGTTTATCGATTGGAGGAGACGACCTAAA


#### Abstract

GTCCCTGACTACACGACATCGACTTCTCACTTTCCTG CCG GAGCTATGTTCCCTAGGTTT TACAGTTTTCAGACAGCAACTACTTCCACAAGTTACAATCCCTATAATCATCAGCAGCCA CGTCATCATCACAGTGGTTACTGTTATCCTCAAATCCCGAGAGAATTTGGATATGGGTAT GTCGTTAGGTCAGTAGATCAGAGGGCGGTGGTGGCTGATCCGTTAGTGATCGAATCTGTG CCGGTGATGATGCACGGAGGAGCTCGAGTGAACCAGGCGGCTGTTGGAACGGCCGGGAAA AGGCTGAGGCTTTTTGGAGTCGATATGGAATGTGGCGAGAGTGGAGGAACAAACAGTACG GAGGAAGAATCTTCATCTTCCGGTGGGAGTTTGCCACGTGGCGGTGCTTCTCCGTCTTCC TCTATGTTTCAGCTGAGGCTTGGAAACAGCAGTGAAGATGATCACTTATTTAAGAAAGGA AAGTCTTCATTGCCTTTTAATTTGGATCAATAA

Bra040478 Cover 96\% identity 48\%


SEQ ID NO: 112
MMTNLSLAREGEAQVKKPI EEVEREHMFDKVVTPSDVGKLNRLVIPKQHAERYFPLDSSS NEKGLLLNFEDLTGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRCVGDS RLFI DWRRRPKVPDYPTSTAHFAAGAMFPRFYSFPTATTSTCYDLYNHQPPRHHHIGYGY PQI PREFGYGYFVRSVDQRAVVADPLVI ESVPVMMRGGARVSQEVVGTAGKRLRLFGVDM EEESSSSGGSLPRAGGGGASSSSSLFQLRLGSSCEDDH FSKKGKSSLPFDLDQ

QID NO: 113
ATGATGACCAACTTGTCTCTTGCAAGGGAAGGAGAAGCACAAGTAAAGAAGCCCATAGAA GAAGTTGAGAGAGAGCACATGTTCGACAAAGTGGTGACTCCAAGCGACGTAGGGAAACTA AACAGACTCGTGATCCCAAAGCAACACGCAGAGAGATACTTCCCTCTAGATTCATCCTCA AACGAGAAAGGTTTGCTTCTAAACTTTGAAGATCTAACAGGAAAGTCATGGAGGTTCCGT TACTCTTACTGGAACAGTAGCCAGAGCTATGTCATGACTAAAGGTTGGAGTCGTTTCGTT AAAGACAAGAAGCTTGACGCCGGAGATATTGTCTCTTTCCAGAGATGTGTCGGAGACAGC CGCTTGTTTATCGATTGGAGGAGACGACCTAAAGTCCCTGACTATCCGACATCGACTGCT CACTTTG CTG CAGGAG CTATGTTCCCTAGGTTTTACAGTTTTCCG ACAGCAACTACTTCG ACATGTTACGATCTGTACAATCATCAGCCGCCACGTCATCATCACATTGGTTACGGTTAT CCACAGATTCCGAGAGAATTTGGATACGGGTATTTCGTTAGGTCAGTGGACCAGAGAGCG GTGGTGGCTGATCCGTTGGTGATCGAATCTGTGCCGGTGATGATGCGCGGAGGAGCTCGA GTTAGTCAG GAG GTTGTTGGAACGGCCGGGAAGAGG CTGAGG CTTTTTGGAGTCGATATG GAGGAAGAATCTTCATCTTCCGGTGGGAGTTTGCCGCGTGCCGGAGGTGGCGGTGCTTCT TCATCTTCCTCTTTGTTTCAGCTGAGACTTGGGAGCAGCTGTGAAGATGATCACTTCTCT AAGAAAGGAAAGTCTTCATTGCCTTTTGATTTGGATCAATAA

Bra004501
Cover 74\% identity 45\%

SEQ ID NO: 114
M M MTNLSLSREGEEEEEEEQEEAKKPM EEVEREHM FDKVVTPSDVGKLNRLVI PKQYAER YFPLDSSTNEKGLLLNFEDLAGKSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVS FQRCVGDSGRDSRLFIDWRRRPKVPDHPTSIAHFAAGSM FPRFYSFPTATSYNLYNYQQP RHHHHSGYNYPQI PREFGYGYLVDQRAVVADPLVIESVPVMM HGGAQVSQAVVGTAGKRL RLFGVDMEEESSSSGGSLPRGDASPSSSLFQLRLGSSSEDDH FSKKGKSSLPFDLDQ

SEQ ID NO: 133
ATGATGATGACAAACTTGTCTCTTTCAAGAGAAGGAGAAGAGGAGGAAGAAGAAGAACAA GAAGAGGCCAAGAAGCCCATGGAAGAAGTAGAGAGAGAGCACATGTTCGACAAAGTGGTG ACTCCAAGCGATGTTGGTAAACTAAACCGGCTCGTGATCCCAAAGCAATACGCAGAGAGA


#### Abstract

TACTTCCCTTTAGATTCATCCACAAACGAGAAAGGTTTGCTTCTAAACTTCGAAGATCTC GCAGGAAAGTCATGGAGGTTCCGTTACTCTTACTGGAACAGTAGTCAGAGCTATGTCATG ACTAAAGGTTGGAGCCGTTTCGTTAAAGACAAAAAGCTAGACGCCGGAGATATTGTCTCT TTCCAGAGATGTGTCGGAGATTCAGGAAGAGACAGCCGCTTGTTTATTGATTGGAGGAGA AGACCTAAAGTTCCTGACCATCCGACATCGATTGCTCACTTTGCTGCCGGATCTATGTTT CCTAGGTTTTACAGTTTTCCGACAGCAACTAGTTACAATCTTTACAACTATCAGCAGCCA CGTCATCATCATCACAGTGGTTATAATTATCCTCAAATTCCGAGAGAATTTGGATACGGG TACTTGGTGGATCAAAGAGCCGTGGTGGCTGATCCGTTGGTGATTGAATCTGTGCCGGTG ATGATGCACGGAGGAGCTCAAGTTAGTCAGGCGGTTGTTGGAACGGCCGGGAAGAGGCTG AGGCTTTTTGGAGTCGATATGGAGGAAGAATCTTCATCTTCCGGTGGGAGTTTGCCACGT GGTGACGCTTCTCCGTCTTCCTCTTTGTTTCAGCTGAGACTTGGAAGCAGCAGTGAAGAT GATCACTTCTCTAAGAAAGGAAAGTCCTCATTGCCTTTTGATTTGGATCAATAA


Bra003482
Cover 79\% identity 44\%

SEQ ID NO: 115
MNQEEENPVEKASSM EREHM FEKVVTPSDVGKLNRLVIPKQHAERYFPLDNNSDSSKGLL LNFEDRTGNSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRDPGNKDKLFI D WRRRPKI PDHHHQFAGAMFPRFYSFSHPQNLYHRYQQDLGIGYYVSSMERNDPTAVI ESV PLI MQRRAAHVAAI PSSRGEKRLRLFGVDM ECGGGGGSVNSTEEESSSSGGGGGVSMASV GSLLQLRLVSSDDESLVAMEAASVDEDHHLFTKKGKSSLSFDLDRK

## SEQ ID NO: 116

ATGAATCAAGAAGAAGAGAATCCTGTGGAAAAAGCCTCTTCAATGGAGAGAGAGCACATG TTTGAAAAAGTAGTAACACCAAGCGACGTAGGCAAACTAAACCGACTCGTGATCCCAAAG CAACACGCGGAGAGATACTTCCCTTTAGACAACAATTCTGACAGCAGCAAAGGTTTGCTT CTAAACTTCGAAGACCGAACAGGAAACTCATGGAGATTCCGTTACTCTTACTGGAACAGT AGCCAGAGTTATGTCATGACAAAAGGTTGGAGCCGCTTCGTCAAAGACAAGAAGCTTGAT GCTGGCGACATCGTTTCTTTTCAGAGAGATCCTGGTAATAAAGACAAGCTTTTCATTGAT TGGAGGAGACGACCAAAGATTCCAGATCATCATCATCAATTCGCTGGAGCTATGTTCCCT AGGTTTTACTCTTTCTCTCATCCTCAGAACCTTTATCATCGATATCAACAAGATCTTGGA ATTGGGTATTATGTGAGTTCAATGGAGAGAAATGATCCAACGGCTGTAATTGAATCTGTG CCGTTGATAATGCAAAGGAGAGCAGCACACGTGGCTGCTATACCTTCATCAAGAGGAGAG AAGAGGTTAAGGCTGTTTGGAGTGGACATGGAGTGCGGCGGCGGCGGAGGAAGTGTGAAT AGCACGGAGGAAGAGTCGTCGTCTTCCGGTGGTGGCGGCGGCGTTTCTATGGCTAGTGTT GGTTCTCTTCTCCAATTGAGGCTAGTGAGCAGTGATGATGAGTCTTTGGTAGCAATGGAA GCTG CAAGTGTCGATGAGGATCATCACTTGTTTACAAAGAAAG GAAAGTCTTCTTTGTCT TTCGATTTGGATAGAAAATGA

Bra007646
Cover 74\% identity 45\%

SEQ ID NO: 117
MNQEN KKPLEEASTSMEREN MFDKVVTPSDVGKLNRLVIPKQHAERYFPLDNSSTNNKGL LLDFEDRTGSSWRFRYSYWNSSQSYVMTKGWSRFVKDKKLDAGDIVSFQRDPCNKDKLYI DWRRRPKIPDHHQFAGAMFPRFYSFPHPQM PTSFESSHNLYHHRFQRDLGIGYYPTAVIE SVPVI MQRREAQVAN MASSRGEKRLRLFGVDVECGGGGGGSVNSTEEESSSSGGSMSRGG VSMAGVGSLLQLRLVSSDDESLVAMEGATVDEDHH LFTTKKGKSSLSFDLDI

CDS SEQ ID NO: 118
ATGAATCAAGAAAACAAGAAGCCTTTGGAAGAAGCTTCGACTTCAATGGAGAGAGAGAAC ATGTTCGACAAAGTAGTAACACCAAGCGACGTAGGGAAACTAAACCGACTCGTGATCCCA AAGCAACACGCAGAGAGATACTTCCCTTTAGACAACTCCTCAACAAACAACAAAGGGTTG CTTCTAGACTTCGAAGACCGTACAGGAAGCTCATGGAGATTCCGTTACTCTTACTGGAAC AGTAGCCAAAGTTATGTCATGACAAAAGGTTGGAGCCGTTTTGTCAAAGACAAGAAGCTT GATGCTGGTGACATCGTGTCTTTTCAAAGAGATCCCTGTAATAAAGACAAGCTTTACATA GATTGGAGGAGACGACCAAAGATTCCAGATCATCATCAGTTCGCCGGAGCTATGTTCCCT AGGTTTTACTCTTTCCCTCACCCTCAGATGCCGACAAGTTTTGAAAGTAGTCACAACCTT TATCATCATCGGTTTCAACGAGATCTTGGAATTGGGTATTATCCAACGGCTGTGATTGAA TCTGTGCCGGTGATAATGCAAAGGAGAGAAGCACAAGTGGCTAATATGGCTTCATCAAGA GGAGAGAAGAGGTTAAGGCTGTTTGGAGTGGACGTGGAGTGCGGCGGCGGAGGAGGAGGA AGTGTGAATAGCACGGAGGAAGAGTCGTCGTCTTCCGGTGGTAGTATGTCACGTGGCGGC GTTTCTATGGCTGGTGTTGGTTCTCTCCTTCAGTTGAGGTTAGTGAGCAGTGATGATGAG TCTTTAGTAGCGATGGAAGGTGCTACTGTCGATGAGGATCATCACTTGTTTACAACTAAG AAAGGAAAGTCTTCTTTGTCTTTCGATTTGGATATATGA

Bra014415
Cover 48\% identity 60\%
SEQ ID NO: 119
MERKSNDLERSENI DSQNKKMNLEEERPVQEASSMEREHM FDKVVTPSDVGKLNRLVI PK QHAERYFPLDNNSSDNN KGLLLNFEDRIGI LWSFRYSYWNSSQSYVMTKGWSRFVKDKKL DAGDIVSFHRGSCNKDKLFI DWKRRPKI PDHQVVGAM FPRFYSYPYPQIQASYERHNLYH RYQRDIGIGYYVRSM ERYDPTAVIESVPVIMQRRAHVATMASSRGEKRLRLFGVDM ECVR GGRGGGGSVNSTEEESSTSGGSISRGGVSMAGVGSPLQLRLVSSDGDDQSLVARGAARVD EDHH LFTKKGKSSLSFDLDK

CDS SEQ ID NO: 120
ATGGAGAGGAAGTCCAATGATCTTGAGAGATCTGAGAATATTGATTCTCAAAACAAGAAG ATGAATCTAGAAGAAGAGAGGCCTGTACAAGAAGCTTCTTCGATGGAGAGAGAGCACATG TTCGACAAAGTAGTAACACCAAGCGACGTTGGGAAACTAAACCGGCTGGTGATCCCAAAG CAACACGCAGAGCGATACTTCCCTTTAGACAATAATTCCTCAGACAACAACAAAGGTTTG CTTCTAAACTTCGAAGATCGAATAGGAATCTTATGGAGTTTCCGTTACTCCTACTGGAAC AGTAGCCAAAGTTATGTAATGACTAAAGGCTGGAGCCGTTTCGTCAAAGACAAGAAGCTT GATGCTGGCGACATAGTTTCTTTTCATAGAGGTTCTTGTAATAAAGACAAGCTTTTCATT GATTGGAAGAGACGACCAAAGATTCCTGATCACCAAGTCGTCGGAGCTATGTTCCCTAGG TTTTACTCTTACCCTTATCCTCAGATACAGGCTAGTTATGAACGTCACAACCTTTATCAT CGATATCAACGAGATATAGGAATTGGGTATTATGTGAGGTCAATGGAGAGATATGATCCA ACGGCTGTAATTGAATCTGTGCCGGTGATAATGCAAAGGAGAGCACATGTGGCTACTATG GCTTCATCAAGAGGAGAGAAGAGGTTAAGGCTTTTTGGAGTGGATATGGAGTGCGTCAGA GGCGGCCGAGGAGGAGGAGGAAGTGTGAATAGCACGGAGGAAGAGTCTTCGACTTCCGGT GGTAGTATCTCACGTGGCGGCGTTTCTATGGCTGGTGTTGGCTCTCCACTCCAGTTGAGG TTAGTGAGCAGTGACGGTGATGATCAGTCTCTAGTAGCTAGGGGAGCTGCTAGGGTTGAT GAGGATCATCACTTGTTTACAAAGAAAGGAAAGTCTTCTTTGTCTTTCGATTTGGATAAA TGA

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

DSRKLSSSKYKGVVPQPNGRWGAQIYVKHQRVWLGTFCDEEEAAHSYDIAARKFRGRDAV VNFKTFLASEDDNGELCFLEAHSKAEIVDM LRKHTYADELAQSNKRSGANTNTNTTQSHT VSRTREVLFEKVVTPSDVGKLNRLVI PKQHAEKYFPLPSLSVTKGVLI NFEDVTGKVWRF RYSYWNSSQSYVLTKGWSRFVKEKNLRAGDVVTFERSTGSDRQLYIDWKI RSGPSKNPVQ VVVRLFGVDI FNVTSAKPSNVVDACGGKRSRDVDM FALRCSKKHAIINAL

CDS SEQ ID NO: 122
ATGGTATTCAGTTGCATAGACGAGAGCTCTTCCACTTCAGAATCTTTTTCACCCGCAACC GCAACCGCAACCGCAACCGCCACAAAGTTCTCTGCTCCTCCGCTTCCACCGTTACGCCTC AACCGGATGAGAAGCGGTGGAAGCAACGTCGTGTTGGATTCAAAGAATGGCGTAGATATT GATTCACGGAAGCTATCGTCGTCAAAGTACAAAGGCGTGGTTCCTCAGCCCAACGGAAGA TGGGGAGCTCAGATTTACGTGAAGCACCAGCGAGTTTGGCTGGGCACTTTCTGCGATGAA GAGGAAGCTGCTCACTCCTACGACATAGCCGCCCGTAAATTCCGTGGCCGTGACGCCGTT GTCAACTTCAAAACCTTCCTCGCCTCAGAGGACGACAACGGCGAGTTATGTTTCCTTGAA GCTCACTCCAAGGCCGAGATCGTCGACATGTTGAGGAAACACACTTACGCTGACGAGCTT GCGCAGAGCAATAAACGCAGCGGAGCGAATACGAATACGAATACGACTCAAAGCCACACC GTTTCGAGAACACGTGAAGTGCTTTTCGAGAAGGTTGTCACGCCTAGCGACGTTGGTAAG CTAAACCGCCTCGTGATACCTAAACAGCACGCGGAGAAATATTTTCCGTTACCGTCACTG TCGGTGACTAAAGGCGTTCTGATCAACTTCGAAGACGTGACGGGTAAGGTGTGGCGGTTC CGTTACTCATACTGGAACAGTAGTCAAAGTTACGTGTTGACCAAGGGATGGAGTCGGTTC GTTAAGGAGAAGAATCTCCGAGCCGGTGATGTCGTTACTTTCGAGAGATCGACCGGTTCA GACCGGCAGCTTTATATTGATTGGAAAATCCGGTCTGGTCCGAGCAAAAACCCTGTTCAG GTTGTGGTTAGG CTTTTCG GAGTTG ACATCTTCAACGTGACAAGCG CGAAG CCGAGCAAC GTtGTAGACGCGTGCGGTGGAAAGAGATCTCGGGATGTTGATATGTTTGCGCTACGGTGT TCCAAAAAACACGCTATAATCAATGCTTTGTGA

Zea mays GRMZM2G053008
Cover 74\% identity 47\%

SEQ ID NO: 123
MAASPSSPLTAPPEPVTPPSPWTITDGAISGTLPAAEAFAVHYPGYPSSPARAARTLGGL PGLAKVRSSDPGARLELRFRPEDPYCHPAFGQSRASTGLLLRLSKRKGAAAPCAHVVARV RTAYYFEGMADFQHVVPVHAAQTRKRKHSDSQNDNENFGSDKTGHDEADGDVM MLVPPLF SVKDRPTKIALVPSSNAISKTMH RGVVQERWEMNVGPTLALPFNTQVVPEKINWEDHI RK NSVEWGWQMAVCKLFDERPVWPRQSLYERFLDDNVHVSQNQFKRLLFRAGYYFSTGPFGK FWI RRGYDPRKDSESQIYQRI DFRM PPELRYLLRLKNSESRKWADMCKLETMPSQSFIYL QLYELKDDFIQAEI RKPSYQSVCSRSTGWFSKPM IKTLRLQVSI RLLSLLHNEEAKNLLR NAHELI ERSKKQEALSRSELSI EYNDADQVSAAHTGTEDQVGPNNSDSEDVDDEEEEEEL EGYDSPPMADDI HEFTLGDSYAFGEGFSNGYLEEVLRSLPLQEDGQKKLCDAPINADASD

CDS SEQ ID NO: 124
ATGGCCGCCTCGCCCTCTTCACCCTTGACAGCGCCGCCAGAGCCGGTGACCCCGCCGTCC CCATGGACCATCACAGACGGAGCCATCTCTGGCACGCTCCCAGCAGCCGAGGCCTTCGCA GTGCACTACCCGGGCTACCCCTCCTCTCCCGCCCGCGCCGCCCGCACCCTCGGCGGTCTC CCCGGCCTCGCCAAGGTCCGGAGTTCCGATCCCGGCGCCCGCCTCGAGCTCCGCTTCCGC CCCGAGGACCCCTACTGCCATCCAGCCTTTGGCCAGTCCCGCGCCTCCACTGGCCTTCTG CTGCGCCTCTCCAAGCGCAAAGGAGCTGCGGCACCTTGTGCCCATGTGGTCGCTCGTGTC CGGACTGCTTACTACTTCGAAGGTATGGCAGATTTTCAACATGTTGTTCCAGTGCATGCT GCACAAACAAGAAAAAGAAAACACTCAGATTCTCAAAATGATAATGAGAATTTTGGTAGT GATAAGACAGGACATGATGAAGCAGATGGAGATGTCATGATGTTGGTACCCCCTCTCTTT TCAGTGAAG GATAGGCCAACAAAG ATAGCGCTTGTACCATCGTCCAATGCCATATCTAAA

## 84

ACCATGCACAGGGGAGTTGTACAAGAACGGTGGGAGATGAATGTTGGACCAACTCTGGCG CTTCCGTTCAACACTCAAGTTGTCCCGGAGAAGATTAATTGGGAAGACCACATTAGAAAG AATTCTGTAGAATGGGGTTGGCAAATGGCTGTTTGCAAATTGTTTGATGAGCGCCCTGTG TGGCCAAGGCAATCACTTTATGAGCGGTTCCTTGATGATAATGTGCATGTCTCTCAAAAC

GRMZM2G102059_T01
Cover 47\% identity 62\% SEQ ID NO: 125 M EFASSSSRFSREEDEEEEQEEEEEEEEASPREIPFMTAAATADTGAAASSSSPSAAASS GPAAAPRSSDGAGASGSGGGGSDDVQVI EKEHM FDKVVTPSDVGKLNRLVIPKQHAEKYF PLDAAAN EKGQLLSFEDRAGKLWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDTVSFC RGAGDTARDRLFIDWKRRADSRDPH RM PRLPLPMAPVASPYGPWGGGGGGGAGGFFM PPA PPATLYEHHRFRQALDFRNI NAAAAPARQLLFFGSAGM PPRASMPQQQQPPPPPHPPLHS IM LVQPSPAPPTASVPM LLDSVPLVNSPTAASKRVRLFGVNLDNPQPGTSAESSQDANAL SLRTPGWQRPGPLRFFESPQRGAESSAASSPSSSSSSKREAHSSLDLDL

CDS SEQ ID NO: 126
ATGGAGTTCGCGAGCTCTTCGAGTAGGTTTTCCAGGGAGGAGGACGAGGAGGAAGAGCAG GAGGAAGAGGAGGAGGAGGAGGAGGCGTCTCCGCGCGAGATCCCCTTCATGACAGCGGCA GCGACGGCCGACACCGGAGCCGCCGCCTCCTCGTCCTCGCCTTCCGCGGCGGCCTCATCG GGTCCTGCTGCTGCCCCCCGCTCGAGCGACGGCGCCGGGGCGTCCGGGAGCGGCGGCGGC GGGAGCGACGACGTGCAGGTGATCGAGAAGGAGCACATGTTCGACAAGGTGGTGACGCCC AGCGACGTGGGGAAGCTCAACCGGCTGGTGATCCCGAAGCAGCACGCGGAGAAGTACTTC CCGCTGGACGCGGCGGCCAACGAGAAGGGCCAGCTGCTCAGCTTCGAGGACCGCGCCGGT AAGCTCTGGCGCTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTACGTCATGACCAAG GGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGCGACACCGTCTCCTTCTGC CGCGGCGCCGGCGACACCGCGCGGGACCGCCTCTTCATCGACTGGAAGCGCCGCGCCGAC TCCCGCGACCCGCACCGCATGCCGCGCCTCCCGCTCCCCATGGCGCCCGTCGCGTCGCCC TACGGCCCCTGGGGCGGCGGCGGCGGCGGCGGCGCGGGCGGTTTCTTCATGCCGCCCGCG CCGCCCGCCACACTCTACGAGCACCACCGCTTCCGCCAGGCCCTCGACTTCCGCAACATC AACGCCGCGGCCGCGCCGGCCAGGCAGCTCCTCTTCTTCGGCTCAGCCGGCATGCCCCCG CGCGCGTCCATGCCGCAGCAGCAGCAGCCGCCTCCGCCCCCGCACCCGCCTCTGCACAGC ATTATGTTGGTGCAACCCAGCCCCGCGCCGCCCACGGCCAGCGTGCCCATGCTTCTCGAC TCGGTACCGCTCGTCAACAGCCCAACGGCAGCGTCGAAGCGCGTCCGCCTGTTTGGGGTC AACCTCGACAACCCGCAACCAGGCACAAGTGCGGAGTCAAGCCAAGATGCCAACGCATTG TCGCTGAGGACACCGGGATGGCAAAGGCCGGGGCCGTTGAGGTTCTTCGAATCGCCTCAA CGCGGCGCCGAGTCATCTGCAGCCTCCTCGCCGTCGTCATCGTCGTCCTCCAAGAGAGAA

GCGCACTCGTCCTTGGATCTCGATCTGTGA

GRMZM2G098443_T01
Cover 47\% identity 63\%

SEQ ID NO: 127
M EFTTPPPATRSGGGEERAAAEHNQHHQQQHATVEKEHM FDKVVTPSDVGKLNRLVIPKQ HAEKYFPLDAAANEKGLLLSFEDRTGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAG DTVSFGRGISEAARDRLFIDWRCRPDPPVVHHQYHH RLPLPSAVVPYAPWAAHAHHHHYP ADGHTEPVTPCLCATLVATEM RASSSQLSLTRSNLSRPPQPRIARVDGAQPRPSSSPRRQP QSLWCRSCQPQPRRTADVP

CDS SEQ ID NO: 128
ATGGAGTTCACCACTCCCCCGCCCGCGACCCGGTCGGGCGGCGGAGAGGAGAGGGCGGCT GCTGAGCACAACCAGCACCACCAGCAGCAGCATGCGACGGTGGAGAAGGAGCACATGTTC GACAAGGTGGTGACGCCGAGCGACGTCGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAG CACGCGGAGAAGTACTTCCCGCTGGACGCGGCGGCGAACGAGAAGGGCCTCCTGCTCAGC TTCGAGGACCGCACGGGGAAGCCCTGGCGCTTCCGCTACTCCTACTGGAACAGTAGCCAG AGCTACGTGATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGG GACACAGTCTCCTTCGGCCGCGGCATCAGCGAGGCGGCGCGCGACAGGCTTTTCATCGAC TGGCGGTGCCGACCCGACCCGCCCGTCGTGCACCACCAGTACCACCACCGCCTCCCTCTC CCCTCCGCCGTCGTCCCCTACGCGCCGTGGGCGGCGCACGCGCACCACCACCACTACCCA GCAGATGGGCACACGGAACCAGTAACACCTTGCCTGTGCGCCACACTCGTTGCCACTGAA ATGAGAGCATCATCTTCGCAACTGTCACTCACACGCTCCAACCTCTCCAGGCCGCCACAA CCTAGAATAGCCAGAGTCGATGGCGCCCAGCCACGGCCGTCGTCGTCACCACGCCAGCCA CAGTCGTTGTGGTGCCGGTCGTGCCAACCGCAACCACGGCGAACGGCCGACGTTCCTTGA

GRMZM2G082227_T01
Cover $45 \%$ identity 64\%

SEQ ID NO: 129
M EFTAPPPATRSGGGEERAAAEHHQQQQQATVEKEHM FDKVVTPSDVGKLNRLVIPKQHA ERYFPLDAAANDKGLLLSFEDRAGKPWRFRYSYWNSSQSYVMTKGWSRFVKEKRLDAGDT VSFGRGVGEAARGRLFIDWRRRPDPPVVHHQYHH HRLPLPSAVVPYAPWAAAAHAHHHHY PAAGVGAARTTTTTTTTVLH HLPPSPSPLYLDTRRRHVGYDAYGAGTRQLLFYRPHQQPS TTVM LDSVPVRLPPTPGQHAEPPPPAVASSASKRVRLFGVNLDCAAAAGSEEENVGGWRT SAPPTQQASSSSSYSSGKARCSLNLDL

CDS SEQ ID NO: 130
ATGGAGTTCACCGCTCCCCCGCCCGCGACCCGGTCGGGCGGCGGCGAGGAGAGGGCGGCT GCTGAGCACCACCAGCAGCAGCAGCAGGCGACGGTGGAGAAGGAGCACATGTTCGACAAG GTGGTGACGCCGAGCGACGTCGGGAAGCTGAACCGGCTGGTGATCCCGAAGCAGCACGCG GAGAGGTACTTCCCGCTGGACGCGGCGGCGAACGACAAGGGCCTGCTGCTCAGCTTCGAG GACCGCGCGGGGAAGCCCTGGCGCTTCCGCTACTCCTACTGGAACAGCAGCCAGAGCTAC GTGATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGACGCCGGGGACACC GTCTCCTTCGGCCGCGGCGTCGGCGAGGCGGCGCGCGGCAGGCTCTTCATCGACTGGCGG CGCCGACCCGACCCGCCCGTCGTGCACCACCAGTACCACCACCACCGCCTCCCTCTCCCC TCCGCCGTCGTCCCCTACGCGCCGTGGGCGGCGGCGGCGCACGCGCACCACCACCACTAC CCAGCAGCTGGGGTCGGTGCCGCCAGGACGACGACGACGACGACGACGACGGTGCTCCAC CACCTGCCGCCCTCGCCCTCCCCGCTCTACCTTGACACCCGCCGCCGCCACGTCGGCTAC GACGCCTACGGGGCCGGCACCAGGCAACTTCTCTTCTACAGGCCGCACCAGCAGCCCTCC ACGACGGTGATGCTGGACTCCGTGCCGGTACGGTTACCGCCAACGCCAGGGCAGCACGCC GAGCCGCCGCCCCCCGCCGTGGCGTCGTCAGCCTCGAAGCGGGTGCGCCTGTTCGGGGTG

## AACCTCGACTGCGCCGCCGCCGCCGGCTCAGAGGAGGAGAACGTCGGCGGGTGGAGGACT AGTGCGCCGCCGACGCAGCAGGCGTCCTCCTCCTCATCCTACTCTTCCGGGAAAGCGAGG TGCTCCTTGAACCTTGACTTGTGA

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Cover $46 \%$ identity $63 \%$
SEQ ID NO: 131
M DQFAASGRFSREEEADEEQEDASNSMREISFMPPAAASSSSAAASASASASTSASACAS GSSSAPFRSASASGDAAGASGSGGPADADAEAEAVEKEHMFDKVVTPSDVGKLNRLVI PK QYAEKYFPLDAAANEKGLLLSFEDSAGKHWRFRYSYWNSSQSYVMTKGWSRFVKEKRLVA GDTVSFSRAAAEDARHRLFIDWKRRVDTRGPLRFSGLALPM PLPSSHYGGPHHYSPWGFG GGGGGGGGFFMPPSPPATLYEHRLRQGLDFRSMTTTYPAPTVGRQLLFFGSARM PPHHAP PPQPRPFSLPLH HYTVQPSAAGVTAASRPVLLDSVPVI ESPTTAAKRVRLFGVNLDNN PD GGGEASHQGDALSLQMPGWQQRTPTLRLLELPRHGGESSAASSPSSSSSSKREARSALDL DL

CDS SEQ ID NO: 132 ATGGACCAGTTCGCCGCGAGCGGGAGGTTCTCTAGAGAGGAGGAGGCGGACGAGGAGCAG GAGGATGCGTCCAATTCCATGCGCGAGATCTCCTTCATGCCGCCGGCTGCGGCCTCGTCA TCTTCGGCGGCTGCTTCCGCGTCCGCGTCCGCCTCCACCAGCGCATCCGCGTGTGCATCG GGAAGCAGCAGCGCCCCCTTCCGCTCCGCCTCCGCGTCGGGGGATGCCGCCGGAGCGTCG GGGAGCGGCGGCCCAGCGGACGCGGACGCGGAGGCGGAGGCGGTGGAGAAGGAGCACATG TTCGACAAGGTGGTCACGCCGAGCGACGTGGGGAAGCTCAACCGGCTGGTGATCCCGAAG CAGTACGCGGAGAAGTACTTCCCGCTGGACGCGGCGGCCAACGAGAAGGGCCTCCTCCTC AGCTTCGAGGACAGCGCCGGCAAGCACTGGCGCTTCCGCTACTCCTACTGGAACAGCAGC CAGAGCTACGTCATGACCAAGGGCTGGAGCCGCTTCGTCAAGGAGAAGCGCCTCGTCGCC GGGGACACCGTCTCCTTCTCCCGCGCCGCCGCCGAGGACGCGCGCCACCGCCTCTTCATC GACTGGAAGCGCCGGGTCGACACCCGCGGCCCGCTTCGTTTCTCCGGCCTCGCGCTGCCG ATGCCGCTGCCGTCGTCGCACTACGGCGGGCCCCACCACTACAGCCCGTGGGGCTTCGGC GGCGGCGGCGGCGGCGGCGGCGGATTCTTCATGCCGCCCTCGCCGCCCGCCACGCTCTAC GAGCACCGCCTCAGACAGGGCCTCGACTTCCGCAGCATGACGACGACCTACCCCGCGCCG ACCGTGGGGAGGCAGCTCCTGTTTTTCGGCTCGGCCAGGATGCCTCCTCATCACGCGCCG CCGCCCCAGCCGCGCCCGTTCTCGCTGCCGCTGCATCACTACACGGTGCAACCGAGCGCC GCCGGCGTCACCGCCGCGTCACGGCCGGTCCTTCTTGACTCGGTGCCGGTCATCGAGAGC CCGACGACCGCCGCGAAGCGCGTGCGGCTGTTCGGCGTCAACCTGGACAACAACCCAGAT GGCGGCGGCGAGGCTAGCCATCAGGGCGATGCATTGTCATTGCAGATGCCCGGGTGGCAG CAAAGGACTCCAACTCTAAGGCTACTAGAATTGCCTCGCCATGGCGGGGAGTCCTCCGCG GCGTCGTCTCCGTCGTCGTCGTCTTCCTCCAAGAGGGAGGCGCGTTCAGCTTTGGATCTC GATCTGTGA

GRMZM2G328742_T01
Cover 55\% identity 64\%
SEQ ID NO: 134
MATN HLSQGQHQHPQAWPWGVAMYTNLHYH HQQH HHYEKEH LFEKPLTPSDVGKLNRLVI PKQHAERYFPLSSSGAGDKGLILCFEDDDDDEAAAANKPWRFRYSYWTSSQSYVLTKGWS RYVKEKQLDAGDVVRFQRM RGFGM PDRLFISHSRRGETTATAATTVPPAAAAVRVVVAPA QSAGADHQQQQQPSPWSPMCYSTSGSYSYPTSSPANSQHAYHRHSADH DHSNNMQHAGES QSDRDNRSCSAASAPPPPSRRLRLFGVN LDCGPGPEPETPTAMYGYMHQSPYAYNNWGSP YQHDEEI

CDS 135

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GRMZM2G142999_T01
Cover $44 \%$ identity $64 \%$

SEQ ID NO: 136
MEFTPAHAHARVVEDSERPRGGVAWVEKEHM FEKVVTPSDVGKLNRLVIPKQHAERYFPA LDASSAAAAAAAAAAGGGKGLVLSFEDRAGKAWRFRYSYWNSSQSYVMTKGWSRFVKEKR LGAGDTVLFARGAGGARGRFFI DFRRRRQDLAFLQPTLASAQRLLPLPSVPICPWQDYGA SAPAPNRHVLFLRPQVPAAVVLKSVPVHVAASAVEATMSKRVRLFGVNLDCPPDAEDSAT VPRGRAASTTLLQLPSPSSSTSSSTAGKDVCCLDLGL

CDS SEQ ID NO: 137
ATGGAGTTCACGCCCGCGCATGCGCATGCCCGTGTCGTTGAGGATTCCGAGAGGCCTCGC GGCGGCGTGGCCTGGGTGGAGAAGGAGCACATGTTCGAGAAGGTGGTCACCCCGAGCGAC GTGGGGAAGCTCAATCGCCTGGTCATCCCAAAGCAGCACGCGGAGCGCTACTTCCCCGCG CTGGACGCCTCGTCCGCCGCGGCGGCGGCGGCGGCAGCAGCCGCGGGAGGCGGGAAGGGG CTGGTGCTCAGCTTCGAGGACCGGGCGGGGAAGGCGTGGCGCTTCCGCTACTCGTACTGG AACAGCAGCCAGAGCTACGTGATGACCAAAGGTTGGAGCCGCTTCGTGAAGGAGAAGCGC CTCGGTGCCGGGGACACAGTCTTGTTCGCGCGCGGCGCGGGCGGCGCGCGCGGCCGCTTC TTCATCGATTTCCGCCGCCGTCGCCAGGATCTCGCGTTCCTGCAGCCGACGCTGGCGTCT GCGCAGCGACTCCTGCCGCTGCCGTCGGTGCCCATCTGCCCGTGGCAGGACTACGGCGCC TCGGCTCCGGCGCCCAACCGGCACGTGCTGTTCCTGCGGCCGCAGGTGCCGGCCGCCGTA GTGCTCAAGTCGGTCCCCGTGCACGTTGCTGCATCCGCGGTGGAGGCGACCATGTCGAAG CGCGTCCGCCTGTTCGGGGTGAACCTCGACTGCCCGCCGGACGCCGAAGACAGCGCCACA GTCCCCCGGGGCCGGGCGGCGTCGACGACGCTTCTGCAACTGCCCTCGCCATCGTCGTCA ACATCCTCCTCGACGGCAGGGAAGGACGTGTGCTGTTTGGATCTTGGACTGTGA

GRMZM2G125095_T01
Cover 85\% identity 40\%
SEQ ID NO: 138
M EFRPAHARVFEDSERPRGGVAWLEKEHMFEKVVTPSDVGKLNRLVI PKQHAERYFPALD ASAAAASASASAGGGKAGLVLSFEDRAGKAWRFRYSYWNSSQSYVMTKGWSRFVKEKRLG AGDTVLFARGAGATRGRFFI DFRRRRH ELAFLQPPLASAQRLLPLPSVPICPWQGYGASA PAPSRHVLFLRPQVPAAVVLTSVPVRVAASAVEEATRSKRVRLFGVNLDCPPDAEDGATA

TRTPSTLLQLPSPSSSTSSSTGGKDVRSLDLGL

CDS SEQ ID NO: 139
ATGGAGTTCAGGCCCGCGCATGCCCGTGTCTTCGAGGATTCCGAGAGGCCTCGCGGCGGC GTGGCGTGGCTGGAGAAGGAGCACATGTTCGAGAAAGTGGTCACCCCGAGCGACGTGGGG AAGCTCAATCGCCTGGTCATCCCGAAGCAGCACGCCGAGCGCTACTTCCCCGCGCTGGAC GCCTCGGCCGCCGCGGCGTCGGCATCGGCGTCGGCGGGCGGCGGGAAGGCGGGGCTGGTG CTCAGCTTCGAGGACCGGGCGGGGAAGGCGTGGCGCTTCCGCTACTCGTACTGGAACAGC AGCCAGAGCTACGTGATGACCAAGGGATGGAGCCGCTTCGTGAAAGAGAAGCGCCTCGGT GCCGGGGACACGGTATTGTTCGCGCGCGGCGCGGGCGCCACGCGCGGCCGCTTCTTCATC GATTTCCGCCGCCGCCGCCACGAGCTCGCGTTCCTGCAGCCGCCGCTGGCGTCTGCGCAG CGCCTCCTGCCGCTCCCGTCGGTGCCCATCTGCCCGTGGCAGGGCTACGGCGCCTCCGCT CCGGCGCCAAGCCGGCACGTGCTGTTCCTGCGGCCGCAGGTGCCGGCCGCCGTAGTGCTC ACGTCGGTGCCCGTGCGCGTCGCCGCATCCGCGGTGGAGGAGGCGACGAGGTCGAAGCGC GTCCGCCTGTTCGGGGTGAACCTCGACTGCCCGCCGGACGCCGAAGACGGTGCCACAGCC ACCCGGACGCCGTCGACGCTTCTGCAGCTGCCCTCGCCATCGTCGTCAACATCCTCCTCC ACGGGAGGCAAGGATGTGCGTTCTTTGGATCTTGGACTTTGA

Tricum aeseirum

TRAES3BF098300010CFD_tl
Cover: 42\% ident 60\%

SEQ ID NO: 140
MGVEI LSSMVEHSFQYSSGVSTATTESGTAGTPPRPLSLPVAIADESVTSRSASSRFKGVVPQPNGRWGAQIYERH ARVWLGTFPDQDSAARAYDVASLRYRGRDVAFNFPCAAVEGELAFLAAHSKAEIVDMLRKQTYADELRQGLRRG RGMGARAQPTPSWAREPLFEKAVTPSDVGKLNRLVVPKQHAEKH FPLKRTPETPTTTGKGVLLNFEDGEGKVWR FRYSYWNSSQSYVLTKGWSRFVREKGLGAGDSILFSCSLYEQEKQFFIDCKKNTSMNGGKSASPLPVGVTTKGEQV RVVRLFGVDISGVKRGRAATATAEQGLQELFKRQCVAPGQHSPALGAFAL

CDS SEQ ID NO: 141
ATGGGGGTGGAAATCCTGAGCTCCATGGTGGAGCACTCCTTCCAGTACTCTTCCGGCGTG TCCACGGCCACGACGGAGTCAGGCACCGCCGGAACACCGCCGAGGCCTTTGAGCCTACCT GTCGCCATCGCCGACGAGTCCGTGACCTCGCGGTCGGCGTCGTCTCGGTTCAAGGGCGTG GTGCCGCAGCCAAACGGGCGATGGGGCGCCCAGATCTACGAGCGCCACGCTCGCGTCTGG CTCGGCACGTTCCCAGACCAGGACTCGGCGGCGCGCGCCTACGACGTAGCCTCGCTCAGG TACCGCGGCCGCGACGTCGCCTTCAACTTCCCGTGCGCGGCCGTGGAGGGGGAGCTCGCC TTCCTGGCGGCGCACTCCAAGGCTGAGATAGTGGACATGCTCCGGAAGCAGACCTACGCC GATGAACTCCGCCAGGGCCTGCGGCGCGGCCGTGGCATGGGGGCGCGCGCGCAGCCGACG CCGTCGTGGGCGCGGGAGCCCCTTTTCGAGAAGGCCGTGACCCCTAGCGATGTCGGCAAG CTCAATCGCCTCGTAGTGCCGAAGCAGCACGCCGAGAAGCACTTCCCCCTGAAGCGCACG CCGGAGACGCCGACCACCACCGGCAAGGGCGTGCTGCTCAACTTCGAGGACGGCGAGGGG
AAGGTGTGGAGGTTCCGGTACTCGTACTGGAACAGCAGCCAGAGCTACGTGCTCACCAAA GGCTGGAGCCGCTTCGTCCGGGAGAAGGGCCTAGGTGCCGGCGACTCCATCCTATTCTCG TGCTCGCTGTACGAACAGGAGAAGCAGTTCTTCATCGACTGCAAGAAGAACACTAGCATG AACGGAGGCAAATCGGCGTCGCCGCTGCCAGTGGGGGTGACTACCAAAGGAGAACAAGTT CGCGTCGTTAGGCTATTCGGTGTCGACATCTCGGGAGTGAAGAGGGGGCGAGCGGCGACG GCAACGGCGGAGCAAGGCCTGCAGGAGTTGTTCAAGAGGCAATGCGTGGCACCCGGCCAG CACTCTCCTGCCCTAGGTGCCTTCGCCTTATAG

TRAES3BF062700040CFD_tl
Cover 47\% ident 55\%

SEQ ID NO: 142
MASGKPTNHGMEDDNDM EYSSAESGAEDAAEPSSSPVLAPPRAAPSSRFKGVVPQPNGRW GAQIYEKHSRVWLGTFPDEDAAVRAYDVAALRFRGPDAVI NHQRPTAAEEAGSSSSRSEL DPELGFLADHSKAEIVDMLRKHTYDDELRQGLRRGRGRAQPTPAWARELLFEKAVTPSDV GKLNRLVVPKQQAEKHFPPTTAAATGSNGKGVLLNFEDGEGKVWRFRYSYWNSSQSYVLT KGWSRFVKETGLRAGDTVAFYRSAYGNDTEDQLFIDYKKMNKNDDAADAAISDENETGHV AVKLFGVDIAGGGMAGSSGG

CDS SEQ ID NO: 143
ATGGCATCTGGCAAGCCGACAAACCACGGGATGGAGGACGACAACGACATGGAGTACTCC TCCGCGGAATCGGGGGCCGAGGACGCGGCGGAGCCGTCGTCGTCGCCGGTGCTGGCGCCG CCCCGGGCGGCTCCATCGTCGCGGTTCAAGGGCGTCGTGCCGCAGCCCAACGGGCGGTGG GGAGCGCAGATCTACGAGAAGCACTCGCGGGTGTGGCTCGGAACGTTCCCCGACGAGGAC GCCGCCGTGCGCGCCTACGACGTGGCCGCGCTCCGCTTCCGCGGCCCGGACGCCGTCATC AACCACCAGCGACCGACGGCCGCGGAGGAGGCCGGCTCGTCGTCGTCCAGGAGCGAGCTG GATCCAGAGCTCGGCTTCCTTGCCGACCACTCCAAGGCCGAGATCGTCGACATGCTCCGG AAGCACACCTACGACGACGAGCTCCGTCAGGGCCTGCGCCGCGGCCGCGGGCGCGCGCAG CCGACGCCGGCGTGGGCACGAGAGCTCCTCTTCGAGAAGGCCGTGACCCCGAGCGACGTC GGCAAGCTCAACCGCCTCGTGGTGCCGAAGCAGCAGGCCGAGAAGCACTTCCCTCCGACC ACTGCGGCGGCCACCGGCAGCAACGGCAAGGGCGTGCTGCTCAACTTCGAGGACGGCGAA GGGAAGGTGTGGCGCTTCCGGTACTCGTACTGGAACAGCAGCCAGAGCTACGTGCTCACC AAGGGCTGGAGCCGCTTCGTCAAGGAGACGGGCCTCCGCGCCGGCGACACCGTGGCGTTC TACCGGTCGGCGTACGGGAATGACACGGAGGATCAGCTCTTCATCGACTACAAGAAGATG AACAAGAATGACGATGCTGCGGACGCGGCGATTTCCGATGAGAATGAGACAGGCCATGTC GCCGTCAAGCTCTTCGGCGTTGACATTGCCGGTGGAGGGATGGCGGGATCATCAGGTGGC TGA

TRAES3BF062600010CFD_tl
Cover $43 \%$ ident $58 \%$

SEQ ID NO: 144
MASGKPTNHGMEDDNDM EYSSAESGAEDAAEPSSSPVLAPPRAAPSSRFKGVVPQPNGRW GAQIYEKHSRVWLGTFPDEDAAARAYDVAALRFRGPDAVI NHQRPTAAEEAGSSSSRSEL DPELGFLADHSKAEIVDMLRKHTYDDELRQGLRRGRGRAQPTPAWARELLFEKAVTPSDV GKLNRLVVPKQQAEKHFPPTTAAATGSNGKGVLLNFEDGEGKVWRFRYSYWNSSQSYVLT KGWSRFVKETGLRAGDTVAFYRSAYGNDTEDQLFIDYKKMNKNDDAADAAISDENETGHV AVKLFGVDIAGGGMAGSSGG

CDS SEQ ID NO: 145
ATGGCATCTGGCAAGCCGACAAACCACGGGATGGAGGACGACAACGACATGGAGTACTCC TCCGCGGAATCGGGGGCCGAGGACGCGGCGGAGCCGTCGTCGTCGCCGGTGCTGGCGCCG CCCCGGGCGGCTCCATCGTCGCGGTTCAAGGGCGTCGTGCCGCAGCCCAACGGGCGGTGG GGAGCGCAGATCTACGAGAAGCACTCGCGGGTGTGGCTCGGAACGTTCCCCGACGAGGAC GCCGCCGCGCGCGCCTACGACGTGGCCGCGCTCCGCTTCCGCGGCCCGGACGCCGTCATC

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AACCACCAGCGACCGACGGCCGCGGAGGAGGCCGGCTCGTCGTCGTCCAGGAGCGAGCTG GATCCAGAGCTCGGCTTCCTCGCCGACCACTCCAAGGCCGAGATCGTCGACATGCTCCGG AAGCACACCTACGACGACGAGCTCCGTCAGGGCCTGCGCCGCGGCCGCGGGCGCGCGCAG CCGACGCCGGCGTGGGCACGAGAGCTCCTCTTCGAGAAGGCCGTGACCCCGAGCGACGTC

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## CLAIMS:

1. A plant wherein said plant does not produce a functional NGAL2 polypeptide or does not produce functional NGAL2 and NGAL3 polypeptides.
2. 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.
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.
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.
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 ld 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:49145.
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 TDNA 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


Figure 3


$E$

$F$


Figure 4


Figure 5


Figure 6


Figure 7


Figure 8






Figure 9


Figure 10


Figure 11
A



Figure 12

## A

|  | EKG |
| :---: | :---: |
| Brassica rapa2 1 | AE: Y/PLNN--CGGGGDVTAE\% EKG* |
| ine max.At3g11580-like1 | LTPSDVGKLNRLVIPKOHAEKYFPLS\%--------DSGG\%ECKGLLLSFEDES |
| lycine max At5g06250-like1 | PLS\%G-------DSG\% ECKKLLLSFEDES |
| Glycine max At2g36080-like1 | VGKLNRLVIPKOHAEKYFPLDSS------GGD\#AAAKGLLLSFEEDES |
| za sativa | E\#YFPLG\%--------GD\%GE-KGLLLSFEDES |
| t5g06250/NAGL3 | VIPKQHAEKYFPIN\%VLVS-SAAADT\%\%/ EKG\% ILSFEDES |
| um vulgare1 | LD\%-----------AANEK |
| ea mays Os02g0683500 | ID. |
| a mays |  |
| um vulgare2 |  |
| ium hirsutum R | LQS---------G\%A\%SKG\% LINFEDV\% |
| riticum aestivum | FKA\% ${ }^{\text {PPSDVGKLNRLV/ FKQHAEKHEPLKRTP------ETP\%\%.GKG\% }}$ |


SEQ ID NO 164
SEQ ID No 165
SEQ ID No 166
SEQ ID NO 167
SEQ ID No 168
SEQ ID No 169
SEQ ID No 170
SEQ ID No 171
SEQ ID No 172
SEQ ID No 173
SEQ ID No 174
SEQ ID No 175
SEQ ID No 176
SEQ ID No 177
SEQ ID No 178

LOC Os04g49230
DRLFIDWKRR
Bra007646
sGmLoc100795470
Bra000434
Bra040478
Bra004501
Bra003482
Bra014415
GmLoc100818164
GmLoc100802734
GmLoc100781489
GmLoc100778733
Bra005301
Bra017262
GmLoc102660503

RLFGVD
RLFGVD
RLFGVD
RLFGVD
RLFGVD
RLFGVD
RLFGVD
RLFGVN
RLFGVN
RLFGVN
RRLGVN
RLFGVN
RLFGVN
1 RLFGVC: T

| SEQ ID No 179 | HvMLOC_7940 | 1 VRLFGVD\%A- |
| :---: | :---: | :---: |
| SEQ ID No 180 | HvMLOC 56567 | 1 VRLEGVD\%A- |
| SEQ ID No 181 | Bra038346 | 1 VRLFGVD\%F- |
| SEQ ID No 182 | TRAES 3BF098300010CFD_t1 | 1 VRLEGVD\%S- |
| SEQ ID No 183 | GmLoc100776987 | 1 VRLFGVN\% |
| SEQ ID No 184 | GmLoc100801107 | 1 VRLEGVN\% ${ }^{\text {L }}$ |
| SEQ ID No 185 | os01g0693400 | 1 VRLFGVDIL- |
| SEQ ID No 186 | GmLoc100789009 | 1 VRLFGVDIL- |
| SEQ ID No 187 | HvMLOC44012 | 1 VRLFGVDIL- |
| SEQ ID No 188 | HvMLOC_38822 | 1 VRLFGVDIL- |
| 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_Osllg 05740.1 | 1 \%RLFGVNL |
| SEQ ID No 196 | GRMZM2G328742_TO1 | 1 RRLEGVNL |
| SEQ ID No 197 | os 02 g 0683500 | 1 VRLFGVNL\%- |
| SEQ ID No 198 | LOC_Os 03g 02900 | 1 VRLFGVNL\%- |
| SEQ ID No 199 | Os 10 g 0537100 | 1 VRLFGVNL\%- |
| SEQ ID No 200 | HvMLOC_66387 | 1 VRLFGVNI: |
| SEQ ID No 201 | GRMZM2 G102059_T01 | 1 VRLFGVNL\%- |
| SEQ ID No 202 | GRMZM2 G082227_T01 | 1 VRLFGVNL\%- |
| SEQ ID No 203 | GRMZM2G024948_TO1 | 1 VRLEGVNL\%- |
| SEQ ID No 204 | GRMZM2G142999_T01 | 1 VRLFGVNL\%- |
| SEQ ID No 205 | GRMZM2 G125095_T01 | 1 VRLFGVNL\%- |

Figure 13

| GRM2M2G053008 | SEQ ID NO 2071 |  |
| :---: | :---: | :---: |
| HvMLOC_57250 | SEQ ID NO 2081 |  |
| Osl2g0157000 | SEQ ID NO 2091 |  |
| GmLoc100778733 | SEQ ID NO 210 1 |  |
| Bra004501 | SEQ ID NO 2111 |  |
| Bra000434 | SEQ ID NO 2121 |  |
| Bra040478 | SEQ ID NO 213 |  |
| Bra014415 | SEQ ID NO 2141 |  |
| Bra003482 | SEQ ID NO 2151 |  |
| Bra007646 | SEQ ID NO 216 |  |
| GlycinemaxLoc100781489 | SEQ ID NO 2171 |  |
| GRM2M2G024948_T01 | SEQ ID NO 2181 |  |
| Os02g0683500 | SEQ ID NO 219 |  |
| HvMLOC_66387 | SEQ ID NO 220 1 |  |
| OS04g0581400 | SEQ ID NO 2211 |  |
| GRM2M2G102059_T01 | SEQ ID NO 2221 |  |
| Os 10 g 0537100 | SEQ ID NO 223 |  |
| GRM2M2G142999_T01 | SEQ ID NO 2241 |  |
| GRM2M2G125095_T01 | SEQ ID NO 225 |  |
| -s03g0120900 | SEQ ID NO 226 |  |
| GRM2M2G098443_T01 | SEQ ID NO 227 |  |
| GRM2M2G082227_T01 | SEQ ID NO 228 |  |
| Osllg0156000 | SEQ ID NO 229 |  |
| GRM2M2G328742_T01 | SEQ ID NO 230 |  |
| GmLoc100802734 | SEQ ID NO 2311 |  |
| GmLoc100795470 | SEQ ID NO 2321 |  |
| GmLoc100818164 | SEQ ID NO 2331 |  |
| Bra017262 | SEQ ID NO 2341 |  |
| At2g36080 | SEQ ID NO 2351 |  |
| Bra005301 | SEQ ID NO 236 |  |
| At3g11580 | SEQ ID NO 237 |  |
| BraLoc103849927 | SEQ ID NO 238 |  |
| BrassicarapaBra034828 | SEQ ID NO 239 |  |
| At5g06250 | SEQ ID NO 240 |  |
| Bra005886 | SEQ ID NO 2411 |  |
| GmLoc102660503 | SEQ ID NO 2421 |  |
| HvMLOC_38822 | SEQ ID NO 243 |  |
| os01g0693400 | SEQ ID NO 244 | --MDSS-SCLVDDTNSGGSS---------TDKL---------RALAAAAAET |
| HvMLOC44012 | SEQ ID NO 245 |  |
| HvMLOC_7940 | SEQ ID NO 246 | -MGVEIL-SSTGEHSS------QYSSGAASTATT-------ESGVGGRPPTAP |
| HvMLOC_75135 | SEQ ID NO 247 | -MGMEIL-SSTVEHCS-----QYSSSA-STATT-------ESGAAGRSTTAL |
| TRAECDM81004 | SEQ ID NO 248 | -MGVEIL-SSMVEDS5-----QYSSGA-STATT------ESGTTGRALTAL |
| HVMLOC_56567 | SEQ ID NO 2491 | -MGVEIL-SSMVEHSE------QYSSGA-SSATA-------ESGAVGTPPRHL |
| TRAES3BF098300010CFD_ | 1 SEQ ID NO 2501 | --------MGVEIL-SSMVEHSE------QYSSGV-STATT-------ESGTAGTPPRPL |
| HvMLOC_63261 | SEQ ID NO 2511 | MASSKPTNP--EVD-ND-----M------ECSS-------P-------ESGAEDAV-ESS |
| TRAES3BF062700040CFD_t | 1 SEQ ID NO 2521 | MASGKPTNHGMEDD-ND-----M------EYSS--------A-------ESGAEDAA-EPS |
| TRAES3BF062600010CFD | 1 SEQ ID NO 2531 | 1 MASGKPTNHGMEDD-ND-----M------EYSS-------A-------ESGAEDAA-EPS |
| Bra038346 | SEQ ID NO 254 | -MVF-SCIDESSST---SESESPAT-ATAT-------ATATKFSAPPLPP |
| GmLoc732601 | SEQ ID NO 255 | MDGG-CVTDETT-TSSDS---------------------LSV-------PP- |
| GmLoc100789009 | SEQ ID NO 256 |  |
| GmLoc100776987 | SEQ ID NO 2571 | -MDAI-SCLDESTTTESLSIS--------QAKPSSTIMSSEKASPSPPPP |
| GmLoc100801107 | SEQ ID NO 258 | -MDAI-SCMDESTTTESLSISLSPTS-SSEKAKPSSMITSSEKVSLSPPPS |




GRM2M2G053008
HvMLOC 57250
Os12g0157000
GmLoc100778733
Bra004501
Bra000434
Bra04047

Bra007646
GlycinemaxLoc100781489
GRM2M2G024948 T01
g0683500
HvMLOC 66387
0s04g0581400
GRM2M2G102059 T01
Os 10 g 0537100
GRMZM2G142999 T01
GRM2M2G125095_T01
os03g0120900
GRM2M2G098443_T01
GRM2M2G082227 T01
Os 11 g 0156000
GRMZM2G328742_T01
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
11580

BrassicarapaBra034828
At5g06250
GmLoc102660503
HvMLOC_38822
os01g0693400
MLOC44012

HvMLOC 7940
-
HvMLOC 56567
TRAES3BF098300010CFD_t1 LOC 63261 TRAES3BF062600010CFD t1 Bra038346

EmLoc 732601

GmLoc100776987
GmLoc100801107



| GRM2M2G053008 | 141 | AOT䒴KR－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－KHSDSONDNENE |
| :---: | :---: | :---: |
| HVMLOC＿57250 | 1 | －MYC－SR－－－－－－GRIDPAE－－－－－－E |
| Os 12 g 0157000 | 1 |  |
| GmLoc100778733 | 63 |  |
| Bra004501 | 1 |  |
| Bra000434 | 1 |  |
| Bra040478 | 1 |  |
| Bra014415 | 1 | －－－－－MERKSNDL－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－ERSSE－－NI－－－DSQ |
| Bra003482 | 1 |  |
| Bra007646 | 1 |  |
| GlycinemaxLoc100781489 | 25 |  |
| GRMZM2G024948＿T01 | 25 | NSM 敞EISFMPPAAA．SSSSAAA．SA．SA－－－－SASTSA．SACA．SGSSSAPFRSASA．SG－－－DAA |
| OS02g0683500 | 21 |  |
| HvMLOC＿66387 | 24 |  |
| Os04g0581400 | 37 | ASPPEIPFMTSAAAATASSSSPTSV－SPSATASAAA－STSASGSPFRSS－－－－－－－DGA |
| GRM2M2G102059＿T01 | 29 | A．SP需EIPFMTAAATADTGAAASSSS－－－PSA－－－－－A－ASSGPAAAPRSS－－－－－－－DGA． |
| Os 10 g 0537100 | 1 |  |
| GRM2M2G142999＿T01 | 1 | MEFTPAH－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－AH－ARVVE－－－－－－－ |
| GRM2M2G125095＿T01 | 1 | －MEFRPA－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－H－ARVFE－－－－－－－－－－－－ |
| os03g0120900 | 1 | －MEFITPIVR－－－－－－－－－－－－－－－－－－－－－－－－－－－－－PASAAAGGGEV－－－QE－ |
| GRM2M2G098443＿T01 | 1 | MEFTTPP－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－PATRSGGGEE－－－RA－ |
| GRM2M2G082227＿T01 | 1 | MEFTAPP－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－PATRSGGGEE－－－RA－ |
| Os11g0156000 | 21 | A |
| GRM2M2G328742＿T01 | 22 | AM |
| GmLoc100802734 | 20 | QQQAAMWLSNSH－－－－－－－－－－－－－－－－－－－－－－－－－－－－－TPRFNLNDEEEEEEDDV |
| GmLoc100795470 | 22 |  |
| GmLoc100818164 | 22 |  |
| Bra017262 | 22 | $\mathrm{O}--\mathrm{H}$ |
| At2g36080 | 22 | QO－QQ |
| Bra005301 | 22 | HRFH ${ }^{\text {H }}$ |
| At3g11580 | 14 | HHH |
| BraLoc103849927 | 18 | － HH |
| BrassicarapaBra034828 | 14 | NHH |
| At5g06250 | 21 | HRHTT |
| Bra005886 | 23 | NLHTT |
| GmLoc102660503 | 155 |  |
| HvMLOC＿38822 | 14 |  |
| Os01g0693400 | 176 |  |
| HvMLOC44012 | 14 |  |
| HvMLOC＿7940 | 152 | －GL\％̌KRGMGAR |
| HvMLOC＿75135 | 151 | －GL\％RGRGMGAR－ |
| TRAECDM81004 | 147 | －GL\％RGRGMGAR |
| HvMLOC＿56567 | 146 | －GL\％RGRGMGVR－ |
| TRAES3BF098300010CFD＿t1 | 146 | －GI：RGRGMGAR |
| HvMLOC＿63261 | 138 | －GL\％RGHG－－－R |
| TRAES3BF062700040CFD＿t1 | 151 | －GL\％RGRG－－－R |
| TRAES3BF062600010CFD＿t1 | 151 | －GL\％RGRG－－－R |
| Bra038346 | 163 | －SN\％RSGANTN－ |
| GmLoc 732601 | 145 | －ST\％GGR－－RR－ |
| GmLoc100789009 | 151 | －ST\％GGR－－RR－ |
| GmLoc100776987 | 170 |  |
| GmLoc100801107 | 176 |  |

GRMZM2G053008
HVMLOC 57250
Os 12 g 0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489
GRM2M2G024948_T01
Os02g0683500
HvMLOC 66387
$0: 0490581400$
GRMZM2G102059 T01
Os 10 g 0537100
GRMZM2G142999 T01
GRM2M2G125095_T01
os03g0120900
GRMZM2G098443_T01
GRMZM2G082227 T01
Os11g0156000
GRM2M2G328742 TO1
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At $3 g 11580$
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HVMLOC_38822
0s01g0693400
HvMLOC44012
HvMLOC_7940
HvMLOC 75135
TRAECDM81004
HvMLOC 56567
TRAES3BF098300010CFD_t1
HvMLOC_63261
TRAES3BF062700040CFD_t1 TRAES3BE062600010CFD_t1 Bra038346
GmLoc732601
GmLoc 100789009
GmLoc100776987
GmLoc100801107


GRM2M2G053008
HvMLOC_57250 Os 12 g 0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489
GRM2M2G024948_T01
os02g0683500
HvMLOC 66387
$0: 0490581400$
GPMZM2G102059 T01
Os 10 g 0537100
GRMZM2G142999 T01
GRMZM2G125095_T01
os03g0120900
GRMZM2G098443_T01
GRMZM2G082227_T01
Os 11 g 0156000
GRM2M2G328742_TO1
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At 3911580
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HVMLOC_38822
0.s01g0693400

HvMLOC44012
HvMLOC_7940
HvMLOC_75135
TRAECDM81004
HvMLOC 56567
TRAES3BF098300010CFD_t1
HvMLOC 63261
TRAES3BF062700040CFD_t1
TRAES3BF062600010CFD_t1
Bra038346
GmLoc732601
GmLoc100789009
GmLoc100776987
GmLoc100801107


GRMZM2G053008
HVMLOC 57250
Os 12 g 0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489
GRMZM2G024948_TO1
os 02 g 0683500
HvMLOC 66387
$0 \leq 04 \mathrm{~g} 0581400$
GRMZM2G102059 T01
Os 10 g 0537100
GRMZM2G142999 T01
GRMZM2G125095_T01
os03g0120900
GRMZM2G098443 T01
GRMZM2G082227_T01
$0 s 11 \mathrm{~g} 0156000$
GRMZM2G328742_T01
GmLoc 100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At 3 g 11580
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HvMLOC_38822
0s0190693400
HVMLOC44012
HvMLOC_7940
HvMLOC_75135
TRAECDM8 1004
HvMLOC 56567
TRAES3BF098300010CFD_t1
HVMLOC_63261
TRAES3BF062700040CFD_t1
TRAES 3BF062600010CFD_t1
Bra038346
GmLoc732601
GmLoc100789009
GmLoc100776987
GmLoc100801107

|  |  |
| :---: | :---: |
| 79 |  |
| 49 |  |
| 169 |  |
| 74 |  |
| 71 |  |
| 66 |  |
| 81 |  |
| 60 |  |
| 61 |  |
| 126 |  |
| 139 |  |
| 134 | SINEERR------AGEWRFRYSYWNSSQSY - |
| 132 |  |
| 148 |  |
| 13 |  |
| 86 | ISEEDR-----TGXATEFRYSYWNSSQSY - |
| 82 |  |
| 80 |  |
| 75 |  |
| 78 | IISEEDR----TGZ |
| 76 |  |
| 77 |  |
| 82 | LCEEDDDDEAAARNW |
| 105 |  |
| 114 |  |
| 117 |  |
| 78 | SICEEDE-----EGZPWRERYSYWNSSQSY VITKGWSF VKEKGLDAGD ULEHER |
| 79 |  |
| 80 |  |
| 78 |  |
| 78 |  |
| 71 |  |
| 94 | SSEEDE------SGKSTRERYSYWNSSQSY-VITKGWSREVK |
|  |  |
| 227 |  |
| 94 |  |
| 257 |  |
| 91 |  |
| 218 |  |
| 217 |  |
| 21 |  |
| 21 |  |
| 212 |  |
| 200 |  |
| 213 | IINEETG------EGRYWFERYSYWNSSQSY-VITKGWSREVKEIGITRGITUAEYESA |
| 213 |  |
| 227 |  |
| 221 |  |
| 227 |  |
| 251 |  |
| 263 | INEEDV-----GGYWRERYSYWNSSQSY-VLTKGWSREVKEKNUKAGTTVCEHEST |

GRM2M2G053008
HvMLOC 57250
Os12g0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489
GRMZM2G024948 T01
os02g0683500
HvMLOC 66387
0s04g0581400
GRMZM2G102059 T01
Os 10 g 0537100
GRM2M2G142999 TOI
GRM2M2G125095 T01
os03g0120900
GRMZM2G098443_T01
GRM2M2G082227 T01
Osllg0156000
GRM2M2G328742 T01
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At3g11580
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HvMLOC_38822
os01g0693400
HvMLOCA4012
HvMLOC 7940
HvMLOC 75135
TRAECDM81004
HvMLOC_56567
TRAES3BF098300010CFD_t1
HvMLOC_63261
TRAES3BF062700040CFD_t1
TRAES3BF062600010CFD_t1 Bra038346
GmLoc732601
GmLoc100789009
GmLoc100776987
GmLoc100801107


| GRMZM2G053008 | 338 | ------SESRKWADMCKLETMPSQSFIYLQLYELKDDFIQAEIRKPSYQSVCSRSTGWFS |
| :---: | :---: | :---: |
| HVMLOC 57250 |  |  |
| Os 1290157000 | 109 | -------NGGWMCYSTSG---SSYDT--S |
| GmLoc100778733 | 275 | RLYSLPSPTPPRHEEH---------LNYNNA - -----------MYH |
| Bra004501 | 162 | RFYSEPTAT---SYNL---------YNYQQP- |
| Bra000434 | 159 | RFYSFQTATTSTSYNE---------YNHOQP |
| Bra040478 | 150 | RFYSEPTATTSTCYDL---------YNHQPP |
| Bra014415 | 160 | RFYSYPYPQIQASYER- |
| Bra003482 | 141 | RFYSFSHPQN |
| Bra007646 | 141 | RFYSEPHPQMPTSFES |
| GlycinemaxLoc100781489 | 213 | RLYSLPPTMPPRYHHDHHFH---HHLNYNNLF- |
| GRM2M2G024948_T01 | 226 |  |
| os02g0683500 | 222 |  |
| HvMLOC_66387 | 218 |  |
| Os04g0581400 | 232 |  |
| GRM2M2G102059_T01 | 219 |  |
| Os 10 g 0537100 | 185 | ------VPLCPWRDYTTAYG---GGYGY |
| GRM2M2G142999_T01 | 170 | ------VPICPWQDYG |
| GRM2M2G125095_T01 | 168 | ------VPICPWQGYG- |
| os03g0120900 | 163 | ------IPFAPWAHHH---G---H-------G-------------AAAAA-AAAAGARFLL |
| GRM2M2G098443_T01 | 165 | ------VPYAPWAA--HAHH---HHYPADGHT------------EPVTPCLCATLVATEM |
| GRM2M2G082227_T01 | 164 | -VFYAPWAAAAHAHH---HHYPAAGVG------------AARTTTTTTTTVLHHL |
| Os11g0156000 | 164 | -ARQNAGEQQPWSPMCYSTS---GGGSY |
| GRMZM2G328742_T01 | 186 | -DHQQQQQPSSPWSPMCYSTS---G.SYSY |
| GmLoc100802734 | 183 | --SSASFYSAH---PP--Y- |
| GmLoc100795470 | 202 | SNK-----NEGWTRGFYSAH---HP--Y |
| GmLoc100818164 | 204 | SSKNEGDVGVGWTRGFYPAH---HP--Y |
| Bra017262 | 157 |  |
| At2g36080 | 158 |  |
| Bra005301 | 159 |  |
| At3g11580 | 160 | VNITAYWSGLT-------TP--Y |
| BraLoc103849927 | 160 | -VNTTAYWSGLT-------TP--Y |
| BrassicarapaBra034828 | 153 | ANITAYWNGLT-------TP |
| At5g06250 | 176 | SS------MGA-------LS |
| Bra005886 | 170 | ---5.5------MT |
| GmLoc102660503 | 318 |  |
| HVMLOC_38822 | 173 |  |
| Os0190693400 | 336 |  |
| HvMLOC44012 | 172 |  |
| HvMLOC_7940 | 305 |  |
| HvMLOC_75135 | 298 |  |
| TRAECDM81004 | 294 |  |
| HvMLOC_56567 | 293 |  |
| TRAES3BF098300010CFD_t1 | 293 |  |
| HvMLOC_63261 | 280 |  |
| TRAES3BF062700040CFD_t1 | 292 |  |
| TRAES3BF062600010CFD_t1 | 292 |  |
| Bra038346 | 298 |  |
| GmLoc732601 | 301 |  |
| GmLoc100789009 | 307 |  |
| GmLoc100776987 | 325 |  |
| GmLoc100801107 | 341 |  |


| GRMZM2G053008 | 392 |  |
| :---: | :---: | :---: |
| HvMLOC_57250 |  |  |
| Os 12g0157000 | 127 | --ANSYAYHRSV--------- |
| GmLoc100778733 | 301 | ------P-FHHHGAGSGINATTHHYNNYHEMSSTTT--SGSAGSVFYHRS-TPPISMPLA |
| Bra004501 | 181 | --------RHHHHSG----------YNYPQIPRE--------EGYGYLV- |
| Bra000434 | 181 | -RH-HHSG----------YCYPQIPRE-------EGYGYVVRS-V- |
| Bra040478 | 172 | RH-HHIG----------YGYPQIPRE-------EGYGYFVRS-V- |
| Bra014415 | 176 |  |
| Bra003482 | 151 | -LYHRYQQD------LGIGYYVSS-M- |
| Bra007646 | 157 | ------------SHN----------LYHHRFQRD------LGIGYY |
| GlycinemaxLoc100781489 | 243 | --------FQQHQYQOLGAATTTHHNNYGY-------QNSGSGSLYYLRSSMSMGG---- |
| GRM2M2G024948_T01 | 252 | PFSPPATLYEH-RLR---Q-----GLDFRSMTTTYPAPTVGRQLLFFGSARMPPHHAPPP |
| OS02g0683500 | 238 | QPSPPATLYEH-RLR---Q-----GLDFRAFNPA-A--AMGRQVLLFGSAR-IPPQAP-- |
| HvMLOC_66387 | 235 | PFSPPATLYEH-RLR---Q-----GFDFRGMNPSYP--TMGRQVILFGSAARMPPHGPAP |
| OS0490581400 | 251 | PPRSTSI----TAFA---R-----AST----SATS------TPLCRRGSSS----SSAPQ |
| GRM2M2G102059_T01 | 238 | PPAPPATLYEHHRFR---Q-----ALDFRNINAAA---A.PARQLLFFGSAGMPPRASMPQ |
| Os 10 g 0537100 | 204 | -GYG----GGSTPA.SSRHVLFL |
| GRMZM2G142999_T01 | 180 | -A.SAPA.PNRHVLFL |
| GRM2M2G125095_T01 | 178 | ASAPAPSRHVLFL |
| Os03g0120900 | 191 | PFSST-PIYDHHRRH-------AHAVGYDAYA-----AATSRQVLFY- |
| GRM2M2G098443_T01 | 202 | RASSS-QLSLTRSNLS--RPPQPRIARVDGAQPRPSSSPRQPQSLWC- |
| GRM2M2G082227_T01 | 203 | PRSPS-PLYLDTRRR---------HVGYDAY------GAGTRQLLFY |
| Os11g0156000 | 188 | PT-----------SPANSY-----------------A. C |
| GRM2M2G328742_T01 | 210 | PT------------SSPANSQH----------------AYH |
| GmLoc100802734 | 195 | PA-----------HH------------------------ |
| GmLoc100795470 | 220 | PT------------HH-----------------------LHH |
| GmLoc100818164 | 227 | PT------------HH- |
| Bra017262 | 157 |  |
| At2g36080 | 158 |  |
| Bra005301 | 159 |  |
| At3g11580 | 174 |  |
| BraLoc103849927 | 174 | RQ------------VH----------------------A.ST |
| BrassicarapaBra034828 | 167 | RQ-----------VH----------------------A.ST |
| At5g06250 | 184 | HQ-----------IH-----------------------ATS |
| Bra005886 | 174 | AP-----------------------PYS |
| GmLoc102660503 | 318 |  |
| HvMLOC_38822 | 173 |  |
| Os01g0693400 | 336 |  |
| HvMLOC44012 | 172 |  |
| HvMLOC_7940 | 305 |  |
| HvMLOC_75135 | 298 |  |
| TRAECDM81004 | 294 |  |
| HvMLOC_56567 | 293 |  |
| TRAES3BF098300010CFD_t1 | 293 |  |
| HVMLOC_63261 | 280 |  |
| TRAES3BF062700040CFD_t1 | 292 |  |
| TRAES3BF062600010CFD_t1 | 292 |  |
| Bra038346 | 298 |  |
| GmLoc732601 | 301 |  |
| GmLoc100789009 | 307 |  |
| GmLoc100776987 | 325 |  |
| GmLoc100801107 | 341 |  |

GRMZM2G053008
HvMLOC_57250
Os12g0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLocl00781489 GRMZM2G024948 T01
os02g0683500
HvMLOC 66387
0s04g0581400
GRMZM2G102059 T01
Os 10 g 0537100
GRM2M2G142999 T01
GRM2M2G125095 T01
os03g0120900
GRMZM2G098443_T01
GRM2M2G082227_T01
Os11g0156000
GRM2M2G328742 T01
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At3g11580
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HvMLOC_38822
os01g0693400
HvMLOCAA012
HvMLOC 7940
HvMLOC 75135
TRAECDM81004
HvMLOC_56567
TRAES3BF098300010CFD_t1
HvMLOC_63261
TRAES3BF062700040CFD t1
TRAES3BF062600010CFD_t1
Bra030346
GmLoc732601
GmLoc100789009
GmLoc100776987
GmLoc100801107


GRMZM2G053008
HvMLOC_57250
Os 12 g 0157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489 GRMZM2G024948_TO1 OS02g0683500 HvMLOC 66387 $0: 04 \mathrm{~g} 0581400$ GRMZM2G102059 T01 Os 10 g 0537100 GRMZM2G142999 T01 GRMZM2G125095_T01 os03g0120900 GRMZM2G098443_T01 GRMZM2G082227_T01 $0 s 11 \mathrm{~g} 0156000$ GRMZM2G328742 TO1
GmLoc 10080273 4
GmLoc 100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At 3 g 11580
BraLOC103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HvMLOC_38822
os01g0693400
HvMLOC44012
HVMLOC_7940
HvMLOC_75135
TRAECDM8 1004
HvMLOC 56567
TRAES3BF098300010CFD_t1
HvMLOC_63261
TRAES3BF062700040CFD_t1 TRAES 3BF062600010CFD_t1 Bra038346
GmLoc732601
GmLoc100789009
GmLoc100776987
GmLoc100801107


GRM2M2G053008
HvMLOC_57250
Os 1290157000
GmLoc100778733
Bra004501
Bra000434
Bra040478
Bra014415
Bra003482
Bra007646
GlycinemaxLoc100781489
GPMZM2G024948 T01
os02g0683500
HvMLOC_66387
os04g0581400
GRMZM2G102059 T01
Os 1000537100
GRMZM2G142999_T01
GRM2M2G125095_T01
os03g0120900 GRMZM2G098443_T01
GRM2M2G082227_T01
Os11g0156000
GRMZM2G328742_T01
GmLoc100802734
GmLoc100795470
GmLoc100818164
Bra017262
At2g36080
Bra005301
At 3 g 11580
BraLoc103849927
BrassicarapaBra034828
At5g06250
Bra005886
GmLoc102660503
HvMLOC 38822
os01g0693400
HvMLOC44012
HVMLOC_7940
HvMLOC 75135
TRAECDM81004
HvMLOC 56567
TRAES3BF098300010CFD t1 HvMLOC 63261 TRAES3BF062700040CFD t1 TRAES3BF062600010CFD t1 Bra038345
GmLoc 732601
GmLoc100789009
GmLoc100776987 GmLoc100801107

484 --------------DSP-------------------PMADDIHEFTLG---DSYAFGEGFSNG

181 YAA---VSTVNY\%SV
442 --S-SMANSNSQPRLQLLREDTLSSSS-----AREGD-----QRGVGEPSMLFD\#, DPSLQ


255 GGG-GASSSSS; \% QLRLGSSCEDDH--------------------FSKKGKSSLPFD\%DQ---

234 GV--SMA.SVGS\%IQLRLVSSD--DESLVAMEAA.SVDEDHHLET-KKGKSSLSED; DRK--

370 HQR-LRV------PVPVPLEDPLSSSA--AAAAREG----DHKGASTGTSLLFD\% DPSLQ
378 GWQ-QRTPTLP\% LELPRHG---GESSA--ASSPSSS----SSSKREARSALDLD\%-----
365 AWM-RRDPTLR LELPPHHHHGAESSA--ASSESSS----SSSKRDAHSALDLD ${ }^{3}$------
368 AWR-PRDHTLR芯LEFPSHGA------E--ASSPSSS----SSSKREAHSGLDLDॠ-----
GWQ-RPGP-LRF\%. ESPQR---GAESSA--ASSPSSS---SSSKREAHSSLDLD.

243 -RG-RAAS-TT\%LQLPSP-------------SSSTS----SSTAGKDVCCLDLG\% ${ }_{3}^{*}----$
242 -R----TP-ST\%LQLPSP-------------SSSTS----SSTGGKDVRSLDLG\%-------
284 ----TAPP-----PLPSP-------------P-SSS----SSSSGKARCSLNLD\%



269 --------------QTSS-------------YSSSSN--------------PHHHM, PQQP-

308 TQGTDIHSHLNF\%QOOQT-------------SNSKPP--------------PHHMM, RHOPY

213 --NHD---QFHF\%RQQQH-----------------YPP----------------PYYMDSSETGD




|  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |







327 -----ELGPPE $\%$ KRQSV----------------------------------AHGCGRM---NYI









| GRM2M2G053008 | 511 | YLEEVLRSLPLQEDGQKKL-CDAPINADASD |
| :---: | :---: | :---: |
| HvMLOC 57250 |  |  |
| Os 1290157000 |  |  |
| GmLoc100778733 | 489 | YRQ- |
| Bra004501 |  |  |
| Bra000434 |  |  |
| Bra040478 |  |  |
| Bra014415 |  |  |
| Bra003482 |  |  |
| Bra007646 |  |  |
| GlycinemaxLoc100781489 | 417 | YHRH |
| GRM2M2G024948_T01 |  |  |
| Os02g0683500 |  |  |
| HvMLOC 66387 |  |  |
| OS04g0581400 |  |  |
| GRM2M2G102059_T01 |  |  |
| Os 10 g 0537100 |  |  |
| GRMZM2G142999_T01 |  |  |
| GRM2M2G125095_T01 |  |  |
| os03g0120900 |  |  |
| GRM2M2G098443_TO1 |  |  |
| GRM2M2G082227_T01 |  |  |
| Osllg0156000 |  |  |
| GRMZM2G328742_T01 |  |  |
| GmLoc100802734 |  |  |
| GmLoc100795470 | 336 | YY- |
| GmLoc100818164 | 343 | YY |
| Bra017262 | 237 | VHQTSS-Q-----QG |
| At2g36080 | 240 | MNRTS |
| Bra005301 | 240 | VHQTRS-P-----QG |
| At3g11580 | 258 | ALEQVGDG-----RG |
| BraLOC103849927 | 255 | AMEQVGDG-----RR- |
| BrassicarapaBra034828 | 249 | AMEQVGDG-----RG |
| At5g06250 | 283 |  |
| Bra005886 |  |  |
| GmLoc102650503 |  |  |
| HVMLOC_38822 | 248 | CPRSRDQL-----EGVQAAGSTEAL------ |
| os01g0693400 |  |  |
| HvMLOC44012 |  |  |
| HvMLOC 7940 | 352 | - - GAFVL |
| HVMLOC-75135 | 350 | CYSIG------------TI-GPLMLN----- |
| TRAECDM81004 | 346 | --L-GAFVL------ |
| HvMLOC_56567 | 343 | ----------L-GDFAL------ |
| TRAES3BF098300010CFD_t1 | 345 | ----------L-GAFAL------ |
| HvMLOC_63261 |  |  |
| TRAES3BF062700040CFD_t1 |  |  |
| TRAES3BF062600010CFD_t1 |  | ------------------------------ |
| Bra038346 | 345 | ----A------------II-NAL-------- |
| GmLoc732601 | 346 | ---K------------VI-GAL-------- |
| GmLoc100789009 | 357 | --K------------VI-GAL--------- |
| GmLoc100776987 | 379 | ---K------------II-GAL-------- |
| GmLoc100801107 | 396 | -K------------II-GAL----- |

Figure 14


[^0]```
os11g01560000
```

A-KI
GGACTGGGGTTGCTCCTGGGACACAAGCGACAGCGCGCGGG (SEQ ID NO: 147)
$\mathrm{A}-2$
CCCAGGAGCA ACCCCAGTCCGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 148)
B-RI
TGCTATESCTAGCTGTAAAACACACAAGCGACAGCGCGCGGG (SEQ ID NO: 149)
$3-\mathrm{F}_{2}$
GCCCEGGACGCCCAGTGACGGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 150)
Os12g0157000
CMR
GGGGGTGCCCCTGGGCGAGAACACAAGCGACAGCGCGCGGG (SEQ ID NO: 152)
CH 2
TCTCGCCCAGCGGCACCCCCGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 153)
OM ${ }^{2}$
CTCGTAGYGGGGTGCTAGTACACAAGCGACAGCGCGCGGG (SEQ ID NO: 154)
3-E2
ACTACCACCACGACTACGAGGTTTTAGAGCTAGAAATAGCA (SEQ ID NO: 155)


Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
a.
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) for the purposes of international search only in the form of an Annex C/ST. 25 text file.
c. $£$ furnished subsequent to the international filing date for the purposes of international search only:

1
in the form of an Annex C/ST. 25 text file (Rule 13fer1 (a)).
上1 on paper or in the form of an image file (Rule 13fer1 (b) and Administrative Instructions, Section 713).
2. $\square$

In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

| C(Continua | on). DOCUMENTS CONSIDERED TO BE RELEVANT |  |
| :---: | :---: | :---: |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Information on patent family members
International application No
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[^0]:    gacggccagtgccaagcttCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGCCCTTVEATEA
    
    
    
     AGATTGGTTTAGAGCTAGAAATAGCAAGTYAAAATAAGGGTAGTGGTTATCAACTYGAAAA AGTOGCACGGAGTGGTGCTTTTTTTGTCCCTTCGAAGGGCAATTCTGCAGATATCCATCACACT GGCGGCCGCTCGAGGTCGaagettgcatgcctgcagg

