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(54) Title: METHODS OF CONTROLLING PLANT SEED AND ORGAN SIZE COMPRISING MANIPULATION OF DA
PROTEIN EXPRESSION

(57) **Abrégé/Abstract:**

This invention relates to the identification of a regulator protein (termed DA) which controls the size of plant seeds and organs in Arabidopsis and other plants. Manipulation of DA protein expression may be useful, for example, in improving crop yield and increasing plant biomass.

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(54) Title: METHODS OF CONTROLLING PLANT SEED AND ORGAN SIZE

(57) Abstract: This invention relates to the identification of a regulator protein (termed DA) which controls the size of plant seeds and organs in Arabidopsis and other plants. Manipulation of DA protein expression may be useful, for example, in improving crop yield and increasing plant biomass.

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METHODS OF CONTROLLING PLANT SEED AND ORGAN SIZE
COMPRISING MANIPULATION OF DA PROTEIN EXPRESSION

Field of Invention

This invention relates to methods of controlling the size of the
 5 seeds and organs of plants.

Background of Invention

The size of seeds and organs is an agronomically and ecologically
 important trait that is under genetic control (Alonso-Blanco, C.
 10 *Proc Natl Acad Sci U S A* 96, 4710-7 (1999); Song, X.J. *Nat Genet* 39,
 623-30 (2007); Weiss, J. *Int J Dev Biol* 49, 513-25 (2005); Dinneny,
 J.R. *Development* 131, 1101-10 (2004); Disch, S. *Curr Biol* 16, 272-9
 (2006); *Science* 289, 85-8 (2000); Horiguchi, G. *Plant J* 43, 68-78
 (2005); Hu, Y. *Plant J* 47, 1-9 (2006); Hu, Y. *Plant Cell* 15, 1951-61
 15 (2003); Krizek, B.A. *Dev Genet* 25, 224-36 (1999); Mizukami, Y. *Proc*
Natl Acad Sci U S A 97, 942-7 (2000); Nath, U. *Science* 299, 1404-7
 (2003); Ohno, C.K. *Development* 131, 1111-22 (2004); Szecsi, J. *Embo J*
 25, 3912-20 (2006); White, D.W. *Proc Natl Acad Sci U S A* 103, 13238-
 43 (2006); Horvath, B.M. *Embo J* 25, 4909-20 (2006); Garcia, D. *Plant*
 20 *Cell* 17, 52-60 (2005). The final size of seeds and organs is
 constant within a given species, whereas interspecies seed and organ
 size variation is remarkably large, suggesting that plants have
 regulatory mechanisms that control seed and organ growth in a
 coordinated and timely manner. Despite the importance of seed and
 25 organ size, however, little is known about the molecular and genetic
 mechanisms that control final organ and seed size in plants.

The genetic regulation of seed size has been investigated in plants,
 including in tomato, soybean, maize, and rice, using quantitative
 30 trait locus (QTL) mapping. To date, in the published literature, two
 genes (Song, X.J. *Nat Genet* 39, 623-30 (2007); Fan, C. *Theor. Appl.*
Genet. 112, 1164-1171 (2006)), underlying two major QTLs for rice
 grain size, have been identified, although the molecular mechanisms
 of these genes remain to be elucidated. In *Arabidopsis*, eleven loci

affecting seed weight and/or length in crosses between the accessions Ler and Cvi, have been mapped {Alonso-Blanco, 1999 supra}, but the corresponding genes have not been identified. Recent studies have revealed that AP2 and ARF2 are involved in control of seed size. Unfortunately, however, ap2 and arf2 mutants have lower fertility than wild type (Schruff, M.C. *Development* 137, 251-261 (2006); Ohto, M.A. *Proc. Natnl Acad. Sci USA* 102, 3123-3128 (2005); Jofuku, K.D. *Proc. Natnl Acad. Sci. USA* 102, 3117-3122 (2005)). In addition, studies using mutant plants have identified several positive and negative regulators that influence organ size by acting on cell proliferation or expansion {Krizek, B.A. *Dev Genet* 25, 224-36 (1999); Mizukami, Y. *Proc Natl Acad Sci U S A* 97, 942-7 (2000); Nath, U. *Science* 299, 1404-7 (2003); Ohno, C.K. *Development* 131, 1111-22 (2004); Szecsi, J. *Embo J* 25, 3912-20 (2006); White, D.W. *Proc Natl Acad Sci U S A* 103, 13238-43 (2006); Horvath, B.M. *Embo J* 25, 4909-20 (2006); Garcia, D. *Plant Cell* 17, 52-60 (2005). Horiguchi, G. *Plant J* 43, 68-78 (2005); Hu, Y *Plant J* 47, 1-9 (2006) Dinneny, J.R. *Development* 131, 1101-10 (2004)).

Identification of a factor or factors that control the final size of both seeds and organs will not only advance understanding of the mechanisms of size control in plants, but may also have substantial practical applications for example in improving crop yield and plant biomass for generating biofuel.

Summary of Invention

The present inventors have identified a UIM and LIM domain-containing protein (termed DA1) which is a key regulator in controlling the final size of seeds and organs by restricting the duration of proliferative growth. An allele (termed the *dal-1* allele) is shown herein to act as a dominant negative interfering mutation for DARs or DA1-related proteins. Over-expression of the *dal-1* mutant gene (R358K) in wild type causes an increase in seed and organ size in wild type plants, indicating that the *dal-1* allele interferes with DARs in a dosage dependent manner. Mutations that reduce or abolish the function of *EOD1/BB*, which encodes an E3

ubiquitin ligase, synergistically enhance the phenotypes of *dal-1*, indicating that *DA1* acts in parallel with *EOD1/BB* to limit the size of seeds and organs. The functional characterization of *DA1* and *EOD1/BB* provides insight into the mechanism of control of the final seed and organ size and may be a valuable tool for improving crop yield and increasing plant biomass.

Aspects of the invention provide an isolated protein which is *DA1* and an isolated nucleic acid encoding a protein which is *DA1*. Also provided are *DA1*-related proteins and encoding nucleic acid. *DA1* and *DA1*-related proteins (*DARs*) are collectively referred to herein as *DA* proteins.

Other aspects of the invention provide an isolated protein (*DA1R358K*) which interferes with the function of *DA1* and *DA1*-related proteins and an isolated nucleic acid encoding such a protein.

Another aspect of the invention provides a method for producing plants having normal fertility but which have one or more features selected from longer life-span, enlarged organ size, enlarged seed size.

Another aspect of the invention provides a plant having normal fertility but which has a feature selected from longer life-span, enlarged organ size, enlarged seed size, and combinations of these features

Brief Description of Drawings

Figure 1 shows that *dal-1* has large seeds and organs. (A and B) Dry seeds of Col-0 (A) and *dal-1* (B). (C and D) Mature embryos of Col-0 (C) and *dal-1*(D). (E and F) 9d- old seedlings of Col-0 (E) and *dal-1* (F). *dal-1* has larger cotyledons than WT. (G) The fifth leaves of Col-0 (left) and *dal-1* (right). *dal-1* has larger and rounder leaves compared with wild type Col-0. (H and I) Flowers of Col-0 (H) and

dal-1(I). (J and K) Siliques of Col-0 (J) and *dal-1* (K). (L) Average seed weight of Col-0, *dal-1*, *dal-ko1*, *dar1-1*, and *dal-koldar1-1* is given in mg per 100 seeds. Standard deviations (SD) are shown (n=5). Plants were grown under identical conditions. (M-O) stem diameter (M), epidermal cell number in stem cross sections (N), and petal area (O) of Col-0, *dal-1*, *DA1COM#2*, and *35S::DA1^{R358K}#5*. (P and Q) Mass of 5 fresh flowers (stage 14) (P) and leaves (1st -7th) of 35d-old plants (Q). (R) Cell area of embryos (E), petals (P) and leaves (L) in Col-0 and *dal-1*. Values are given as mean $\pm$ SD relative to the respective wild type value, set at 100%. (S) Relative expression levels of *DA1* in Col-0 and *35S::DA1^{R358K}#5* seedlings were measured by quantitative real-time RT-PCR. Scale bars: 200 μ m (A and B), 100 μ m (C and D), 1mm (E and F), 0.5cm (G), 1mm (H to K).

Figure 2 shows kinematic analysis of petal and leaf growth. (A) Growth of Col-0 and *dal-1* mutant petals. The largest petals of each series are from opened flowers (stage 14). (B) Mitotic index in WT and *dal-1* mutant petals. Time axis in (B) corresponds to the one in (A). (C) Growth of the fifth leaf of Col-0, *dal-1*, *DA1COM#2*, and *35S::DA1^{R358K}#5* over time. DAE is days after emergence.

Figure 3 shows the identification and expression of the *DA1* gene. (A) *DA1* gene structure, showing the mutated sites of *dal-1*, *sod1-1*, *sod1-2*, and *sod1-3* alleles. The start codon (ATG) and the stop codon (TGA) are indicated. Closed boxes indicate the coding sequence and lines between boxes indicate introns. T-DNA insertion sites (*dal-ko1*, *dal-ko2* and *dal-ko3*) in *DA1* gene are shown. (B to G) *DA1* promoter activity monitored by *pDA1::GUS* transgene expression. GUS staining in seedlings (B and C), an embryo (D), roots (E), and petals (F and G). (H and I) The flowers of Col-0 (H) and *dal-koldar1-1* double mutant (I). (J) Siliques of Col-0 (left) and *dal-koldar1-1* double mutant (right). (K) Petal area of Col-0, *dal-ko1*, *dar1-1*, *dal-koldar1-1* double mutants. The *dal-koldar1-1* double mutant displays a *dal-1* phenotype including large flowers and petals, wide and flattened siliques, and short styles. (L)

Quantitative RT-PCR analysis revealed that expression of *DA1* is slowly induced by ABA. 7d- old WT seedlings were treated with 10µm ABA for 2, 4, 6, 18 and 30 hours. (M and N) Wild type Col-0 and *dal-1* seeds were grown on MS medium with 2 µm ABA under constant light conditions. The *dal-1* mutant (N) exhibits ABA- insensitive seedling establishment compared with wild type Col-0 (M). (O) 4d- old seedlings of Col-0 (left), *dal-1*(middle) and *DA1COM* #2(right) were transferred to MS medium with 5 µm ABA for 3 weeks. *dal-1* mutant seedlings continue to grow in the presence of low levels of ABA that inhibit the growth of wild type Col-0 seedlings. Scale bars: 1mm (B, H, I, M, and N), 50 µm (D and E), 0.5mm (C and J), 0.1mm (F and G), 0.5cm (O).

Figure 4 show mutations in *EOD1/BB* synergistically enhance the phenotypes of *dal-1*. (A) Flowers of Col-0, *dal-1*, *eod1-2* and *eod1-2dal-1* double mutants. (B) Soil grown plants of Col-0, *eod1-2*, *eod1-2* and *eod1-2dal-1* double mutants. (C) Average seed weights of Col-0, *dal-1*, *eod1-2* and *eod1-2dal-1* double mutants are shown as mg per 100 seeds. Standard deviations are shown (n=5). Plants were grown under identical conditions. (D) Petal area of Col-0, *dal-1*, *eod1-2* and *eod1-2dal-1* double mutant. Standard deviation values are shown (n>50), (E) A model of *DA1* and *EOD1/BB* in controlling seed and organ size. Scale bars: 2mm (A), 50mm (B).

Figure 5 shows that *dal-1* has large seeds. Prewieghed batches of wild type Col-0 (A), *dal-1* (B), *DA1COM*#2 (C), *35S::DA1^{R358K}*#5 (D) ,and *dal-koldal-1* mutant seeds from individual plants were passed through a series of wire sieves of decreasing mesh size (in µm) as described in Supplementary methods. (E) The average seed weight per plant. Standard deviation values was given (n=5). Plants were grown under identical conditions.

Figure 6 shows seed development in wild type and *dal-1* plants.

(A to L), Cleared ovules (A,B) and seeds (C to L) of wild type (A, C, E, G, I and K), and *dal-1* (B, D, F, H, J and L) imaged with differential contrast optics. Scale bars: 50µm (A to L).

5 Figure 7 shows that *dal-1* plant has large flower with extra petals and deformed silique with extra carpels. (A) Wild type flower. (B and C) *dal-1* flowers with extra petals. (D) Wild type silique, (E to G) *dal-1* siliques with extra carpels. Scale bars: 1mm (A to C), 2mm (D to G).

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Figure 8 shows that *dal-1* mutant has the prolonged cell proliferation. (A and B) *pCyclinB1;1::GUS* activity in the first leaves (9 days after germination) of wild type (A) and *dal-1* (B) seedlings grown on MS medium containing 1% glucose. (C) Expression level of *SAG12* gene in the fifth leaves of wild type Col-0 and *dal-1* plants was detected by using Quantitative real-time RT-PCR analysis. DAE is days after emergence.

Figure 9 shows map-based cloning of *DA1*. (A) Fine mapping of the *DA1* locus. The *DA1* locus was mapped to chromosome 1 (Chr 1) between markers T16N11 and CER451450. The *DA1* locus was further narrowed to a 30-kb genomic DNA region between markers T29M8-26 and F18O14-52 and co-segregated with CAPS marker *DA1CAPS*. The number of recombinants identified from F₂ plants is shown. (B) The mutation in *dal-1* was identified using the CAPS marker *DA1CAPS1*. (C to E) Expression levels of *DA1* (C) and *DAR1* (D) in wild type and T-DNA lines were revealed by RT-PCR analysis.

Figure 10 shows the identification of *DA1*-related proteins in *Arabidopsis* and homologs of *DA1* in other species. *DA1*-related proteins in *Arabidopsis* are shown in Figure 10A and *DA1*-related proteins in other species are shown in Figure 10B.

Figure 11 shows that the R358K mutation in *DA1* is responsible for increased seed and organ size. (A) Petal area of Col-0, *dal-1*, *dal-*

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kol1, *dal1-ko2*, *dal1-ko3*, *dal1-1/Col-0* F₁, *dal1-kol1/dal1-1* F₁, *dal1-ko2/dal1-1* F₁, *dal1-ko3/dal1-1* F₁, *dal1-kol1/Col-0* F₁, *dal1-ko2/Col-0* F₁, *dal1-ko3/Col-0* F₁, and *dal1-kol1/dal1-1* F₁. Standard deviation values are given (n>50). (B) Average seed weight of *Col-0*, *dal1-1*, *dal1-kol1*, *dal1-kol1/dal1-1* F₁, and *dal1-kol1/Col-0* F₁ is given in mg per 100 seeds. Standard deviation values are given (n=5). Plants were grown under identical conditions.

Figure 12 shows that mutations in an enhancer of *dal1-1* (*EOD1/BB*) synergistically enhance the large seed and organ phenotypes of *dal1-1*. (A) The *eod1-1dal1-1* double mutant has an increased seed weight compared with *dal1-1*. Average seed weight of *dal1-1* and *eod1-1dal1-1* double mutant is given in mg per 100 seeds. Standard deviation values are shown (n=5). Plants were grown under identical conditions. (B) The *eod1-1dal1-1* double mutant has larger flower than *dal1-1*. (C) *EOD1/BB* gene structure, showing the mutated sites of the two *eod1* alleles. The start codon (ATG) and the stop codon (TGA) are indicated. Closed boxes indicate the coding sequence and lines between boxes indicate introns. The mutated site in *eod1-1* and T-DNA insertion site in *eod1-2* also are shown. (D) Eight week old plants of *Col-0*, *dal1-1*, *eod1-2*, and *eod1-2dal1-1* plants are shown. The *eod1-2dal1-1* plant has a longer growing period than *dal1-1*. (E) Eight week old plants of *Ler*, *dal1-1^{Ler}*, *bb-1*, and *bb-1dal1-1^{Ler}* plants are shown. The *bb-1dal1-1^{Ler}* plant has a longer growing period than *dal1-1^{Ler}*. Scale bars: 1mm (B), 5cm (D and E). (F) Petal areas of *Ler*, *dal1-1^{Ler}*, *bb-1*, and *bb-1dal1-1^{Ler}* double mutants. Standard deviation values are shown (n>50). Mutations in *BB* synergistically enhance the petal size phenotype of *dal1-1*, suggesting that *DA1* and *BB* act in parallel pathways.

Figure 13 shows that genetic analysis between *dal1-1* and *ant-5*, *axr1-12*, *ap2-7*, and *arf2-7*. (A and B) The petal size phenotype of *ant-5dal1-1^{Ler}* and *axr1-12dal1-1* double mutant is essentially additive, compared to their parental lines. (C and D) The seed size phenotype

of *ap2-7dal-1* and *arf2-7dal-1* double mutants is also essentially additive, compared to their parental lines.

Figure 14 shows a phylogenetic analysis of DA1-like proteins. Left graph: A distance matrix phylogenetic tree was created using PHYLIP software (VERSION 3.66) with the default settings (the JTT model of protein sequence evolution and the neighbour-joining algorithm). The tree was then imported into MEGA 4.0 software to rearrange.

Bootstrap values (the numbers on the branches indicate the number of times the partition of the species into the two sets which are separated by that branch occurred among the trees, only shown over 70) were obtained by 100 replicates. The data for the tree was the C-terminal 250 amino acid region of full length DA1-like protein sequences. The right graph shows a simplified overview of plant evolution based on the hyperbolic tree presented at (<http://ucjeps.berkeley.edu/TreeofLife/hyperbolic.php>). Clades that are related with text are retained in the graph. Species that were analysed are underlined.

Figure 15 (A-D) shows siliques of Col-0, *BrDA1aCOM* (35S::*BrDA1a* transgenic line), *OsDA1COM* (35S::*OsDA1* transgenic line) and *dal-1*. (E-H) Rosette leaves of Col-0, *dal-1*, *BrDA1bCOM* (35S::*BrDA1b* transgenic line) and 35S::*BrDA1a^{R/K}* (overexpressing 35S::*BrDA1a^{R/K}* in Col-0). (I) *DA1* gene structure showing the mutated sites of *dal-1* and T-DNA insertion sites (*dal-ko1*, *dal-ko2* and *dal-ko3*).

Detailed Description of Embodiments of the Invention

In various aspects, the invention provides isolated DA polypeptides encoded by *DA* genes and nucleic acid sequences described herein.

DA polypeptides include both DA-1 polypeptides and DA-1 related (DAR) polypeptides, and functional homologues thereof, as described herein.

DA polypeptides, including DA-1 polypeptides and DA-1 related (DAR) polypeptides, possess a characteristic domain structure.

A DA polypeptide may comprise a UIM1 domain and a UIM2 domain. A UIM1 domain may consist of the sequence of SEQ ID NO: 3 and a UIM2 domain may consist of the sequence of SEQ ID NO: 4.

p---pLpbAl pb.Sbp-.pp p (SEQ ID NO: 3)

10 p---pLpbAl pb.Sbp-spp p (SEQ ID NO:4)

wherein;

p is a polar amino acid residue, for example, C, D, E, H, K, N, Q, R, S or T;

15 b is a big amino acid residue, for example, E, F, H, I, K, L, M, Q, R, W or Y;

s is a small amino acid residue, for example, A, C, D, G, N, P, S, T or V;

l is an aliphatic amino acid residue, for example, I, L or V;

20 . is absent or is any amino acid, and

- is any amino acid.

Examples of suitable UIM1 and UIM2 domain sequences are set out below. Further examples of UIM1 and UIM2 domain sequences may be identified using standard sequence analysis techniques as described herein (e.g. Simple Modular Architecture Research Tool (SMART); EMBL Heidelberg, DE).

A DA polypeptide may comprise an LIM domain. An LIM domain may consist of the sequence of SEQ ID NO: 5;

pCs.CscsIh s.....bhlp tb.sp.aH.. .pCFpCs..p CppsLss... .p.ab.pcsp
baCpps... (SEQ ID NO: 5)

35 wherein;

c is a charged amino acid residue, for example, D, E, H, K, R;

10

p is a polar amino acid residue, for example, C, D, E, H, K, N, Q, R, S or T;

h is a hydrophobic amino acid residue, for example, A, C, F, G, H, I, L, M, T, V, W and Y;

5 t is a tiny amino acid residue, for example, A, G or S;

a is an aromatic amino acid residue, for example, F, H, W or Y;

b is a big amino acid residue, for example, E, F, H, I, K, L, M, Q, R, W or Y;

10 s is a small amino acid residue, for example, A, C, D, G, N, P, S, T or V;

l is an aliphatic amino acid residue, for example, I, L or V;

. is absent or is any amino acid; and

- is any amino acid.

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Examples of suitable LIM domain sequences are set out below. Further examples of LIM domain sequences may be identified using standard sequence analysis techniques (e.g. Simple Modular Architecture Research Tool (SMART); EMBL Heidelberg, DE).

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A DA polypeptide may comprise a carboxyl terminal region having at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% amino acid identity to residues 250 to 532 of SEQ ID NO: 1 that
25 define the C terminal domain of DA1.

A DA polypeptide may further comprise R at a position equivalent to position 358 of SEQ ID NO: 1.

30 A position in an amino acid sequence which is equivalent to position 358 of SEQ ID NO: 1 can be readily identified using standard sequence analysis tools. Examples of sequences with an R residue at a position equivalent to position 358 of SEQ ID NO: 1 are shown elsewhere herein.

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In some preferred embodiments, a DA polypeptide may comprise;

a UIM domain of SEQ ID NO:3

a UIM domain of SEQ ID NO:4

a LIM domain of SEQ ID NO:5, and

5 a C terminal region having at least 20% sequence identity to residues 250 to 532 of SEQ ID NO: 1.

A preferred DA polypeptide may further comprise R at a position equivalent to position 358 of SEQ ID NO: 1.

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For example, a DA polypeptide may comprise an amino acid sequence set out in a database entry selected from the group consisting of SGN-U317073, SGN-U277808, SGN-U325242, AT4G36860, SGN-U209255, ABO82378.1, AT2G39830, CAN69394.1, OS03G16090, 9234.M000024, 15 29235.M000021, AT5G66620, AT5G66630, AT5G66610, AT5G66640, AT5G17890, SGN-U320806, ABO96533.1, CAL53532.1, OS06G08400, SGN-U328968, OS03G42820 and OS12G40490 or may be variant or a fragment of one of these sequences which retains DA activity.

20 A DA polypeptide may comprise an amino acid sequence of AtDA1, AtDAR1, AtDAR2, AtDAR3, AtDAR4, AtDAR5, AtDAR6, AtDAR7, BrDA1a, BrDA1b, BrDAR1, BrDAR2, BrDAR3-7, BrDAL1, BrDAL2, BrDAL3, OsDA1, OsDAR2, OsDAL3, OsDAL5, PpDAL1, PpDAL2, PpDAL3, PpDAL4, PpDAL5, PpDAL6, PpDAL7, PpDAL8, SmDAL1 and SmDAL2 (as shown in Alignment E).

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Other examples of database entries of sequences of DA polypeptides are shown in Table 6 and Table 11. Other DA polypeptide sequences which include the characteristic features set out above may be identified using standard sequence analysis tools.

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In some preferred embodiments, a DA polypeptide may comprise the amino acid sequence of SEQ ID NO: 1 (AT1G19270; NP_173361.1 GI: 15221983) or may be a fragment or variant of this sequence which retains DA activity.

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A DA polypeptide which is a variant of a reference DA sequence, such as SEQ ID NO: 1 or a sequence shown in alignment E, may comprise an amino acid sequence having at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% sequence identity to the reference sequence.

A DA polypeptide which is a variant of SEQ ID NO: 1 may comprise a UIM1 domain having the sequence QENEDIDRAIALSLLEENQE (SEQ ID NO: 6) and a UIM2 domain having the sequence DEDEQIARALQESMVVGNSP (SEQ ID NO: 7).

A DA polypeptide which is a variant of SEQ ID NO: 1 may comprise a LIM domain having the sequence:

ICAGCNMEIGHGRFLNCLNSLWHPECFRCYGCSPISEYEFSTSGNYPFHKAC (SEQ ID NO: 8)

Particular amino acid sequence variants may differ from the DA-1 polypeptide of SEQ ID NO:1 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20, 20-30, 30-50, or more than 50 amino acids.

Sequence similarity and identity are commonly defined with reference to the algorithm GAP (Wisconsin Package, Accelrys, San Diego USA).

GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4.

Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul et al. (1990) *J. Mol. Biol.* 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) *J. Mol Biol.* 147: 195-197), or the TBLASTN program, of Altschul et al. (1990) *supra*, generally

employing default parameters. In particular, the psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used.

Sequence comparison may be made over the full-length of the relevant
5 sequence described herein.

In various aspects, the invention provides DA genes and nucleic acid sequences which encode DA polypeptides, as described herein.

10 For example, a nucleic acid encoding a DA polypeptide may comprise a nucleotide sequence set out in a database entry selected from the group consisting of SGN-U317073, SGN-U277808, SGN-U325242, AT4G36860, SGN-U209255, ABO82378.1, AT2G39830, CAN69394.1, OS03G16090, 9234.M000024, 29235.M000021, AT5G66620, AT5G66630,
15 AT5G66610, AT5G66640, AT5G17890, SGN-U320806, ABO96533.1, CAL53532.1, OS06G08400, SGN-U328968, OS03G42820 and OS12G40490 or may be variant or a fragment of one of these sequences.

Other database entries of nucleic acid sequences which encode DA
20 polypeptides are shown in Table 7.

In some preferred embodiments, a nucleic acid encoding a DA polypeptide may comprise the nucleotide sequence of SEQ ID NO: 2 or any one of SEQ ID NOS: 11 to 16 or may be a variant or fragment of
25 this sequence which encodes a polypeptide which retains DA activity.

A variant sequence may be a mutant, homologue, or allele of a reference DA sequence, such as SEQ ID NO: 2; any one of SEQ ID NOS: 11 to 16; or a sequence having a database entry set out above, and
30 may differ from the reference DA sequence by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide. Of course, changes to the nucleic acid that make no
35 difference to the encoded amino acid sequence are included. A

nucleic acid encoding a *DA* polypeptide may comprise a sequence having at least 20% or at least 30% sequence identity with the reference *DA* nucleic acid sequence, preferably at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 80%,
5 at least 90%, at least 95% or at least 98%. Sequence identity is described above.

A fragment or variant may comprise a sequence which encodes a functional *DA* polypeptide i.e. a polypeptide which retains one or
10 more functional characteristics of the polypeptide encoded by the wild-type *DA* gene, for example, the ability to modulate the duration of proliferative growth.

A nucleic acid comprising a nucleotide sequence which is a variant
15 of a reference *DA* nucleic acid sequence, such as SEQ ID NO: 2 or any one of SEQ ID NOS: 11 to 16, may selectively hybridise under stringent conditions with this nucleic acid sequence or the complement thereof.

Stringent conditions include, e.g. for hybridization of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na₂HPO₄, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include
20 hybridization overnight at 65°C in 0.25M Na₂HPO₄, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

An alternative, which may be particularly appropriate with plant nucleic acid preparations, is a solution of 5x SSPE (final 0.9 M
30 NaCl, 0.05M sodium phosphate, 0.005M EDTA pH 7.7), 5X Denhardt's solution, 0.5% SDS, at 50°C or 65°C overnight. Washes may be performed in 0.2x SSC/0.1% SDS at 65°C or at 50-60°C in 1x SSC/0.1% SDS, as required.

Nucleic acids as described herein may be wholly or partially synthetic. In particular, they may be recombinant in that nucleic acid sequences which are not found together in nature (do not run contiguously) have been ligated or otherwise combined artificially.

5 Alternatively, they may have been synthesised directly e.g. using an automated synthesiser.

The nucleic acid may of course be double- or single-stranded, cDNA or genomic DNA, or RNA. The nucleic acid may be wholly or partially
10 synthetic, depending on design. Naturally, the skilled person will understand that where the nucleic acid includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

15 In various aspects, the invention provides dominant negative DA polypeptides and encoding nucleic acids. A dominant negative DA polypeptide may increase one or more of organ size, seed size or longevity without affecting fertility, upon expression in a plant.

20 A dominant negative allele of a DA polypeptide may comprise a DA polypeptide having a mutation, e.g. a substitution or deletion, at a position equivalent to position 358 of SEQ ID NO: 1.

For example, a dominant negative allele of a DA polypeptide may
25 comprise a mutation of the conserved R residue at a position equivalent to position 358 of SEQ ID NO: 1. In preferred embodiments, the conserved R residue may be substituted for K. Position R358 of SEQ ID NO: 1 is located within the conserved C terminal region (amino acids 250 to 532 of SEQ ID NO: 1). An R
30 residue at a position in a DA polypeptide sequence which is equivalent to position 358 of SEQ ID NO: 1 may be identified by aligning these conserved C terminal regions using standard sequence analysis and alignment tools.

Nucleic acid which encodes a dominant negative allele of a DA protein may be produced by any convenient technique. For example, site directed mutagenesis may be employed on a nucleic acid encoding a DA polypeptide to alter the conserved R residue at the equivalent position to R358 in SEQ ID NO: 1, for example to K. Reagents and kits for *in vitro* mutagenesis are commercially available. The mutated nucleic acid encoding the dominant negative allele of a DA protein and may be further cloned into an expression vector and expressed in plant cells as described below to alter plant phenotype.

The nucleic acid encoding the DA polypeptide may be expressed in the same plant species or variety from which it was originally isolated or in a different plant species or variety (i.e. a heterologous plant).

"Heterologous" indicates that the gene/sequence of nucleotides in question or a sequence regulating the gene/sequence in question, has been introduced into said cells of the plant or an ancestor thereof, using genetic engineering or recombinant means, i.e. by human intervention. Nucleotide sequences which are heterologous to a plant cell may be non-naturally occurring in cells of that type, variety or species (i.e. exogenous or foreign) or may be sequences which are non-naturally occurring in that sub-cellular or genomic environment of the cells or may be sequences which are non-naturally regulated in the cells i.e. operably linked to a non-natural regulatory element. "Isolated" indicate that the isolated molecule (e.g. polypeptide or nucleic acid) exists in an environment which is distinct from the environment in which it occurs in nature. For example, an isolated nucleic acid may be substantially isolated with respect to the genomic environment in which it naturally occurs. An isolated nucleic acid may exist in an environment other than the environment in which it occurs in nature.

A nucleic acid encoding a DA 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.

5

Many suitable regulatory sequences are known in the art and may be used in accordance with the invention. Examples of suitable regulatory sequences may be derived from a plant virus, for example the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is
10 expressed at a high level in virtually all plant tissues (Benfey et al, (1990) EMBO J 9: 1677-1684). Other suitable constitutive regulatory elements include the cauliflower mosaic virus 19S promoter; the Figwort mosaic virus promoter; and the nopaline synthase (nos) gene promoter (Singer et al., Plant Mol. Biol. 14:433
15 (1990); An, Plant Physiol. 81:86 (1986)).

Constructs for expression of the DA genes under the control of a strong constitutive promoter (the 35S promoter) are exemplified below but those skilled in the art will appreciate that a wide
20 variety of other promoters may be employed to advantage in particular contexts.

A tissue-specific promoter may be employed to express the dominant negative form of the DA polypeptide in a specific tissue or organ to
25 increase size of that tissue or organ relative to tissues or organs in which the tissue-specific promoter is not active and the dominant negative form of the DA polypeptide is not expressed. For example, to increase the size of seeds, the dominant negative form of the DA polypeptide may be preferentially expressed in seed tissue, using a
30 seed specific promoter. For example, the polypeptide may be expressed in developing integument using an integument-specific promoter such as the INO promoter (Meister R.M., Plant Journal 37: 426-438 (2004)) or in embryos using an embryo specific promoter such as the histone H4 promoter (Devic M. Plant Journal 9; 205-215
35 (1996)).

Alternatively, or in addition, one might select an inducible promoter. In this way, for example, the dominant negative form of the DA polypeptide may be expressed at specific times or places in order to obtain desired changes in organ growth. Inducible promoters include the alcohol inducible AlcA gene-expression system (Roslan et al., Plant Journal; 2001 Oct; 28(2):225-35) may be employed.

The DA nucleic acid may be contained on a nucleic acid construct or vector. The construct or vector is preferably suitable for transformation into and/or expression within a plant cell.

A vector is, *inter alia*, any plasmid, cosmid, phage or *Agrobacterium* binary vector in double or single stranded linear or circular form, which may or may not be self transmissible or mobilizable, and which can transform prokaryotic or eukaryotic host, in particular a plant host, either by integration into the cellular genome or exist extrachromasomally (e.g. autonomous replicating plasmid with an origin of replication).

Specifically included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different organisms, which may be selected from *Actinomyces* and related species, bacteria and eukaryotic (e.g. higher plant, mammalia, yeast or fungal) cells.

A construct or vector comprising nucleic acid as described above need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

Constructs and vectors may further comprise selectable genetic markers consisting of genes that confer selectable phenotypes such as resistance to antibiotics such as kanamycin, hygromycin, phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones, glyphosate and d-amino acids.

Those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression, in particular in a plant cell. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 3rd edition, Sambrook & Russell, 2001, Cold Spring Harbor Laboratory Press.

Those skilled in the art can construct vectors and design protocols for recombinant gene expression, for example in a microbial or plant cell. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 3rd edition, Sambrook et al, 2001, Cold Spring Harbor Laboratory Press and *Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds. John Wiley & Sons, 1992. Specific procedures and vectors previously used with wide success upon plants are described by Bevan, Nucl. Acids Res. (1984) 12, 8711-8721), and Guerineau and Mullineaux, (1993) Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148.

When introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct that contains effective regulatory elements that will drive transcription. There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur.

Finally, the target cell type is preferably such that cells can be regenerated into whole plants.

Those skilled in the art will also appreciate that in producing
5 constructs for achieving expression of the genes according to this invention, it is desirable to use a construct and transformation method which enhances expression of the nucleic acid encoding the dominant negative form of the DA polypeptide. Integration of a
10 single copy of the gene into the genome of the plant cell may be beneficial to minimize gene silencing effects. Likewise, control of the complexity of integration may be beneficial in this regard. Of particular interest in this regard is transformation of plant cells utilizing a minimal gene expression construct according to, for example, EP Patent No. EP1407000B1.

15

Techniques well known to those skilled in the art may be used to introduce nucleic acid constructs and vectors into plant cells to produce transgenic plants with the properties described herein.

20

Agrobacterium transformation is one method widely used by those skilled in the art to transform woody plant species, in particular hardwood species such as poplar. Production of stable, fertile transgenic plants is now routine in the art (see for example
25 Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, et al. (1988) *Plant Cell Rep.* 7, 379-384; Zhang, et al. (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, et al. (1989) *Nature* 338, 274-276; Datta, et al. (1990) *Bio/Technology* 8, 736-740; Christou, et al. (1991) *Bio/Technology* 9, 957-962; Peng, et al. (1991) *International*
30 *Rice Research Institute, Manila, Philippines* 563-574; Cao, et al. (1992) *Plant Cell Rep.* 11, 585-591; Li, et al. (1993) *Plant Cell Rep.* 12, 250-255; Rathore, et al. (1993) *Plant Molecular Biology* 21, 871-884; Fromm, et al. (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, et al. (1990) *Plant Cell* 2, 603-618; D'Halluin, et al. (1992)
35 *Plant Cell* 4, 1495-1505; Walters, et al. (1992) *Plant Molecular*

Biology 18, 189-200; Koziel, et al. (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, et al. (1993) *Plant Physiology* 102, 1077-1084; Somers, et al. (1992) *Bio/Technology* 10, 1589-1594; WO92/14828; Nilsson, O. et al (1992) *Transgenic Research* 1, 209-220).

Other methods, such as microprojectile or particle bombardment (US 5100792, EP-A-444882, EP-A-434616), electroporation (EP 290395, WO 8706614), microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant Tissue and Cell Culture*, Academic Press), direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman et al. *Plant Cell Physiol.* 29: 1353 (1984)) or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d)) may be preferred where *Agrobacterium* transformation is inefficient or ineffective, for example in some gymnosperm species.

Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, e.g. bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, II and III, *Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be
5 apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

10 Another aspect of the invention provides a method of altering the phenotype of a plant comprising;

expressing a nucleic acid encoding a dominant-negative DA polypeptide within cells of said plant relative to control plants.

15 Suitable dominant-negative DA polypeptides and methods for expression in plant cells are described above.

A plant with altered phenotype produced as described above may have an extended period of proliferative growth and may display one or
20 more of increased life-span, increased organ size and increased seed size relative to control plants. Preferably, the fertility of plants having the altered phenotype is normal. Methods described herein may be useful, for example, in increasing plant yields, improving grain yield in crop plants, and/or for increasing plant biomass, for
25 example, in the production of biofuels.

The effect of dominant negative alleles of DA proteins is shown herein to be enhanced by reducing or abolishing the expression or function of the *Big Brother* (BB) protein in the plant.

30

Big Brother (BB) is an E3 ubiquitin ligase which is known to repress plant organ growth {Disch, 2006}. A BB protein may comprise the amino acid sequence of SEQ ID NO: 9 (At3g63530 NP_001030922.1 GI: 79316205) or the sequence of a database entry shown in table 9, or
35 may be a fragment or variant of any one of these sequences which

retains BB activity or is capable of interfering with the function of BB.

5 A BB polypeptide which is a variant of a reference BB sequence, for example SEQ ID NO: 9 or the sequence of a database entry shown in Table 9, may comprise an amino acid sequence having at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% sequence identity to the reference sequence. Sequence identity is described
10 in more detail above.

Particular amino acid sequence variants may differ from the BB polypeptide of SEQ ID NO:9 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, or more
15 than 50 amino acids.

In some embodiments, a BB polypeptide may comprise an A at a position corresponding to position 44 of SEQ ID NO: 9.

20 A nucleic acid encoding the BB polypeptide may for example comprise a nucleotide set out in a database entry shown in table 10 or may be a variant or fragment thereof.

In some preferred embodiments, a nucleic acid encoding a BB
25 polypeptide may comprise the nucleotide sequence of SEQ ID NO: 10 (NM_001035845.1 GI: 79316204) or may be a variant or fragment of this sequence which encodes a polypeptide which retains BB activity.

A variant sequence may be a mutant, homologue, or allele of a
30 reference BB sequence, such as SEQ ID NO: 10 or a sequence having a database entry set out in table 10, and may differ from the reference BB sequence by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution
35 of one or more amino acids in the encoded polypeptide. Of course,

changes to the nucleic acid that make no difference to the encoded amino acid sequence are included. A nucleic acid encoding a *BB* polypeptide may comprise a sequence having at least 20% or at least 30% sequence identity with the reference *BB* nucleic acid sequence, preferably at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 80%, at least 90%, at least 95% or at least 98%. Sequence identity is described above.

A fragment or variant may comprise a sequence which encodes a functional *BB* polypeptide i.e. a polypeptide which retains one or more functional characteristics of the polypeptide encoded by the wild-type *BB* gene, for example, E3 ubiquitin ligase activity.

A method of altering a plant phenotype as described herein may further comprise reducing or abolishing the expression or activity of a *BB* polypeptide in said plant.

This may enhance or increase the effect of the expression of a dominant negative *DA* polypeptide on one or more of organ size, seed size or longevity.

Methods for reducing or abolishing the expression or activity of a *BB* polypeptide in said plant are well known in the art and are described in more detail below.

The expression of active protein may be abolished by mutating the nucleic acid sequences in the plant cell which encode the *BB* 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.

For example, an *EOD1* allele of a *BB* polypeptide may be generated by introducing a mutation, such as a deletion, insertion or substitution, at a position corresponding to position 44 of SEQ ID

NO: 9, for example, an A to T substitution. A position in a BB polypeptide sequence which is equivalent to position 44 of SEQ ID NO: 9 may be identified using standard sequence analysis and alignment tools. Others mutations suitable for abolishing expression of an active protein will be readily apparent to the skilled person.

The expression of active protein may be reduced using suppression techniques. The suppression of the expression of target polypeptides in plant cells is well-known in the art. Suitable suppressor nucleic acids may be copies of all or part of the target BB gene inserted in antisense or sense orientation or both relative to the BB gene, to achieve reduction in expression of the BB gene. See, for example, van der Krol et al., (1990) *The Plant Cell* 2, 291-299; Napoli et al., (1990) *The Plant Cell* 2, 279-289; Zhang et al., (1992) *The Plant Cell* 4, 1575-1588, and US-A-5,231,020. Further refinements of this approach may be found in WO95/34668 (Biosource); Angell & Baulcombe (1997) *The EMBO Journal* 16, 12:3675-3684; and Voinnet & Baulcombe (1997) *Nature* 389: pg 553.

In some embodiments, the suppressor nucleic acids may be sense suppressors of expression of the BB polypeptide.

A suitable sense suppressor nucleic acid may be a double stranded RNA (Fire A. et al *Nature*, Vol 391, (1998)). dsRNA mediated silencing is gene specific and is often termed RNA interference (RNAi). RNAi is a two step process. First, dsRNA is cleaved within the cell to yield short interfering RNAs (siRNAs) of about 21-23nt length with 5' terminal phosphate and 3' short overhangs (~2nt). The siRNAs target the corresponding mRNA sequence specifically for destruction (Zamore P.D. *Nature Structural Biology*, 8, 9, 746-750, (2001))

siRNAs (sometimes called microRNAs) down-regulate gene expression by binding to complementary RNAs and either triggering mRNA elimination (RNAi) or arresting mRNA translation into protein. siRNA may be derived by processing of long double stranded RNAs and when found in

nature are typically of exogenous origin. Micro-interfering RNAs (miRNA) are endogenously encoded small non-coding RNAs, derived by processing of short hairpins. Both siRNA and miRNA can inhibit the translation of mRNAs bearing partially complementary target sequences without RNA cleavage and degrade mRNAs bearing fully complementary sequences.

Accordingly, the present invention provides the use of RNAi sequences based on the BB nucleic acid sequence for suppression of the expression of the DA polypeptide. For example, an RNAi sequence may correspond to a fragment of SEQ ID NO: 10 or other BB nucleic acid sequence referred to above, or a variant thereof.

siRNA molecules are typically double stranded and, in order to optimise the effectiveness of RNA mediated down-regulation of the function of a target gene, it is preferred that the length and sequence of the siRNA molecule is chosen to ensure correct recognition of the siRNA by the RISC complex that mediates the recognition by the siRNA of the mRNA target and so that the siRNA is short enough to reduce a host response.

miRNA ligands are typically single stranded and have regions that are partially complementary enabling the ligands to form a hairpin. miRNAs are RNA sequences which are transcribed from DNA, but are not translated into protein. A DNA sequence that codes for a miRNA is longer than the miRNA. This DNA sequence includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a partially double stranded RNA segment. The design of microRNA sequences is discussed on John et al, PLoS Biology, 11(2), 1862-1879, 2004.

Typically, the RNA molecules intended to mimic the effects of siRNA or miRNA have between 10 and 40 ribonucleotides (or synthetic

analogues thereof), more preferably between 17 and 30 ribonucleotides, more preferably between 19 and 25 ribonucleotides and most preferably between 21 and 23 ribonucleotides. In some embodiments of the invention employing double-stranded siRNA, the molecule may have symmetric 3' overhangs, e.g. of one or two (ribo)nucleotides, typically a UU or dTdT 3' overhang. Based on the disclosure provided herein, the skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such as Ambion's siRNA finder, see

http://www.ambion.com/techlib/misc/siRNA_finder.html. siRNA and miRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (e.g. vectors). In a preferred embodiment, the siRNA is synthesized synthetically.

Longer double stranded RNAs may be processed in the cell to produce siRNAs (see for example Myers (2003) *Nature Biotechnology* 21:324-328). The longer dsRNA molecule may have symmetric 3' or 5' overhangs, e.g. of one or two (ribo) nucleotides, or may have blunt ends. The longer dsRNA molecules may be 25 nucleotides or longer. Preferably, the longer dsRNA molecules are between 25 and 30 nucleotides long. More preferably, the longer dsRNA molecules are between 25 and 27 nucleotides long. Most preferably, the longer dsRNA molecules are 27 nucleotides in length. dsRNAs 30 nucleotides or more in length may be expressed using the vector pDECAP (Shinagawa et al., *Genes and Dev.*, 17, 1340-5, 2003).

Another alternative is the expression of a short hairpin RNA molecule (shRNA) in the cell. shRNAs are more stable than synthetic siRNAs. A shRNA consists of short inverted repeats separated by a small loop sequence. One inverted repeat is complementary to the gene target. In the cell the shRNA is processed by DICER into a siRNA which degrades the target gene mRNA and suppresses expression. In a preferred embodiment the shRNA is produced endogenously (within a cell) by transcription from a vector. shRNAs may be produced

within a cell by transfecting the cell with a vector encoding the shRNA sequence under control of a RNA polymerase III promoter such as the human H1 or 7SK promoter or a RNA polymerase II promoter. Alternatively, the shRNA may be synthesised exogenously (in vitro) by transcription from a vector. The shRNA may then be introduced directly into the cell. Preferably, the shRNA molecule comprises a partial sequence of SHR. For example, the shRNA sequence is between 40 and 100 bases in length, more preferably between 40 and 70 bases in length. The stem of the hairpin is preferably between 19 and 30 base pairs in length. The stem may contain G-U pairings to stabilise the hairpin structure.

siRNA molecules, longer dsRNA molecules or miRNA molecules may be made recombinantly by transcription of a nucleic acid sequence, preferably contained within a vector. Preferably, the siRNA molecule, longer dsRNA molecule or miRNA molecule comprises a partial sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 or a variant thereof.

In other embodiments, the suppressor nucleic acids may be anti-sense suppressors of expression of the two or more DA 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. See, for example, Rothstein et al, 1987; Smith et al, (1988) *Nature* 334, 724-726; Zhang et al, (1992) *The Plant Cell* 4, 1575-1588, English et al., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell (1994) *PNAS USA* 91, 3490-3496.

An anti-sense suppressor nucleic acid may comprise an anti-sense sequence of at least 10 nucleotides from a nucleotide sequence is a fragment of SEQ ID NO: 10 or other BB sequence referred to above, or a variant thereof.

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 two or more DA nucleic acids in developing ovules and seeds to increase final seed size.

Nucleic acid which suppresses expression of a *BB* 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 as described above.

The construct or vector may be transformed into plant cells and expressed as described above.

A plant expressing the dominant-negative form of the DA polypeptide and, optionally having reduced or abolished expression of a BB polypeptide, may be sexually or asexually propagated or off-spring or descendants may be grown.

5

Another aspect of the invention provides a method of producing a plant with an altered phenotype comprising:

incorporating a heterologous nucleic acid which encodes a dominant-negative DA polypeptide into a plant cell by means of transformation, and;

10

regenerating the plant from one or more transformed cells.

The altered phenotype of the plant produced by the method is described in more detail above. The method may be useful, for example, in producing plants having increased yields, for example, crop plants having improved grain yield, relative to control plants.

15

In some embodiments, a method may further comprise reducing or abolishing the expression or activity of a BB polypeptide in the plant cell or plant.

20

This may be carried out before, at the same time or after the incorporation of the nucleic acid which encodes the dominant-negative DA polypeptide. For example, in some embodiments, the expression or activity of a BB polypeptide may be abolished or reduced in one or more plant cells which already incorporate the nucleic acid encoding the dominant negative DA polypeptide. In other embodiments, the nucleic acid encoding the dominant negative DA polypeptide may be incorporated into one or more plant cells which have abolished or reduced expression of a BB polypeptide.

25

30

A plant thus produced may comprise a heterologous nucleic acid which encodes a dominant-negative DA polypeptide and may possess abolished or reduced expression or activity of a BB polypeptide in one or more of its plant cells.

35

The expression or activity of a BB polypeptide may be reduced or abolished as described above. For example, a method may comprise incorporating a heterologous nucleic acid into a plant cell by means of transformation, wherein the nucleic acid encodes a suppressor nucleic acid, such as an siRNA or shRNA, which reduces the expression of a BB polypeptide.

The heterologous nucleic acids encoding the dominant negative DA polypeptide and BB suppressor nucleic acid may be on the same or different expression vectors and may be incorporated into the plant cell by conventional techniques.

Dominant-negative DA polypeptides and BB suppressor nucleic acids are described in more detail above.

A plant produced as described above may be sexually or asexually propagated or grown to produce off-spring or descendants. Off-spring or descendants of the plant regenerated from the one or more cells may be sexually or asexually propagated or grown. The plant or its off-spring or descendants may be crossed with other plants or with itself.

A plant suitable for use in the present methods is preferably a higher plant, for example an agricultural plant selected from the group consisting of *Lithospermum erythrorhizon*, *Taxus* spp, tobacco, cucurbits, carrot, vegetable brassica, melons, capsicums, grape vines, lettuce, strawberry, oilseed brassica, sugar beet, wheat, barley, maize, rice, soyabeans, peas, sorghum, sunflower, tomato, potato, pepper, chrysanthemum, carnation, linseed, hemp and rye.

Another aspect of the invention provides a plant which expresses a dominant negative DA polypeptide and optionally has reduced or abolished expression of a BB polypeptide, wherein said plant displays an altered phenotype relative to controls.

The dominant negative DA polypeptide may be heterologous polypeptides.

5 A suitable plant may be produced by a method described herein

As described above, the plant may have one or more of increased life-span, increased organ size, increased duration of proliferative growth and increased seed size relative to control plants. The
10 plant may have normal fertility relative to control plants.

A plant according to the present invention may be one which does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to
15 Plant Breeders Rights.

In addition to a plant expressing a dominant negative DA polypeptide, for example, a plant produced by a method described herein, the invention encompasses any clone of such a plant, seed,
20 selfed or hybrid progeny and descendants, and any part or propagule of any of these, such as cuttings and seed, which may be used in reproduction or propagation, sexual or asexual. Also encompassed by the invention is a plant which is a sexually or asexually propagated off-spring, clone or descendant of such a plant, or any part or
25 propagule of said plant, off-spring, clone or descendant.

The present inventors have shown that reducing or abolishing the expression or activity of two or more DA polypeptides also produces an altered phenotype characterised by normal fertility and one or
30 more of increased life-span, increased organ size, increased duration of proliferative growth and increased seed size.

Another aspect of the invention provides a method of altering the phenotype of a plant comprising;

reducing or abolishing the expression or activity of two or more active DA proteins in one or more cells of the plant.

Another aspect of the invention provides a method of producing a plant with an altered phenotype comprising:

reducing or abolishing the expression or activity of two or more active DA proteins in a plant cell, and;
regenerating the plant from the plant cell.

The phenotype of the plant following reduction or abolition of expression is described in more detail above.

The expression of active protein may be abolished by mutating the nucleic acid sequences in the plant cell which encode the two or more DA proteins 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.

The expression of target polypeptides in plant cells may be reduced by suppression techniques. The use of suppressor nucleic acids to suppress expression of target polypeptides in plant cells is well-known in the art and is described in more detail above.

Suppressor nucleic acids which reduce expression of two or more DA polypeptides may be operably linked to tissue-specific or inducible promoters. For example, integument and seed specific promoters can be used to specifically down-regulate two or more DA nucleic acids in developing ovules and seeds to increase final seed size.

30

Other aspects of the invention relate to the over-expression of DA polypeptides in plant cells. A method of altering the phenotype of a plant may comprise;

expressing a nucleic acid encoding a DA polypeptide within cells of said plant.

The plant may have an altered phenotype characterised by normal fertility and one or more of reduced life-span, reduced organ size, reduced duration of proliferative growth and reduced seed size relative to control plants.

Nucleic acid encoding a DA polypeptide may be expressed in a plant cell as described above *mutatis mutandis* for dominant negative DA polypeptides.

Another aspect of the invention provides a method of identifying a dominant negative DA polypeptide comprising;

providing an isolated nucleic acid encoding a DA polypeptide, incorporating one or more mutations into the nucleic acid, introducing the nucleic acid into a plant cell by means of transformation;

regenerating the plant from one or more transformed cells and, identifying the phenotype of the regenerated plant.

An altered phenotype which includes normal fertility and one or more of increased life-span, increased organ size and increased seed size relative to control plants is indicative that the mutated nucleic acid encodes a dominant negative DA allele.

Another aspect of the invention provides a method of producing a dominant-negative DA polypeptide comprising;

providing a nucleic acid sequence encoding a plant DA polypeptide,

identifying an R residue in the encoded plant DA polypeptide at a position equivalent to position 358 of SEQ ID NO: 1 and mutating the nucleic acid to alter said R residue in the encoded plant DA polypeptide,

the mutant nucleic acid sequence encoding a dominant negative DA polypeptide.

Mutated nucleic acid encoding a dominant negative DA polypeptide which are identified or produced as described above may be used to produce plants having the altered phenotype, as described above.

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

10

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.

15

Having generally described the invention above, certain aspects and embodiments of the invention will now be illustrated by way of example to extend the written description and enablement of the invention, and to ensure adequate disclosure of the best mode of practicing the invention. Those skilled in the art will appreciate, however, that the scope of this invention should not be interpreted as being limited by the specifics of these examples. Rather, variations, extensions, modifications and equivalents of these specifics and generic extensions of these details may be made without departing from the scope of the invention comprehended by this disclosure. Therefore, for an appreciation of the scope of this invention and the exclusive rights claimed herein, reference should be had to the claims appended to this disclosure, including equivalents thereof.

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30

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Examples

5 The data set out below shows that "DA1" is a key regulator in
terminating seed and organ growth, and encodes a novel protein
containing UIM and LIM domains. DA1 is shown to control both seed
and organ size by restricting the duration of proliferative growth.
eod1, an enhancer of dal-1, is allelic to bb, suggesting that the
10 DA1 and the E3 ubiquitin ligase BB, "Big Brother" {Disch, S. Curr
Biol 16, 272-9 (2006); Science 289, 85-8 (2000)) can act in parallel
pathways to control the final size of seeds and plant organs. It is
possible that DA1 and EOD1/BB may share down stream components that
control seed and organ size.

15

Previous study has shown that BB acts a negative regulator of organ
growth, most likely by marking cellular proteins for degradation
(Disch, S. Curr. Biol. 16, 272-279 (2006)). DA1 contains two
predicted UIM motifs, which may have the function of binding
20 ubiquitin and promoting ubiquitination (Hurley, J.H. Biochem. J.
399,361-372 (2006)).

Expression of the DA1 gene is induced by the phytohormone abscisic
acid (ABA), and the dal-1 mutant is insensitive to ABA, providing
25 indication that ABA negatively regulates organ growth through DA1.
The inhibitory effects of ABA on growth have long been recognized as
resulting from an inhibition of cell division (Lui, J.H. Planta 194,
368-373 (1994), consistent with the fact that ABA can induce the
expression of a cyclin-dependent kinase inhibitor (ICK1), an
30 important regulator of cell cycle progression (Wang, H. Cell Biol.
Int. 27, 297-299 (2003)). In seed development, the transition from
developing seeds to mature seeds is also correlated with an increase
in seed ABA content (Finkelstein, R.R. Plant Cell 14 Suppl. S15-45
(2002)), which suggests that ABA may be one of environmental cues
35 sensed by plants to control the final size of seeds and organs, by

inducing negative growth regulators such as DA1. We herein report that one such negative growth regulator is DA1.

By conducting genetic analysis of *abi4-1dal-1* and *abi5-1dal-1* double mutants, we found that the large organ size phenotype of *dal-1* is independent of ABI4 and ABI5 pathways.

We also show herein that suppressors of *dal-1* (*sod1*) are molecules which have a second site mutation in the *dal-1* mutant gene that are predicted to reduce gene function, indicating that the R358K mutation in DA1 is responsible for increased seed size and that the *dal-1* allele interferes with activities of DARs.

We also show herein that the *dal-1* R358K allele also interferes with DA1 functions in a dosage dependent manner, as evidenced by the fact that plants overexpressing *dal-1* allele (35S::DA1R358K) in wild type have large seed and organ size. This result also demonstrates that the *dal-1* mutant gene (DA1R358K) may be used to genetically engineer significant increases in seed weight and biomass.

To date, some mutants (e.g., *ap2* and *arf2*) exhibiting large seeds usually have strong negative effects on their fertility and growth (Schruff, M.C. Development 137, 251-261 (2006); Ohto, M.A. Proc. Natnl Acad. Sci USA 102, 3123-3128 (2005); Jofuku, K.D. Proc. Natnl Acad. Sci. USA 102, 3117-3122 (2005)). However, the experiments set out below show that *dal-1* has increased seed mass, large organ size, but normal fertility, compared with wild type.

Methods

Plant materials

The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild type. All mutants are in the Col-0 background, except for *dal-1Ler* and *bb-1*, which are in Landsberg erecta background. Before analysis, *dal-1* and *dal-1Ler* were backcrossed into Col-0 and Ler six

times, respectively. T-DNA insertion lines were obtained from SIGnAL (Salk Institute) and NASC (Nottingham).

Genetic screen and map-based cloning

5 *dal-1* was identified as a novel seed and organ size mutant from an ethyl-methanesulphonate (EMS)- treated M2 populations of Col-0 accession. *sod1-1*, *sod1-2*, *sod1-3* and *eod1-1* were identified as suppressor and enhancer of *dal-1* from an ethyl-methanesulphonate (EMS)- treated M2 populations of *dal-1*, respectively. F2 mapping
10 populations were generated from a single cross of Ler/*dal-1*, Ler/*sod1-3dal-1*, and *dal-1*Ler/*eod1-1dal-1*. A list of primer sequences is provided in Table 2.

Plasmids and transgenic plants

15 The following constructs were generated: DA1COM, 35S::*DA1*-HA, 35S::*GFP-DA1*, 35S::*DA1*-GFP, 35S::*DA1R358K*, pDA1::*GUS*, and 35S::*EOD1*.

Morphological and cellular analysis

Sample preparation, measurement, microscopy, and histochemical
20 staining for β -glucuronidase activity used standard methods (Jefferson R., EMBO J. Embo J 6, 3901-3907 (1987)).

DA1 limits the size of seeds and organs

To identify repressors of seed and/or organ growth, we screened for
25 *da* mutants (*DA* means 'large' in Chinese) with large seed and/or organ size from an EMS mutagenized population in the Col-0 accession of *Arabidopsis thaliana*. *dal-1* mutant has large seed and organ size, but normal fertility, compared with wild type (Fig.1a-1p), providing indication that seed and organ growth share common regulatory
30 mechanisms. Genetic analysis with reciprocal crosses between *dal-1* and wild type plants revealed that *dal-1* possesses a mutation in a single nuclear locus.

To reveal differences in seed size between wild type and *dal-1*
35 mutant, we examined *dal-1* mutant seed size by fractionating seeds

produced by individual wild type and *dal-1* plants by using a series wire mesh screens. Seeds from wild type were retained only in 180-300µm aperture meshes while the mutant seeds displayed a shift in range to larger exclusion sizes, 180- 355 µm (Fig. 5a and 5b). More than 80% of the wild type seeds were retained in 250µm aperture meshes, whereas about 70% *dal-1* seeds were retained in 300µm aperture meshes. To determine whether the increase in seed size in *dal-1* reflected an alternation in embryo size, we isolated mature embryos from wild type and *dal-1* mutant seeds. *dal-1* mature embryos were significantly fatter and longer than those of wild type (Fig. 1c and 1d). We observed that the seed cavity in *dal-1* seeds is larger throughout development than that in wild type (Fig. 6). In addition, the average seed mass of *dal-1* mutant is increased to 132% of that of wild type (Table 5).

The fertility of *dal-1* plant was found to be normal and the average seed weight per *dal-1* plant is higher than that per wild type plant (Fig. 5). Therefore, we concluded that DA1 contributes to the determination of seed size and seed weight in Arabidopsis. We also identified examples of DA1 and DAR- related genes from crop plants that demonstrate related genes with related functions can be targeted by making the R358K dominant interfering mutation, or reducing expression of selected DA1- and DAR- related proteins using RNA interference methods described above.

We investigated whether DA1 acts maternally or zygotically. As shown in Table 1, the effect of the *dal-1* mutation on seed mass was observed only when maternal plants were homozygous for the mutation. Seeds produced by a *dal-1* mother, regardless of the genotype of the pollen donor, were consistently heavier than those produced by maternal wild type plants. In contrast, *dal-1* mutant and wild type pollen produced seeds whose weight was comparable to that of wild type maternal plants. These results show that *dal-1* is a maternal effect mutation that affects seed mass.

In addition to the increased seed mass, the *dal-1* mutant exhibited larger organ size than wild type (Fig. 1e-m and o). Compared with wild type, *dal-1* plants have large flowers (Fig. 1h and i) that frequently have extra petals and carpels (Fig. 7). The average size of *dal-1* petals was about 1.6-fold that of comparable wild-type petals (Fig. 1o). Siliques of the *dal-1* mutant were wide, deformed and flattened, compared with the narrow, smooth, cylindrical shape of wild type siliques (Fig. 1j and 1k). *dal-1* mutant also forms larger cotyledons and leaves, as well as thicker stems than wild type (Fig. 1e,-g , i, and m). Consistent with this, *dal-1* mutant accumulates more biomass in the form of flowers and leaves than wild type plants (Fig. 1p and q). Taken together, these results indicated that *DA1* limits the size of seeds and organs in *Arabidopsis*.

DA1 restricts the duration of proliferative growth

Seed and organ size is determined by both cell number and/or size. To understand which parameter is responsible for large seed and organ size in *dal-1* mutant, we analyzed cell size of embryos, petals and leaves. As shown in Fig.1r, the size of cells from *dal-1* embryos, petals and leaves, were comparable with that of corresponding wild type cells. The epidermal cell number of stem in *dal-1* mutant is increased to 180% that of wild type stems (Fig. 1n). These results indicate that *dal-1* induced effects on seed mass and organ size are due to the increased cell number.

To determine how *DA1* limits seed and organ size, we performed a kinematic analysis of embryos, petals, and leaves in wild type and the *dal-1* mutant. We manually pollinated *dal-1* mutant and wild type plants with their own pollen grains and examined the duration of seed development. Most of the wild-type seeds developed into desiccation stage in 8 days after fertilization, whereas most of the *dal-1* seeds developed into mature stage in 10 days after fertilization in our growth conditions, suggesting that the period of seed development of *dal-1* mutant was prolonged. Plotting the size of petal primordia and leaves over time revealed that the organ

enlargement in *dal-1* mutant is mainly due to a longer growing period of time (Fig. 2a, c). Consistent with this, *dal-1* plants have younger and fresher organs in early developmental stages (Fig. 1g) and longer lifespan than wild type (Fig. 12 e, f).

5

To determine how cell division contributes to the observed growth dynamic, we measured the mitotic index of petals and leaves in wild type and mutant. A transgene marker of cell- cycle progression, a *pCYCB1:1::GUS* fusion, was used to compare the extent of cell proliferation in developing petals of wild type and *dal-1* plants. The cells in *dal-1* petals continue to proliferate for a longer time than those in wild- type petals (Fig. 2b). Similarly, the arrest of cell cycling in the cells of leaves was also delayed (Fig. 8). The analysis of ploidy level also indicated that *dal-1* mutant exits cell cycle later than wild type. This result provided indication that *dal-1* exhibits prolonged cell proliferation.

15

DA1 encodes a novel protein containing UIM and LIM domains

The *DA1* locus was fine-mapped to an about 30-kilobase (kb) region using polymerase chain reaction-based markers (Fig. 9). DNA sequencing revealed that the *dal-1* allele carries a single nucleotide mutation from G to A in the *At1g19270* gene which cosegregated with the *dal-1* phenotype and results in a change of an arginine (R) to a lysine (K) at amino acid 358 of the predicted protein (Fig.3a and Fig. 9a,b). A binary plasmid (*DA1COM*) carrying a 6.4-kb wild- type genomic fragment containing the entire ORF and a plasmid (*35S::DA1*) carrying 35S promoter plus *At1g19270* cDNA were able to rescue the phenotypes of the *dal-1* mutant (Fig.1i-q and Fig.2a), confirming that that *At1g19270* is indeed the *DA1* gene.

25

DA1 is predicted to encode a 532-amino acid protein containing two ubiquitin interaction motifs (UIM) (Hiyama, H. J. Bio. Chem. 274, 28019-28025 (1999)) and one zinc-binding domain (LIM) present in Lin-11, Isl-1, Mec-3 (Freyd, G. Nature 344, 876-879 (1990) at the N terminus (see Sequences). The UIM is a short peptide motif with the

30

dual function of binding ubiquitin and promoting ubiquitination. This motif is conserved throughout eukaryotes and is present in numerous proteins involved in a wide variety of cellular processes including endocytosis, protein trafficking, and signal transduction (Hurley J.H. Biochem. J. 399, 361-372 (2006)). The LIM domain is a protein-protein interaction motif critically involved in a variety of fundamental biological process, including cytoskeleton organization, organ development and signal transduction (Dawid, I.B. Trends Genet. 14, 156-162 (1998); Dawid, I.B. CR Acad. Sci. III 318, 295-306 (1995); Kadrmas, J.L. Nat. Rev. Mol. Cell Biol. 5, 920-931 (2004)) Seven other predicted proteins in *Arabidopsis* share extensive amino acid similarity (>30% identity) with DA1 and have been named DA1-related proteins (DARs) (see sequence alignment D). The conserved regions among DA1 and DARs lie in the C terminal portion of the molecule, indicating that these conserved regions may be crucial for their function. Proteins that share significant homology with DA1 outside the UIM and LIM are also found in plants and green algae, but not animals.

The spatial expression patterns of DA1 were revealed by histochemical assays of β -glucuronidase (GUS) activity of transgenic plants containing DA1 promoter::GUS fusions (pDA1::GUS). Histochemical staining shows DA1 gene expression throughout the plant, including cotyledons, true leaves, flowers, and embryos (Fig. 3b-h), consistent with the large size phenotypes of *dal-1* mutant plants. Relatively high levels of GUS activity were detected in proliferating tissues (Fig. 3c-f). In addition, the DA1 promoter is also active in roots (Fig. 3b, c). Given the effects of hormones on organ growth, we tested whether any major classes of phytohormones (abscisic acid, jasmonic acid, ethylene, auxin, cytokinin, gibberellin, brassinosteroids and glucose) could influence transcription of DA1 gene. The expression level of the DA1 gene was induced slowly by ABA (Fig. 3m), but not by other hormones, suggesting that the ABA signal may participate in regulation of DA1. Consistent with this, *dal-1* mutant is insensitive to ABA (Fig. 3n),

providing indication that ABA may be one of the environmental cues that regulate DA1 gene to limit seed and organ growth.

5 A green fluorescent protein (GFP)-DA1 translational fusion under the control of 35S promoter rescued the *dal-1* phenotype. However, we could not detect GFP signal. Consistent with this, we also could not detect DA1 proteins of transgenic plants overexpressing DA1 with HA (35S::DA1-HA) and GFP (35S::DA1-GFP) tags using western blot providing indication that the DA1 protein is readily degraded or
10 cleaved in plants.

dal-1 acts as a type of dominant negative mutation for DA1-related proteins

To identify the novel components of the DA1 pathway that determines
15 the final size of seed and organ size, we screened for suppressors of the large seed and organ size phenotypes of *dal-1* (*sod*) and found three *sod1* alleles that were mapped to the original DA1 locus. We sequenced the DA1 gene from these lines and found that each harboured a second site mutation that is predicted to reduce or
20 abolish gene function (Fig. 3A). That second site mutations in the *dal-1* mutant gene suppress the *dal-1* phenotype indicates that the (R358K) mutation within the DA1 coding sequence produces the large seed and organ size. Consistent with this, disruptions of the DA1 gene via T-DNA insertions (*dal-ko1*, *dal-ko2* and *dal-ko3*) display no
25 obvious phenotype (Fig. 10). To determine if one amino acid change found in the original *dal-1* allele was necessary for the *dal-1* phenotype, we crossed *dal-1* with wild type or *dal-ko* lines. All heterozygotic lines (F1) from crosses between Col-0 and *dal-1* exhibited the wild type phenotype, whereas all the F1 plants from
30 crosses between *dal-1* and T-DNA lines (*dal-ko1*, *dal-ko2* and *dal-ko3*) exhibited similar phenotypes to *dal-1* (Fig. S9). In addition, the *dal-1* phenotype was also observed in wild type plants carrying the 35S::DA1R358K transgene (Fig. 1i-r). Therefore, the R358K mutation in DA1 is necessary and sufficient to cause the *dal-1* phenotype.

The loss-of-function alleles display no obvious phenotype. We therefore postulated that DA1 may act redundantly with DARs and expression of *dal-1* allele interferes with the ability of DARs to replace DA1. To test this hypothesis, we generated *dal-koldar1-1* double mutants. The *dal-koldar1-1* double mutant displayed the original *dal-1* phenotype (Fig.3i-l, Table 1 and Fig. 4e), indicating that *dal-1* acts as a type of recessive interfering allele for DARs. Large seed and organ size phenotypes of plants overexpressing the *dal-1* allele in Col-0 suggested that the *dal-1* allele also interferes with the activity of DA1 in dosage-dependent manner (Fig. 1i-q).

DA1 acts in parallel with EOD1/BB, independent of ANT, AXR1, ARF2 and AP2

In enhancer screens, we isolated one allele of a recessive enhancer of *dal-1* (*eod1-1*). *eod1-1dal-1* plants exhibits larger seed and organ size, more extra petals and longer lifespan than *dal-1* (Fig. 12a,b). We mapped the *eod1-1* mutation and found that it was linked to Big Brother (BB) gene (At3g63530) that encodes an E3 ubiquitin ligase and represses organ growth in Arabidopsis {Disch, 2006}. Sequencing revealed that the *eod1-1* allele carries a single nucleotide mutation from G to A in the At3g63530 and resulted in a change of an Alanine (A) to a Threonine (T) at amino acid 44 of the predicted BB protein (Fig. 12c). Both T-DNA insertion in the intron (*eod1-2*) and *bb-1* also enhance *dal-1* phenotypes (Fig.4 and Fig. 12d, e). A binary plasmid (35S::EOD1) carrying 35S promoter plus At3g63530 cDNA was able to rescue the phenotype of the *eod1* mutant, indicating that EOD1 is the BB gene. To determine the relationships between EOD1/BB and DA1 in limiting organ size, we analyzed the mRNA expression levels of DA1 in a *bb-1* mutant and of BB in a *dal-1* mutant. Expression of the DA1 gene in a *bb-1* mutant and of the BB gene in a *dal-1* plant is not significantly affected, compared with wild type (Fig. 12a,b).

To understand the genetic relationships between EOD1/BB and DA1, we measured seed and petal size in *eod1-2dal-1* and *bb-1dal-1Ler* double mutants and found that mutations in *EOD1/BB* synergistically enhance the phenotype of *dal-1* (Fig. 4, Fig. 11 d, e and 12a), providing
 5 indication that the two genes act in parallel pathways to limit seed and organ size in plants (Fig. 4e).

aintegumenta (*ant*) alleles exhibit small petals and plants over-expressing ANT exhibit organ enlargement because of a prolonged
 10 period of organ growth {Krizek, B.A. *Dev Genet* 25, 224-36 (1999); Mizukami, Y. *Proc Natl Acad Sci U S A* 97, 942-7 (2000)}, providing indication that DA1 and ANT could function antagonistically in a common pathway. To test this, we analyzed the mRNA expression levels of DA1 in *ant* mutants and of ANT and its downstream target
 15 *CyclinD3;1* in *dal-1* mutant. DA1 mRNA levels do not show robust changes in *ant* mutants (Fig. 12b). Similarly, the levels of both ANT and *Cyclin3;1* mRNA are not significantly affected by the *dal-1* mutation, as is the mRNA level of ARGOS {Hu, Y. *Plant Cell* 15, 1951-61 (2003)} (Fig. 11c, d, e). Genetic analysis also showed that the
 20 petal size phenotype of *ant-5dal-1* mutant was essentially additive, providing indication that DA1 and ANT act in independent pathways. We also generated *axr1-12dal-1*, *arf2-7dal-1* and *ap2-7dal-1* double mutants, since *axr1*, *arf2* and *ap2* mutants have altered organ and/or seed size (Lincoln, C. *Plant Cell* 2, 1071-1080 (1990); Schruoff, M.C.
 25 *Development* 137, 251-261 (2006); Ohto, M.A. *Proc. Natnl Acad. Sci USA* 102, 3123-3128 (2005); Jofuku, K.D. *Proc. Natnl Acad. Sci. USA* 102, 3117-3122 (2005)). Genetic analysis revealed that the petal size phenotype of *axr1-12dal-1* mutant or the seed size phenotype of *arf2-7dal-1* and *ap2-7dal-1* were essentially additive (Fig.
 30 12b,c,d,e), compared with their parental lines. Therefore, we concluded that DA1 appears to act in parallel with EOD1/BB, independent of ANT, ARX1, ARF2 and AP2.

The DA1 protein family in Arabidopsis thaliana

As described above, the *DA1* gene is predicted to encode a 532-amino-acid protein containing two ubiquitin interaction motifs (UIM) typical of ubiquitin receptors and a single zinc-binding LIM domain defined by its conservation with the canonical Lin-11, Isl-1, and
5 Mec-3 domains (Li et al. 2008). In *Arabidopsis*, seven other predicted proteins share extensive C-terminal (outside UIM and LIM domains) amino acid similarity with *DA1* and have been named *DA1*-related (DAR) proteins, of which four (DAR3, DAR5-7) are found in a tandem cluster on chromosome 5. Using SMART (http://smart.embl-heidelberg.de/smart/show_motifs.pl), the different functional domains were
10 characterised (see Table 11).

UIM is the ubiquitin-interacting motif with two conserved serine residues required for binding and forms a short α -helix structure
15 with ubiquitin (Haglund and Dikic 2005). LIM is a cysteine-rich protein interaction motif, has zinc-binding ability (Freyd et al. 1990). NB-ARC (stands for "a nucleotide-binding adaptor shared by APAF-1, certain R gene products and CED-4") links a protein-protein interaction module to an effector domain, it is a novel signalling
20 motif shared by plant resistance gene products and regulators of cell death in animals. LRRs are leucine-rich repeats, they are short sequence motifs and are thought to be involved in protein-protein interactions. RPW8 belongs to a family that consists of several broad-spectrum mildew resistance proteins from *Arabidopsis thaliana*.

25 These diverse protein structures provide indication the family has diverse functions and has functionally diversified recently. Table 11 may be used to guide the formation of double and triple mutants eg *DA1*, *DAR1* are similar and have been shown to function
30 redundantly; it is possible that *DA6* and *DAR7* may also function redundantly with each other and *DA1* and *DAR1* because of their similar structures.

Characterisation of DA1-like (DAL) proteins in Physcomitrella patens, Selaginella moellendorffii, Brassica rapa, Brachypodium distachyon and Oryza sativa

The amino acid sequences of Arabidopsis DA1 and DAR1-7 were used as queries to screen the available *Physcomitrella patens*, *Selaginella moellendorffii*, *Brassica rapa*, *Brachypodium distachyon* and *Oryza sativa* databases. Using different BLAST algorithms, candidate genes were then selected for preliminary phylogenetic analysis.

10 *DA1-like proteins in early land plants, Physcomitrella patens and Selaginella moellendorffii*

The DA1 family orthologs in *P. patens* were searched by using DA1 amino acid sequence as query in NCBI BLAST, and then revised in JGI *P.patens* BLAST(http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Phypa1_1&advanced=1). Eight genes (PpDAL1-8) were selected based on scores, E-values and preliminary phylogenetic analysis. All *P. patens* DA1-like proteins are shorter than DA1 amino acid sequences, due to absence of the two UIM domains at the N-terminal (see Figure 1), according to SMART (Simple Molecular Architecture Research Tool) program (<http://smart.embl-heidelberg.de/>). The *S. moellendorffii* sequencing project provides us an opportunity to investigate DA1 family orthology in first vascular plant. By using JGI *S. moellendorffii* BLAST(<http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Selmol1&advanced=1>) , two orthologs were found,

25 and comparing with *P.patens* DA1-like proteins, they had similar amino acid sequences length with Arabidopsis DA1 family proteins. The regions preceding the LIM domain were predicted to be low-complexity regions by SMART, and no clear UIM protein sequence motifs were found. We can therefore conclude that the characteristic
30 DA1 protein structure is not found in lower plants.

DA1-like proteins in Brassica rapa

Due to the close evolutionary relationships of Arabidopsis and Brassica, nucleotide BLAST methods for identifying Brassica DA1
35 family orthologs were used. In the Brassica database

(<http://www.brassica.bbsrc.ac.uk/>), full length cDNA sequences of Arabidopsis *DA1* and *DAR1-7* were used as queries. Because of a recent entire genome duplication, one Arabidopsis gene probably has 2 or 3 homologous genes in *Brassica rapa*. Two *DA1* orthologs and one *DAR2* orthologs were found (Figure 1). These were called *DAL* (DA- Like) genes. The *DAR3-7* Brassica orthologs were also found in a tandem cluster. The number of Brassica orthologs found is less than predicted probably due to incomplete genome sequencing. The partial sequences of Brassica *DAL* genes were used to design primers for the specific amplification of full-length *DAL* genes from *B. rapa*.

DA1-like proteins in the grasses Brachypodium distachyon and Oryza sativa

In *Brachypodium distachyon*, three major *DA1* family orthologs were identified, and in *Oryza sativa*, four *DA1*-like proteins were found using PROTEIN BLAST at NCBI. Rice *DA1* and *DAR2* orthologs were identified and named *OsDA1* and *OsDAR2*. No rice gene was found to match Arabidopsis *DAR1*.

In the phylogenetic tree of Figure 14 all DA-like proteins from vascular plants form one large clade. In that clade, *S.moellendorffii* DA-like proteins are highly divergent, but it is possible that DA-like proteins might originate from bryophytes, and function as size regulators since the evolution of the first vascular plants (Lycophytes). In the tree, a clade was formed by *AtDAR2*, *BrDAR2*, *BdDAL3* and *OsDAR2*. These protein sequences show greater similarity, suggesting that *DAR2* evolved before monocots originated (see right graph of Figure 14) and is functionally conserve during evolution. Another clade consists of *AtDA1*, *BrDA1a* and *BrDA1b*. The high similarity between them suggests *Brassica rapa* *DA1* proteins might have the same function as *AtDA1*. The clade consisting of *OsDA1*, *BdDAL1* and *BdDAL2* was placed apart from this clade (see Figure 14), indicating that grass *DA1* proteins may be slightly functionally divergent from those in the *Brassicaceae*. All *Brassicaceae* *DAR1* and *DAR3-7* were placed in one clade, indicating these genes probably

arose from *DA1* or *DAR2* in the genome duplication within *Brassicaceae*. This hypothesis has been partially proved by genetic analysis, which demonstrated, in *Arabidopsis*, *DA1* and *DAR1* are functional redundant.

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Functional analysis of DA1-like proteins in Brassica rapa, Oryza sativa

In silico, two *Brassica rapa* *DA1*-like genes (*BrDA1a*, *BrDA1b*) and one rice *DA1*-like gene (*OsDA1*) were identified. Full length cDNAs were synthesised and sequenced using directional TOPO vectors. The predicted biochemical characteristics of *AtDA1*, *BrDA1a*, *BrDA1b* and *OsDA1* are shown in Table 12. The proteins these three genes encode have very similar biochemical characteristics, particularly the two *Brassica* ones. Interestingly, although analysis based on amino acid sequences shown *BrDA1a* is more close to *AtDA1*, *BrDA1b* was predicted to have more similar biochemical features to *AtDA1* (Table 12).

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The phenotypes of dal-1 are rescued by BrDA1a, BrDA1b and OsDA1 genes

Full-length *BrDA1a*, *BrDA1b* and *OsDA1* cDNAs were sub-cloned to TOPO vectors and transferred to pMDC32 binary destination vectors by LR recombination. These vectors express cDNAs from the constitutive 35S promoter. *dal-1* plants were transformed to test whether the wild-type *DAL* genes from *Brassica* and rice could complement the large growth phenotypes of *dal-1* plants. The 35S::*BrDA1a* and 35S::*OsDA1* transgenic plants showed at least partial complementation (see Figure 15A-D). Interestingly, although *BrDA1b* is not the closest homolog to *AtDA1*, the 35S::*BrDA1b* transgenic plants showed full complementation of the *dal-1* phenotype (see Figures 15E-G), consistent with the high biochemical similarity to *AtDA1* (see Table 12). Two rounds of transformants were screened. In the first round, 10 out of 40 35S::*BrDA1a* and 3 out of 11 35S::*OsDA1* T1 plants show the siliques phenotypes in Figure 15A-D. In the second round, 30 out of 150 35S::*BrDA1b* have shown the rosette leaves phenotypes in Figure 15E-G. This is convincing data that *BrDA1b* functions like

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Arabidopsis *DA1*. Consequently we have demonstrated that *DA1* and related genes have similar functions in controlling organ and seed size in Brassica and rice, and probably many other types of plants.

5 *BrDA1a^{R358K} can interfere with AtDA1 function in Col-0 plants*

Site directed mutagenesis was used to generate the equivalent R358K mutation in the *BrDA1a* cDNA in the TOPO vector and then the mutated cDNA was transferred to pMDC32 destination vectors using the gateway system. Typical *dal-1* phenotypes were observed in wildtype Col-0
10 plants expressing 35S:: *BrDA1a^{R358K}* (see Figure 15E,F,H), including large organ phenotypes. In this transformation experiment, 60 T1 transgenic plants were screened and 7 of these were found to have characteristic large organ phenotypes seen in *dal-1* plants.

15 *DA1 protein stability.*

We have observed that transformants expressing fusions of the GFP protein with the C terminus of the full length *DA1* protein complements the *DA1^{R358K}* large organ phenotype, demonstrating that the fusion protein is fully functional. However, we did not detect GFP
20 fluorescence in many transgenic lines, providing indication that *DA1GFP* protein levels are very low. This is supported by the observation that detection of *DA1* protein with a good specific antibody in plant extracts is very difficult. We therefore tested the stability of *DA1* protein in Arabidopsis using *DA1* protein
25 expressed in *E. coli* and cell-free protein extracts from Arabidopsis. Full length *DA1* protein expressed and purified as an N-terminal GST fusion protein, was incubated with Arabidopsis protein extracts and ATP, and subjected to Western analysis using a specific *DA1* antibody. *DA1* protein was found to be rapidly degraded under these
30 conditions. MG132, a specific inhibitor of the proteasome, was found to abolish this degradation. Therefore, *DA1* is rapidly degraded by the proteasome in Arabidopsis. The UIM motifs of *DA1* are predicted, based on knowledge of UIM function in animal cells, to be involved in ubiquitination. It may be that *DA1* is ubiquitinated and targeted
35 for degradation as part of the mechanism of growth control.

Parent		F ₁
Female	male	seed weight
<i>Col-0</i>	<i>Col-0</i>	2.368 ± 0.023
<i>Col-0</i>	<i>da1-1</i>	2.427 ± 0.031
<i>da1-1</i>	<i>Col-0</i>	3.189 ± 0.042
<i>da1-1</i>	<i>da1-1</i>	3.231 ± 0.046

Average seed weight is given in mg per 100 seeds. Standard deviation values was given (n=5). Plants were grown together in the same conditions.

Table 1 *DA1* acts maternally to control seed weight

Marker	Forward Primer	Reverse Primer	Restriction Enzyme
T16N11	TAATTTAATATTTCTTCTTCCCC	TTACTTTGACTCACTTTCACCAC	
F6A14	AATTAGAATAATAATGCAGCGTTG	CGTTTCGGTATCGCTTTGCG	
F14D16-12	TTG GTT TTC GTT GGG TCA AGG	TGTTTCTGCAGAAGCGAGGG	
T29M8-26	AATCACGTGGTGTTCCTTAGCC	ACTCATTTTGGCAGCTTGGTG	
DA1CAPS	GACACCATGCAATGCCAACC	CTTTGAGCCTCATCCACGCA	<i>MnlI</i>
F18O14-78	CTCAGGCTCAGGTAAATGCG	TTCACGTCCGAAACGCATCC	<i>BsaHI</i>
F18O14-52	CTAACACGACCCACATGATGC	CTCGAGTTTCGTGGTTACGG	<i>NspI</i>
T20H2	AGCATCCTCAAGGTATAAGCC	GGTGCTGCATTTCTGTCACC	
CER451450	GGTTGCTCTAAATCACCTAACG	CTCACCAAGAATATGCATATGTG	

Restriction enzymes for CAPS or dCAPS markers are indicated and others are SLP markers

Table 2 List of PCR-based molecular markers.

Gene Name	Forward Primer	Reverse Primer
RT-DA1	ATGGGTTGGTTTAACAAGATCTTT	AACCGGAATCTACCGGTCA
RT-DAR1	ATGGAGTTTCTTCTCTTTCTTGG	TTAAATCCATTTAGGAAATGTACCG
RT-ACTIN	GAGAAGATGACTCAGATC	ATCCTTCCTGATATCGAC
QRT-DA1	GACACCATGCAATGCCAACC	CTTTGAGCCTCATCCACGCA
QRT-TUB6	GGTGAAGGAATGGACGAGAT	GTCTCTGCAGTTGCGTCTT

Table 3 List of Primers for RT-PCR and QRT-PCR

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T-DNA Lines	LP	RP
SALK_126092	AAGCCAGCTAAATATGATTGG	AATCCGTTTGGAACGCTTTG
SALK_110232	GAATTTGGTTCGGTTGGTTTG	TCACATGCCAGAAACAAGAGG
SALK_054295	TCCTCTGGTTGAGAGACAAGC	TCCATTGGGTTCTTAAACCG
SALK_067100	ATTTAGTCGAAGCCATGCATG	TTACAAGGAGCAGCATCATCC
SALK_016122	TGAGGTGGCCTATTTTGATACC	CACAACCTTAGTCACTTCAGAAGG
SALK_045169	GAGCGATGCATCTCTAACCAC	AGTAGGAACAGAAAGCAGGGG
Lba	TGGTTCACGTAGTGGGCCATCG	

Table 4 List of Primers for verifying T-DNA.

Genotype	Average seed weight, mg	% increase over wild type
Col-0	2.206 ± 0.015	
<i>dal-1</i>	2.915 ± 0.039	+32
<i>DA1COM</i> # 2	2.182 ± 0.022	
<i>DA1COM</i> # 3	2.301 ± 0.018	
<i>35S::DA1^{R358K}</i> # 2	2.513 ± 0.026	+14
<i>35S::DA1^{R358K}</i> # 5	2.672 ± 0.019	+21
<i>dal-kol</i>	2.231 ± 0.029	
<i>dar1-1</i>	2.199 ± 0.032	
<i>dal-kol dar1-1</i>	2.727 ± 0.019	+24

Plants were grown under identical conditions.

Average seed weight is given in mg per 100 seeds. Standard deviation values was given (n=5). Percent increases in seed weight were calculated based on comparison with that of wild type seeds produced under similar growth conditions.

Table 5 *DA1* controls seed weight

- 1: CAO61229
unnamed protein product [Vitis vinifera]
gi|157335399|emb|CAO61229.1| [157335399]
- 2: EAZ36049
hypothetical protein OsJ_019532 [Oryza sativa (japonica cultivar-group)]
gi|125596269|gb|EAZ36049.1| [125596269]
- 3: EAY99923
hypothetical protein OsI_021156 [Oryza sativa (indica cultivar-group)]
gi|125554318|gb|EAY99923.1| [125554318]
- 4: NP_001056985
Os06g0182500 [Oryza sativa (japonica cultivar-group)]
gi|115466772|ref|NP_001056985.1| [115466772]
- 5: CAO22922
unnamed protein product [Vitis vinifera]
gi|157348212|emb|CAO22922.1| [157348212]
- 6: EAZ21100
hypothetical protein OsJ_035309 [Oryza sativa (japonica cultivar-group)]
gi|125579954|gb|EAZ21100.1| [125579954]
- 7: NP_001067188
Os12g0596800 [Oryza sativa (japonica cultivar-group)]
gi|115489402|ref|NP_001067188.1| [115489402]
- 8: CAO22921
unnamed protein product [Vitis vinifera]
gi|157348211|emb|CAO22921.1| [157348211]
- 9: AAW34243
putative LIM domain containing protein [Oryza sativa (japonica cultivar-group)]
gi|57164484|gb|AAW34243.1| [57164484]
- 10: AAW34242
putative LIM domain containing protein [Oryza sativa (japonica cultivar-group)]
gi|57164483|gb|AAW34242.1| [57164483]
- 11: NP_001050702

Os03g0626600 [Oryza sativa (japonica cultivar-group)]
gi|115454203|ref|NP_001050702.1| [115454203]

12: EAY83760
hypothetical protein OsI_037719 [Oryza sativa (indica
cultivar-group)]
gi|125537272|gb|EAY83760.1| [125537272]

13: EAZ27845
hypothetical protein OsJ_011328 [Oryza sativa (japonica
cultivar-group)]
gi|125587181|gb|EAZ27845.1| [125587181]

14: NP_001049668
Os03g0267800 [Oryza sativa (japonica cultivar-group)]
gi|115452135|ref|NP_001049668.1| [115452135]

15:
EAY91080
hypothetical protein OsI_012313 [Oryza sativa (indica
cultivar-group)]
gi|125544941|gb|EAY91080.1| [125544941]

16:
AAP06895
hypothetical protein [Oryza sativa (japonica cultivar-group)]
gi|29893641|gb|AAP06895.1| [29893641]

17: EAY89390
hypothetical protein OsI_010623 [Oryza sativa (indica
cultivar-group)]
gi|125543251|gb|EAY89390.1| [125543251]

18:
CAO16347
unnamed protein product [Vitis vinifera]
gi|157346464|emb|CAO16347.1| [157346464]

19: CAN64300
hypothetical protein [Vitis vinifera]
gi|147817187|emb|CAN64300.1| [147817187]

20: CAN69394
hypothetical protein [Vitis vinifera]
gi|147768077|emb|CAN69394.1| [147768077]

Oryza sativa (japonica cultivar-group) Os06g0182500 (Os06g0182500)
mRNA, complete cds

gi|115466771|ref|NM_001063520.1|[115466771]

Oryza sativa (japonica cultivar-group) cDNA clone:001-201-F10, full
insert sequence

gi|32990928|dbj|AK105719.1|[32990928]

Oryza sativa (japonica cultivar-group) cDNA clone:J023004G21, full
insert sequence

gi|32979080|dbj|AK069056.1|[32979080]

Oryza sativa (japonica cultivar-group) Os12g0596800 (Os12g0596800)
mRNA, complete cds

gi|115489401|ref|NM_001073720.1|[115489401]

Oryza sativa (japonica cultivar-group) cDNA clone:J013039D10, full
insert sequence

gi|32975778|dbj|AK065760.1|[32975778]

Oryza sativa (japonica cultivar-group) cDNA clone:J013073O11, full
insert sequence

gi|32976683|dbj|AK066665.1|[32976683]

Oryza sativa (japonica cultivar-group) Os03g0626600 (Os03g0626600)
mRNA, partial cds

gi|115454202|ref|NM_001057237.1|[115454202]

Oryza sativa (japonica cultivar-group) cDNA clone:001-043-H07, full
insert sequence

gi|32972053|dbj|AK062035.1|[32972053]

Oryza sativa (japonica cultivar-group) Os03g0267800 (Os03g0267800)
mRNA, complete cds

gi|115452134|ref|NM_001056203.1|[115452134]

Oryza sativa (japonica cultivar-group) cDNA clone:J023020C05, full
insert sequence

gi|32979610|dbj|AK069586.1|[32979610]

Oryza sativa (japonica cultivar-group) isolate 29050 unknown mRNA

gi|29368349|gb|AY224559.1|[29368349]

Oryza sativa (japonica cultivar-group) isolate 29050 disease
resistance-like protein mRNA, partial cds

gi|29367476|gb|AY224475.1|[29367476]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 6

gi|58531193|dbj|AP008212.1|[58531193]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 6, BAC clone:OSJNBb0036B04

gi|50657316|dbj|AP007226.1|[50657316]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 12

gi|58531199|dbj|AP008218.1|[58531199]

Oryza sativa chromosome 12, . BAC OSJNBa0056D07 of library OSJNBa from chromosome 12 of cultivar Nipponbare of ssp. japonica of Oryza sativa (rice), complete sequence

gi|23897123|emb|AL928754.2|[23897123]

Oryza sativa chromosome 12, . BAC OJ1306_H03 of library Monsanto from chromosome 12 of cultivar Nipponbare of ssp. japonica of Oryza sativa (rice), complete sequence

gi|20513132|emb|AL713904.3|[20513132]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 3

gi|58530789|dbj|AP008209.1|[58530789]

Oryza sativa (japonica cultivar-group) chromosome 3 clone OSJNBa0002I03 map E1419S, complete sequence

gi|57164481|gb|AC091246.8|[57164481]

Oryza sativa (japonica cultivar-group) chromosome 3 clone OJA1364E02, complete sequence

gi|27901829|gb|AC139168.1|[27901829]

Oryza sativa (japonica cultivar-group) chromosome 3 clone OJ1364E02, complete sequence

gi|27901828|gb|AC135208.3|[27901828]

Oryza sativa chromosome 3 BAC OSJNBb0013K08 genomic sequence, complete sequence

gi|16356889|gb|AC092390.3|[16356889]

Oryza sativa (indica cultivar-group) clone OSE-97-192-H5 zn ion binding protein mRNA, partial cds

gi|149390776|gb|EF575818.1|[149390776]

Oryza sativa (indica cultivar-group) cDNA clone:OSIGCRA102J03, full insert sequence

gi|116633496|emb|CT833300.1|[116633496]

Oryza sativa (japonica cultivar-group) Os01g0916000 (Os01g0916000) mRNA, complete cds

gi|115441820|ref|NM_001051725.1|[115441820]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 1

gi|58530787|dbj|AP008207.1|[58530787]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 1, PAC clone:P0413C03

gi|19386744|dbj|AP003451.4|[19386744]

Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 1, PAC clone:P0004D12

gi|20804980|dbj|AP003433.3|[20804980]

Oryza sativa (japonica cultivar-group) cDNA clone:002-101-C04, full insert sequence

gi|32991509|dbj|AK106300.1|[32991509]

Oryza sativa (japonica cultivar-group) cDNA, clone: J100024013, full insert sequence

gi|116012466|dbj|AK243101.1|[116012466]

Zea mays clone EL01N0524A08.d mRNA sequence

gi|54653541|gb|BT018760.1|[54653541]

Zea mays PC0156068 mRNA sequence

gi|21212590|gb|AY109151.1|[21212590]

Zea mays clone EL01N0553E07 mRNA sequence

gi|85540336|gb|BT024085.1|[85540336]

Zea mays clone E04912705F06.c mRNA sequence

gi|54651736|gb|BT016955.1|[54651736]

Zea mays nitrate reductase gene, promoter region

gi|4894987|gb|AF141939.1|AF141939[4894987]

Hordeum vulgare subsp. *vulgare* cDNA clone: FLbaf82h16, mRNA sequence

gi|151419042|dbj|AK250393.1|[151419042]

Vitis vinifera, whole genome shotgun sequence, contig VV78X106678.4, clone ENTAV 115

gi|123680846|emb|AM488121.1|[123680846]

Vitis vinifera contig VV78X222701.5, whole genome shotgun sequence

gi|147817185|emb|AM484789.2|[147817185]

Vitis vinifera, whole genome shotgun sequence, contig VV78X165152.5, clone ENTAV 115

gi|123703056|emb|AM483648.1|[123703056]

Vitis vinifera contig VV78X263569.4, whole genome shotgun sequence

gi|147790377|emb|AM453516.2|[147790377]

Vitis vinifera contig VV78X179395.3, whole genome shotgun sequence
gi|147769864|emb|AM456337.2|[147769864]

Vitis vinifera contig VV78X219892.2, whole genome shotgun sequence
gi|147780236|emb|AM461946.2|[147780236]

Vitis vinifera, whole genome shotgun sequence, contig VV78X014445.8,
clone ENTAV 115
gi|123704690|emb|AM483793.1|[123704690]

Vitis vinifera contig VV78X193742.9, whole genome shotgun sequence
gi|147768076|emb|AM435996.2|[147768076]

Lotus japonicus genomic DNA, chromosome 2, clone:LjB06D23, BM0787,
complete sequence
gi|37591131|dbj|AP006541.1|[37591131]

Agropyron cristatum isolate Bsy1 y-type high-molecular-weight glutenin
subunit pseudogene, complete sequence
gi|71159564|gb|DQ073532.1|[71159564]

Agropyron cristatum isolate Bty1 y-type high-molecular-weight glutenin
subunit pseudogene, complete sequence
gi|71159568|gb|DQ073535.1|[71159568]

Agropyron cristatum isolate Bfy1 y-type high-molecular-weight glutenin
subunit pseudogene, complete sequence
gi|71159563|gb|DQ073531.1|[71159563]

Pinus taeda putative cell wall protein (lp5) gene, complete cds
gi|2317763|gb|AF013805.1|[2317763]

Brassica rapa subsp. pekinensis clone KBrH011G10, complete sequence
gi|110797257|gb|AC189577.1|[110797257]

Brassica rapa subsp. pekinensis clone KBrB032C14, complete sequence
gi|110796986|gb|AC189306.1|[110796986]

Brassica rapa subsp. pekinensis clone KBrB011P07, complete sequence
gi|110744010|gb|AC189225.1|[110744010]

Poplar cDNA sequences
gi|115416791|emb|CT029673.1|[115416791]

Poplar cDNA sequences
gi|115416790|emb|CT029672.1|[115416790]

Coffea arabica microsatellite DNA, clone 26-4CTG

gi|13398992|emb|AJ308799.1|[13398992]

Medicago truncatula clone mth2-34m14, complete sequence

gi|61675739|gb|AC126779.19|[61675739]

Medicago truncatula chromosome 5 clone mth2-5p5, COMPLETE SEQUENCE

gi|119359633|emb|CU302347.1|[119359633]

Medicago truncatula chromosome 8 clone mth2-14m21, complete sequence

gi|50355770|gb|AC148241.21|[50355770]

Solanum lycopersicum cDNA, clone: LEFL1035BC02, HTC in leaf

gi|148538338|dbj|AK247104.1|[148538338]

Mimulus guttatus clone MGBa-44P14, complete sequence

gi|150010729|gb|AC182564.2|[150010729]

Mimulus guttatus clone MGBa-64L10, complete sequence

gi|154350257|gb|AC182570.2|[154350257]

Selaginella moellendorffii clone JGIASXY-5I19, complete sequence

gi|62510100|gb|AC158187.2|[62510100]

M.truncatula DNA sequence from clone MTH2-170H18 on chromosome 3,
complete sequence

gi|115635794|emb|CT967314.8|[115635794]

Vigna unguiculata glutelin 2 mRNA, partial cds

gi|4973069|gb|AF142332.1|AF142332[4973069]

Table 7

Soybean cDNA clones

gb|CX711863.1|CX711863
gb|BM525343.1|BM525343
gb|BG156297.1|BG156297
gb|BM308148.1|BM308148
gb|AI856660.1|AI856660
gb|BF596520.1|BF596520
gb|BI472193.1|BI472193
gb|CO982042.1|CO982042
gb|BM143278.1|BM143278
gb|AW831270.1|AW831270
gb|BE329874.1|BE329874
gb|BG652163.1|BG652163
gb|BI967821.1|BI967821
gb|BI321493.1|BI321493
gb|BU546579.1|BU546579
gb|CO984945.1|CO984945
gb|DW247614.1|DW247614
gb|BG726202.1|BG726202
gb|BI968915.1|BI968915
gb|BG043212.1|BG043212
gb|BG510065.1|BG510065
gb|BG043153.1|BG043153
gb|AW832591.1|AW832591
gb|AI856369.1|AI856369
gb|BI699452.1|BI699452
gb|BG650019.1|BG650019
gb|AW234002.1|AW234002
gb|AW310220.1|AW310220
gb|AW394699.1|AW394699
gb|AW832462.1|AW832462
gb|AW459788.1|AW459788
gb|BM731310.1|BM731310
gb|BI317518.1|BI317518
gb|AI988431.1|AI988431
gb|CA801874.1|CA801874
gb|BE057592.1|BE057592
gb|AW102002.1|AW102002
gb|CA938750.1|CA938750
gb|AW397679.1|AW397679

Table 8

emb|CAO40855.1| unnamed protein product [Vitis vinifera]
 gb|EAY88740.1| hypothetical protein Osl_009973 [Oryza sativa (indica cultivar-group)]
 gb|EAZ25768.1| hypothetical protein OsJ_009251 [Oryza sativa (japonica cultivar-group)]
 ref|NP_001049123.1| Os03g0173900 [Oryza sativa (japonica cultivar-group)]
 emb|CAO21927.1| unnamed protein product [Vitis vinifera]
 emb|CAD41576.3| OSJNBa0088122.8 [Oryza sativa (japonica cultivar-group)]
 gb|EAY95219.1| hypothetical protein Osl_016452 [Oryza sativa (indica cultivar-group)]
 emb|CAH67282.1| OSIGBa0111L12.9 [Oryza sativa (indica cultivar-group)]
 ref|NP_001053604.1| Os04g0571200 [Oryza sativa (japonica cultivar-group)]
 ref|NP_001063719.1| Os09g0525400 [Oryza sativa (japonica cultivar-group)]
 gb|EAZ09811.1| hypothetical protein Osl_031043 [Oryza sativa (indica cultivar-group)]
 gb|EAZ45411.1| hypothetical protein OsJ_028894 [Oryza sativa (japonica cultivar-group)]
 ref|NP_001062434.1| Os08g0548300 [Oryza sativa (japonica cultivar-group)]
 gb|EAZ07897.1| hypothetical protein Osl_029129 [Oryza sativa (indica cultivar-group)]
 ref|NP_001063778.1| Os09g0535100 [Oryza sativa (japonica cultivar-group)]
 emb|CAC10211.1| hypothetical protein [Cicer arietinum]
 emb|CAO44394.1| unnamed protein product [Vitis vinifera]
 gb|ABG73441.1| zinc finger C3HC4 type family protein [Oryza brachyantha]
 ref|NP_001056653.1| Os06g0125800 [Oryza sativa (japonica cultivar-group)]
 gb|EAZ09879.1| hypothetical protein Osl_031111 [Oryza sativa (indica cultivar-group)]
 gb|EAZ45482.1| hypothetical protein OsJ_028965 [Oryza sativa (japonica cultivar-group)]
 dbj|BAD82497.1| RING-H2 finger protein RHG1a-like [Oryza sativa (japonica cultivar-group)]
 emb|CAN71989.1| hypothetical protein [Vitis vinifera]
 dbj|BAD05399.1| DNA binding zinc finger protein-like [Oryza sativa (japonica cultivar-group)]
 emb|CAH65886.1| OSIGBa0148J22.5 [Oryza sativa (indica cultivar-group)]
 emb|CAE02518.2| OSJNBb0003A12.5 [Oryza sativa (japonica cultivar-group)]
 ref|NP_001052192.1| Os04g0185500 [Oryza sativa (japonica cultivar-group)]
 emb|CAO71872.1| unnamed protein product [Vitis vinifera]
 emb|CAO39354.1| unnamed protein product [Vitis vinifera]
 emb|CAE01827.2| OSJNBa0041A02.20 [Oryza sativa (japonica cultivar-group)]
 emb|CAO71869.1| unnamed protein product [Vitis vinifera]
 emb|CAA85320.1| C-terminal zinc-finger [Glycine max]
 gb|EAZ08608.1| hypothetical protein Osl_029840 [Oryza sativa (indica cultivar-group)]
 gb|EAY75305.1| hypothetical protein Osl_003152 [Oryza sativa (indica cultivar-group)]
 ref|NP_001043810.1| Os01g0667700 [Oryza sativa (japonica cultivar-group)]
 dbj|BAD73651.1| RING-finger protein-like [Oryza sativa (japonica cultivar-group)]
 emb|CAO71875.1| unnamed protein product [Vitis vinifera]
 ref|NP_001062870.1| Os09g0323100 [Oryza sativa (japonica cultivar-group)]
 gb|EAZ35180.1| hypothetical protein OsJ_018663 [Oryza sativa (japonica cultivar-group)]
 dbj|BAA74802.1| DNA binding zinc finger protein (Pspzf) [Pisum sativum]
 ref|NP_001056239.1| Os05g0550000 [Oryza sativa (japonica cultivar-group)]
 gb|EAY98923.1| hypothetical protein Osl_020156 [Oryza sativa (indica cultivar-group)]
 emb|CAN83345.1| hypothetical protein [Vitis vinifera]
 emb|CAO43928.1| unnamed protein product [Vitis vinifera]
 emb|CAN79375.1| hypothetical protein [Vitis vinifera]

Table 9 BB polypeptides identified by Blastp

ref|NM_001055658.1| *Oryza sativa* (japonica cultivar-group) Os03g0173900 (Os03g0173900) mRNA, complete cds
 dbj|AK063978.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:001-124-C08, full insert sequence
 dbj|AP006425.1| *Lotus japonicus* genomic DNA, chromosome 1, clone:LjT39B10, TM0315, complete sequence
 gb|AY110224.1| *Zea mays* CL5837_1 mRNA sequence
 ref|NM_001060139.1| *Oryza sativa* (japonica cultivar-group) Os04g0571200 (Os04g0571200) mRNA, partial cds
 dbj|AK071401.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J023097G23, full insert sequence
 ref|NM_001068969.1| *Oryza sativa* (japonica cultivar-group) Os08g0548300 (Os08g0548300) mRNA, complete cds
 dbj|AK073266.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J033029A20, full insert sequence
 emb|CT832808.1| *Oryza sativa* (indica cultivar-group) cDNA clone:OSIGCSN035L02, full insert sequence
 ref|NM_001070254.1| *Oryza sativa* (japonica cultivar-group) Os09g0525400 (Os09g0525400) mRNA, complete cds
 dbj|AK104112.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:006-202-G09, full insert sequence
 dbj|AK066238.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J013059J01, full insert sequence
 emb|CT832015.1| *Oryza sativa* (indica cultivar-group) cDNA clone:OSIGCRA126H24, full insert sequence
 dbj|AK250973.1| *Hordeum vulgare* subsp. *vulgare* cDNA clone: FLbaf101a03, mRNA sequence
 dbj|AK249803.1| *Hordeum vulgare* subsp. *vulgare* cDNA clone: FLbaf58c16, mRNA sequence
 emb|CT832014.1| *Oryza sativa* (indica cultivar-group) cDNA clone:OSIGCRA115D08, full insert sequence
 gb|BT016451.1| *Zea mays* clone Contig284 mRNA sequence
 emb|AJ299062.1| CAR299062 *Cicer arietinum* partial mRNA for hypothetical protein (ORF1), clone Can183
 gb|AY109631.1| *Zea mays* CL5026_1 mRNA sequence
 gb|AY108288.1| *Zea mays* PCO148716 mRNA sequence
 ref|NM_001070313.1| *Oryza sativa* (japonica cultivar-group) Os09g0535100 (Os09g0535100) mRNA, complete cds
 dbj|AK069888.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J023039O04, full insert sequence
 gb|AY103990.1| *Zea mays* PCO093361 mRNA sequence
 emb|AM485242.1| *Vitis vinifera*, whole genome shotgun sequence, contig VV78X218805.2, clone ENTAV 115
 gb|BT018037.1| *Zea mays* clone EL01N0530G02.c mRNA sequence
 emb|CT829435.1| *Oryza sativa* (indica cultivar-group) cDNA clone:OSIGCRA107A15, full insert sequence
 ref|NM_001063188.1| *Oryza sativa* (japonica cultivar-group) Os06g0125800 (Os06g0125800) mRNA, complete cds
 gb|AY225189.1| *Oryza sativa* (indica cultivar-group) zinc finger protein mRNA, complete cds
 gb|AY207044.1| *Oryza sativa* (indica cultivar-group) zinc-finger protein mRNA, complete cds
 dbj|AK104425.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:006-205-F10, full insert sequence
 dbj|AK068302.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J013144A04, full insert sequence
 gb|AY112568.1| *Zea mays* CL32837_1 mRNA sequence
 emb|AM453896.2| *Vitis vinifera* contig VV78X100953.6, whole genome shotgun sequence
 gb|AC157490.18| *Medicago truncatula* clone mth2-123f23, complete sequence
 gb|AC151824.13| *Medicago truncatula* clone mth2-45n18, complete sequence
 ref|NM_001058727.1| *Oryza sativa* (japonica cultivar-group) Os04g0185500 (Os04g0185500) mRNA, complete cds
 gb|BT019187.1| *Zea mays* clone Contig858.F mRNA sequence
 dbj|AK064939.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J013000P06,
 gb|AY110468.1| *Zea mays* CL16240_2 mRNA sequence
 gb|AY110685.1| *Zea mays* CL9024_1 mRNA sequence
 dbj|AK246964.1| *Solanum lycopersicum* cDNA, clone: LEFL1004CA06, HTC in leaf
 dbj|AP008214.1| *Oryza sativa* (japonica cultivar-group) genomic DNA, chromosome
 ref|NM_001050479.1| *Oryza sativa* (japonica cultivar-group) Os01g0692700 (Os01g0692700) mRNA, partial cds
 dbj|AK065626.1| *Oryza sativa* (japonica cultivar-group) cDNA clone:J013028F14,
 dbj|AP004704.3| *Oryza sativa* (japonica cultivar-group) genomic DNA, chromosome 8, PAC clone:P0544G09
 dbj|AP006265.2| *Oryza sativa* (japonica cultivar-group) genomic DNA, chromosome 8, BAC clone:OJ1112_E06

Table 10 nucleic acid encoding BB polypeptides identified by tBlastn

65

	Length	Function Domains
DA1	532aa	Two UIM, One LIM
DAR1	548aa	three UIM, one LIM
DAR2	529aa	One LIM
DAR3	451aa	None
DAR4	1614aa	one NB-ARC, one LRR3, three LRR1, one LIM
DAR5	703aa	One RPW8, one LIM
DAR6	645aa	Seven UIM, one LIM
DAR7	561aa	Three UIM, one LIM

Table 11

5

Analysis	AtDA1	BrDA1a	BrDA1b	OsDA1
Length	533 aa	533 aa	515 aa	487 aa
Molecular Weight	60470.46	60185.30	59041.38	55268.94
Isoelectric Point	5.98	5.89	5.96	6.08
Charge at pH 7	-12.15	-13.24	-13.04	-8.57

Table 12

Sequences

MGWFNKIFKGSNQRLRVGNKHNHNVYYDNYPTASHDDEPSAADTDADNDEPHHTQEPST
 SEDNTSNDQENEDIDRAIALSLLEENQEQTSSISGKYSMPVDEDEQLARALQESMVVGNPS
 5 RHKSGSTYDNGNAYGAGDLYGNHMYGGGNYVANGDIYYPRPITFQMDFRICAGCNMEIG
 HGRFLNCLNSLWHPECFRYGCSPISEYEFSTSGNYPFHKACYRERYHPKCDVCSHFIP
 TNHAGLIEYRAHPFWQKYCPSHEHDATPRCCSCERMEPRNTRYVELNDGRKLCLECLDS
 AVMDTMQCQPLYLQIQNFYEGLNMKVEQEVPLLLVERQALNEAREGEKNGHYHMPETRGL
 CLSEEQTVSTVRKRSKHGTGKWAGNITEPYKLTRQCEVTAILILFGLPRLLTGSILAHM
 10 MHAWMLKGFRTLSQDVEEGICQVMAHKWLD AELAAGSTNSNAASSSSSSQGLKKGPRSQ
 YERKLGEFFKHQIESDASPVYGDGFRAGRLAVHKYGLRKTLEHIQMTGRFPV

SEQ ID NO: 1

15 atggggttggtttaacaagatcttttaaaggctctaaccaaaggctccgggttggaataat
 aagcacaatcacaatgtttattacgataattatccgactgcttcacatgatgatgagcct
 agtgcggcggatacagatgctgataatgatgaacctcatcatactcaggaacctctaca
 tctgaggataatacatcgaatgaccaggaataatgaagacatagaccgtgcaattgcattg
 20 tcgcttttagaagagaatcaagaacagacaagtataagcgggaaatactcgatgccggtg
 gatgaagatgagcaacttgctagagccctacaagaaagtatggtagttgggaattcaccc
 cgtcacaaaagtgggaagtacatatgataatgggaatgcataatggagctggagatttatat
 ggggaatggacatatgtatggaggaggaaatgtatatgcaaatggagatatattattatcca
 agacctattacttttcaaattggatttcaggatttgctgctggctgtaatatggagattggc
 25 catggaagatttctgaattgccttaattcactatggcatccagaatgttttcgatgttat
 ggctgcagtcagccgatttctgagtacgagttttcaacatcaggggaactacccttttcac
 aaggcttggttacaggagagatatcatcctaaatgtgatgtctgcagccactttatacca
 acaaatcatgctggtcttattgaatatagggcacatcctttttgggttcagaagtattgt
 ccttctcacgaacacgatgctaccccgagatggtgcagttgtgaaagaatggagccacgg
 30 aatacagatatgttgaacttaacgatggacggaaactttgccttgagtgtttggactcg
 gcggtcatggacaccatgcaatgccaacctctgtacttgcaaatacaaaaatttctatgaa
 ggactcaacatgaaggtagagcaggaagttccactcctcttggttgagagacaagcactt
 aacgaagccagagaaggtgaaaagaatggtcactatcacatgccagaaacaagaggactc
 tgcctttcagaagaacaaactgttagtactgtaagaaagcgatcaaagcatggcacagga
 35 aaatgggcccgggaatattacagaaccttacaagttaacacggcaatgtgaagttaccgcc
 attctcatcttatttcgggctccctaggttacttactgggttcgattctagctcatgagatg
 atgcatgcgtggatgaggctcaaaggattccgaacactgagccaagatgttgaagaaggt
 atatgtcaagtgatggctcataaatgggttagatgctgagttagctgctggttcaacaaat
 agcaatgctgcatcatcatcctcctcttctcaaggactgaaaaagggaccgagatctcag
 40 tacgagagaaagcttggtgagtttttcaagcaccaaatcgagctgatgcttctccggtt
 tatggagacgggttcagagctgggaggttagctgttcacaagtacgggttgcgaaaaaca
 cttgagcatatacagatgaccggtagattcccgggtttaa

SEQ ID NO: 2

p---pLpbA1 pb.Sbp-.pp p

SEQ ID NO: 3

p---pLpbA1 pb.Sbp-spp p

SEQ ID NO: 4

55 pCs.CscsIh s.....bhlp tb.sp.aH.. .pCFpCs..p CppsLss... .p.ab.pcsp
 baCpps...

Wherein;

c is a charged amino acid residue, for example, D, E, H, K, R;

p is a polar amino acid residue, for example, C, D, E, H, K, N, Q,

5 R, S or T;

h is a hydrophobic amino acid residue, for example, A, C, F, G, H, I, L, M, T, V, W and Y;

t is a tiny amino acid residue, for example, A, G or S

a is an aromatic amino acid residue, for example, F, H, W or Y;

10 b is a big amino acid residue, for example, E, F, H, I, K, L, M, Q, R, W or Y;

s is a small amino acid residue, for example, A, C, D, G, N, P, S, T or V;

l is an aliphatic amino acid residue, for example, I, L or V;

15 . is absent or is any amino acid; and

- is any amino acid.

SEQ ID NO: 5

QENEDIDRAIALSLLEENQE

20 SEQ ID NO: 6

DEDEQIARALQESMVVGNSP

SEQ ID NO: 7

25 ICAGCNMEIGHGRFLNCLNSLWHPECFRCYGCSQPISEYEFSTSGNYPFHKAC

SEQ ID NO: 8

30 1 mngdnrpved ahytetgfpv aatgsymdfy ggaaqgplny dhaatmhpqd nlywtmntna
61 ykfgfsgsdn asfygsydmn dhlsrmsigr tnwdyhpvmv vaddpentva rsvqigdtde
121 hseaeecian ehdpdspqvs wqddidpdtm tyeelevelge avgtesrgls qelietlptk
181 kykfgsifsr kragercvic qlkykigerq mnlpc khvyh seciskwlsi nkvcpcvnse
241 vfgepsih

35 SEQ ID NO: 9

40 1 acactctttc ctctctcttt cttctctctt tctttctctt ctctctcttc tgctctctcg
61 tctctcgtct acagtgcctt ccgcatcacc tttttccttg tcctatgaat ttggtcgaaa
121 tgcccttctc ctctctctcc ttccactaat ctcaaaagat atatccttcg agactctccc
181 ttgccgtctc caattgccac tcaccgctcc aactctcttc gaattagctg aatgaatgg

68

241 agataataga ccagtggaa atgctcatta cacggagaca ggtttccctt atgctgctac
 301 tggaggttac atggactttt atggtggtgc ggctcagggg cctcttaact acgatcatgc
 361 cgcaactatg catcctcagg acaatctgta ctggaccatg aataccaatg catacaagtt
 421 tgggttttca ggatcagata atgcttcttt ctatggttca tatgacatga acgatcattt
 5 481 atcgaggatg tccatagggg gaacaaattg ggactatcat cccatgggtg acgttgctga
 541 tgatcctgaa aacacagttg cacgttccgt ccaaactcga gacacagatg agcactctga
 601 agctgaagaa tgcattgcaa atgagcatga tcccgcagct cctcaggtat cctggcaaga
 661 tgacattgat cctgatacaa tgacctatga ggaattagta gagctggggg aagcagtagg
 721 aacagaaagc aggggggttg ctcaggaact catagaaacg cttcccacta aaaagtataa
 10 781 gtttgggagc atcttctcca ggaaaagagc tggggagagg tgtgtgatat gccagctcaa
 841 gtacaagata ggggagaggc aaatgaatct gccgtgcaag catgtgtatc attctgaatg
 901 catttccaaa tggctaagca tcaacaaggt ttgcccgggtg tgaacacgcg aggtcttttg
 961 ggagcccagc attcattgat cggcacaagg ggctcctcct cttcttttct ttttggcttt
 1021 ttatctcgag gctcatcaag taattgtttt agtgtagtga aaaccccaaa aaatagtcta
 15 1081 aaagatgtcc acactatact ctctcatggt cagtccttct ctgtacatgt aatttttctt
 1141 ctagtcccat tttcgcttgt gtgtgcttta agtttaacag tcactcgtat tgtatactaa
 1201 atgctaagtc aaaaacgctg aatccatat

SEQ ID NO: 10

20 1 atggcctact cctcacggctc ttgtgatcag tgcagtcacg agaggagatc cggcttcatg
 61 aagtggctct gcgctttcct gaaggggagc aaggacggcg aggccaaacc acggcgccct
 121 cgggtgacgg caggagaaga gaccacgctc tgggaagaac cagttagacc aaagaaggaa
 181 gaaccaccta gacataacaa tgaagaaatg gaccatgcac ttgcccttgc tcttgacagc
 25 241 gatgccaaaa atacaaaaga gagaaacatg gacaaggagc aaaacgatga agaactcgct
 301 agagcaatac aggacagtct gaacatgaat ccttaccagc cttacaatcc ttgtgcaccc
 361 tctcagaccc aggccaggctc gagaggatac agggctctgtg ggggttgcaa gcatgagata
 421 gggcatggcc attacttgag ctgcttggga atgtactggc accctcagtg cttccgctgt
 481 tcttctgtgc gccaccctat ccgtgagatg gagttcacct tgctaggtac agatccatac
 30 541 cacaagctgt gctacaagga gcttcacac ccaaagtgtg acgtctgcct tcaatttatc
 601 ccaacgaaca ggactggttt gatagagtac agagcccatc cattctgggg acagaagtat
 661 tgtcctttgc atgagcatga tagaacacct cgttgctgta gctgtgagaa aatggagcca
 721 aggaacacaa agtatatgtc attaggggat ggacgcagct tgtgcatgga atgcctggat
 781 tctgcaatca tggacaccgg tgaatgtcaa ccgctatacc attccatcag agactactac
 35 841 gaagggatga acatgaaact agaccagcag ataccatgc tcttggttga acgtcaagcc
 901 cttaatgaag ctatggaagg agaaagcaaa ggaccgcatc atatgcctga aacacaggcc
 961 ctttgtctgt cagaggagca gactgtgacc agtatactta ggaggcccag aattggtgca
 1021 aatcggttac tagatatgaa aacccaaccg caaaagctaa ctaggagatg cgaagtcact
 1081 gcaattcttg tattgtttgg cctcccagc ctgctaaccg gctccattct tgcccatgaa
 40 1141 ttgatgcatg ggtggttgcg cctcaaaggc taccggaacc taaaggcgga gattgaggaa
 1201 ggtatatgcc aggtcatgtc ttacctgtgg ctggagtcag agatccttcc atccacttca
 1261 agatatggac aggcttcaac atcttacgct tcattctcgt cgtcctcctg tcgaccacca
 1321 ccgtccaaga aggggtgggat ctctcacacc gagaagaagc ttggagaatt cttcctgcat
 1381 cagatcgcca atgacacatc atcagcatac ggcgatgggt tcagagctgc ctatgcagct
 45 1441 gtgaacaagt atggccttcg ccaatcactg aaccatatac ggctaaccgg aggctttcct
 1501 gtgtaa

SEQ ID NO: 11 (OsDAR2)

50 1 atggagtttc ttcttctctt gtttggatc attaagaatg tgtttctctt tgcaggtaag
 61 aggttggttg tgatgccaat ggggtggcct actaagatcc ttaaagggtc tagtcataag
 121 tattcagatg gtcaagctaa cagaagatac aatagagagg atagaagcct ggacactcct
 181 cgttattccg cggaaggatc tgattttgac aaagaagaaa ttgaatgcgc cattgcactc
 241 tccctttctg aacaagaaca tgtgattcca caagatgaca aaggaaagaa agtcatcgga
 55 301 atacaaatct gaaactgaag aagatgatga tgaggatgag gatgaggatg aggaggatga

69

361 tgatgaagaa cacatgagag ctcaggtgga agcagcagaa gaagaggaaa agaaggtagc
 421 tcaagctcaa atagaggaag aagagaaacg aagagctgaa gaagctgagc tagaagagtt
 481 agagaaacag cttgccaaag ctgactaga agaggaagaa gttagacgcg ccaaagctca
 541 acttgaggaa gatgagcagc tcgcaaagcg tattcaagaa agtatgaatg tgggatctcc
 5 601 tcctcctgga tatgattctg gaagtgtgtt tccatcatac cccttccttg ttccttctag
 661 agaatatgca ctggttgccg agctgagatt ggacatggaa ggtttctgag ttgcatgggt
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 841 catccaaaat gtgatgtttg tcataacttt attcctacaa atccagctgg tctcattgag
 10 901 tacagggcac atcccttttg gatgcagaag tattgtcctt cacatgagcg tgatggaaca
 961 cctagatgct gcagctgtga gcgcatggag ccgaaagata caaagtatct gatacttgat
 1021 gatggtagaa aactgtgtct tgaatgtcta gactcagcca ttatggacac taatgaatgc
 1081 caaccgttgt atctcgagat acgtgagttt tatgaaggct tgcacatgaa agtgaacag
 1141 cagataccta tgctcttggt ggagagatca gctttaaacg aagctatgga aggagagaaa
 15 1201 catggacatc atcacttacc tgagactaga ggactctggt tgtctgaaga acaaactgtc
 1261 acaacagtgt tgaggagacc aaagattggg gcaggctaca agttgataga catgatcact
 1321 gagccttgca ggctggtgcg ccgttggtgaa gtcactgcta ttctcatctt atatggactt
 1381 ccccgctttt gttaactgga tcaatcctag ctcagatgat gatgcatgca tggcttcgac
 1441 taaatggggg atccaaatct tagaccagaa gtggaagaag ggatatgtca ggttttagct
 20 1501 cacatgtggg tggaaatctga gacttatgct ggctctacat tgatagatat tgcattcttct
 1561 tcttcgtctt catcatcagc cgctgtggcg attgcatcgt ccaagaaagg tgagaggtct
 1621 gatttttgaga agaaactcgg tgagtttttc aagcaccaga tagagtcaga ttcttcttcg
 1681 gcatatgggg atgggttcag gcaaggtaac caagctgttc ttacgcatgg tctgaagcga
 1741 acccttgatc atattcgctt gaccggtaca tttccttaa

SEQ ID NO:12 (BrDAR1)

1 atggattctt cttcatatgg tgtttctcat gtcagccata tctccaatcc ttgtatcttt
 61 ggggctgggt cgtcgtcttc gccagagaag aaatggaaact tgatgaaatg ggtgagtaaa
 30 121 cttttcaaga gtggctctaa cgggtggcact ggtggtgctc gcactaacgg tcatcctcct
 181 cagtttcaag aggacgagaa tatggtcttt cctttacctc ctctctcttc ggacgatcgg
 241 tcgagagcct cacgggacaa agaagaacta gatcgtgcat tgtcagtttc tctagctgac
 301 gatacgaacc gaccatatgg atatggttgg tctatggata ataattcaga tttccctagg
 361 ccttttcaca gtggattgaa tccatctttc attccacctt atgaaccgtc ctatcaagtc
 35 421 agacgaccac aaagaatatg tggcggttgc aatagcgata ttggattggg gaactatctg
 481 ggatgcatgg gaacattctt tcatcctgat tgcttctggt gtgattcatg tcgttaccct
 541 atcactgagc atgagttctc tctatcagga accaaacctt accatcagat ttgtttcaaa
 601 gagctcactc atcctaaatg cgaagtttgt caccatttta tcccaactaa tgatgctggc
 661 ttgatcgaat atcgatgcca tccgttttgg aacaaaaagt attgcccctc tcacgaacac
 40 721 gatagaaccg ctggttgctg tagctgcgaa cgtttgaggt catgggaggt gagatattac
 781 acgtttagac atgggagaag tttatgttta gaatgcatgg aaactgcatg aaccgacact
 841 ggagattgtc aaccacttta ccatgcaata cgtgactatt acgaaggaat gtacatgaaa
 901 cttgagcaac aaatcccat gcttcttggt cagcgagaag ctctcaacga cgctatcgtc
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 45 1021 caaacagtca caagtgttct taaaagaccg agactgggag ctcaccgtct tgttggtatg
 1081 agaactcagc ctcaaaagct tacacgtaaa tgtgaagtca ctgcgattct cgttctttac
 1141 ggctcccta gactattaac tggagcaatt cttgccacg agctgatgca tggatggcta
 1201 aggcctcaaag ggtataggaa ccttaaccct gaggtagagg aaggtatctg ccaagtcctc
 1261 tcttacatgt ggcttgaatc tgaagtctc tcagatcctt cttcaagaag catgccctca
 50 1321 acatcaactg ccacctcgtc atcatcatca tcatcatctt cttctaaca gaaaggaggg
 1381 aaaacaaacg tggagaagaa acttgagag tttctttaagc atcagatagc tcatgacgca
 1441 tctcctgctt acggaggggg tttcagagca gcaaatgcag cggtttgtaa gtacggtctg
 1501 cgtcgcacac ttgatcatat ccgcttact ggaacgtttc ctttgtaa

55 SEQ ID NO:13 (BrDAR2)

1 atgccattga gaggtagata tctgatggaa gatcggaaaa gaaaaaggaa aaagcttttt

70

5 61 gattttgggca gcggaacttaa ccttaaaccct gcaggatcct tttgaagctg aaactgatata
 121 cgtcaaacaa gtgtcatcga atgatgctca cgttcaagaa gatgaacagc ttgctttggc
 181 cattcaaaaa tctaaagaag acgaagagga aagaaggccc accagggact tagaagagca
 241 tgcacatgag agaggagaaa ggcaaaataa ttatgacaac tcttcttctt tgaaagacaa
 301 aaaagaagga cagacttctg aggagaaaac atgacaacat ttcctctgaa gctcgttgg
 361 atgagaatga ggagcagcgg attatctggg agagtttgaa ggataaagggt caaacaagc
 421 catctgaaga tgaggtcatt cctcctcgta gagcaagtgt ggtggttgcc actctgagat
 481 tgaacaagga ggatcagtgg atgtctttgg tgttccttgg catcctgaat gtttctcttg
 541 tgggtgcttg cgtaacccaa ttgctgtcca cgaggttcaa aaccatgtct caaactcaag
 10 601 aggcaagttc cacaaaaact gctataaccg gtactgctat gtctgccaag agaaagttaa
 661 gattagagag tacaatagcc atcctttctg gaaggagata tactgccctg ctcacgaac
 721 tgatggaact cccaagtgtt gcagctgcga gaggctagag cctagagaaa cggagttcgt
 781 aatgctaagt gatggaagggt ggctatgtct agaattgtat gactcagcgg ttatggatatac
 841 tgacgaagtc cagcctcttc actttgaaat ccttgacttc ttccatggct tgttcttgcc
 15 901 agttgagaaa gagttttctc ttcttttgggt ggagaaacaa gccctgaata aagctgagga
 961 ggaagagaag attgtgtcaa aaggggccaaa gatgggggag aacaagcagc taacaggaaa
 1021 gaccacggaa tctcaaagggt ttgtgagtgg atgcccgggtc actgcaattc tcatcttata
 1081 tggacttctc agaggttact aacaggatct atcatggctc acgagatgat gcatgcttat
 1141 cttagactca atgggacata ataatttgaa caaggttctg gaagaaggaa tatgccaagt
 20 1201 gctagggcac atgtggttgg agactcagag atacgcccct attgatgttg ctgcagcttc
 1261 ttcttcttct tcgtcaaagt cggcaaagaa aggggagtgg tctgaactcg agaagaagct
 1321 ggtggatttt tacaagtatg agatagaaac agatgagtca gctgtctatg gtgaagggtt
 1381 taggaaagtt aactatatgg ttacaaactc cagcctccag gaaaccctca aagagattct
 1441 tccccgccgg ggttga

SEQ ID NO:14 BrDAR3-7

1 atggggttgg taaacaagat cttcaaaggc tctaaccaaa ggcacccctt ggggaatgaa
 61 cactatcatc ataattggcg ctattacgag aactaccgac acgaacattc tgagcctagt
 30 121 gcagagacag atgctgatca tacgcaggag ccatctactt ctgaggagga gacatggaat
 181 ggggaaggaaa atgaagaagt agaccgtgta attgcattgt ctattttaga agaagagaat
 241 caaagaccag agactaatac aggcgcctgg aaacacgcaa tgatggatga cgatgagcaa
 301 cttgctagag ccatacaaga gagtatgata gctaggaatg gaactactta tgactttggg
 361 aatgcatatg ggaatggaca tatgcatgga ggaggcaatg tatatgacaa tgggtgatatt
 35 421 tattatccaa gacctattgc tttctcaatg gacttcagga tctgtgctgg ctgcaatatg
 481 gagattggcc atggaagata tctgaattgc ctcaacgcac tatggcatcc acaatgtttt
 541 cgatgctatg gctgcagtca cccaatctct gagtacgagt tctcaacgctc tgggaattac
 601 cctttttcaca aagcttgtta caggagaggg ttccatccaa aatgtgatgt ctgcagcctc
 661 tttattttcaa caaacatgct tggctcttatt gaatatagag cacatccttt ctgggtccag
 40 721 aagtattgcc cttctcacga acacgatgct acgccaagat gttgcagctg tgaaagaatg
 781 gagccgcgga atacaggata ttttgaaact aacgatggac ggaagctttg ccttgagtgt
 841 ctgactcat cggtgatgga cacttttcaa tgccagcctc tgtacttgca gatacaagag
 901 ttctatgaag gacttaacat gacggttagg caggaggttc cacttctctt agttgagcgg
 961 caggcactta acgaagccag agaagggtgaa aggaatggct actatcacat gccagagaca
 45 1021 agaggactct gtctgtcgga agaacaact gttagaactg tgagaaagag atcgaaggga
 1081 aactggagtg ggaatatgat tacagagcaa ttcaagctaa ctgctcgatg cgaggttact
 1141 gccattctca tcttgtttgg tctccctagg ctactcactg gttcaattct agctcatgag
 1201 atgatgcacg cgtggatgct gctcaaagggt tccggccac ttagccaaga tgttgaagag
 1261 gggatatgtc aagtgatggc tcataagtgg ttagaagctg agttagctgc tggttcaaga
 50 1321 aatagcaatg ctgcatcatc ttcatcatct tcttatggag gagtgaagaa gggaccaagg
 1381 tctcagtacg agaggaagct tgggtgagtt ttcaagcacc agatagagtc tgatgcttct
 1441 ccggtttatg gagatgggtt cagggccggg aggttagcgg ttaacaagta tggtttgtgg
 1501 agaacacttg agcatatata gatgactggg agattcccgg tttaa

55 SEQ ID NO:15 BrDA1b

1 atggggttgg ttaacaagat cttcaaaggc tctaaccaaa ggttccggct tgggaatgac

71

5 61 catgaccaca atggctatta ccagagttat ccacatgatg agcctagtgc tgatactgat
 121 cctgatcctg atcctgatcc tgatgaaact catactcagg aaccatctac ctctgaggag
 181 gatacatccg gccaggaaaa cgaagacata gatcgtgcaa tcgcatgtgc tcttatagaa
 241 aacagtcaag gacagactaa taatacatgc gctgccaacg cagggagta cgcaatggtg
 301 gatgaagatg agcaacttgc tagagccata caagagagca tggtagttgg gaatacaccg
 361 cgtcagaagc atgggaagtag ttatgatatt ggggaatgcat atggggctgg agacgtttac
 421 ggggaatggac atatgcatgg aggtggaaat gtatatgccca atggagatat ttattatcca
 481 agacctactg ctttcccaat ggatttcagg atttgtgctg gctgcaatat ggagattgga
 541 catggaagat atctgaattg cttgaatgca ctatggcatc cagaatgttt tcgatgttat
 10 601 ggctgtaggc accccatttc tgagtacgag ttctcaacgt ctgggaacta cctttttcac
 661 aaagcttggt atagggagag ataccatcca aaatgtgatg tctgcagcct ctttattcca
 721 acaaaccatg ctggtcttat tggatatagg gcacatcctt tttgggtcca gaagtattgc
 781 ccttctcacg aacacgatgc taccccaaga tgttgagtt gcgaaagaat ggaaccacgc
 841 aatacaggat atgttgaact taacgatgga cggaaacttt gccttgaatg tctggactca
 15 901 gcggtgatgg acacttttca atgccaacct ctgtatctgc agatacaaga attctacgaa
 961 ggtccttttca tgaaggtaga gcaggacgtt ccacttcttt tagttgagag gcaagcactc
 1021 aacgaagcca gagaagggtga aaagaatggt cactatcaca tgccagagac aagaggactc
 1081 tgccctttcag aagagcaaac tgtttagcact gtaagaaaga gatcgaagca tggcacagga
 1141 aactgggctg ggaatatgat tacagagcct tacaagttga cacgtcaatg cgaggttact
 20 1201 gccattctca tcttgtttgg gctccctagg ctactcaccg gttcgattct agctcatgag
 1261 atgatgcacg cgtggatgcg gctcaaggga ttccggacgc tgagccaaga cgttgaagaa
 1321 ggaatatgtc aagtgatggc tcataagtgg ttggaagcag agttagctgc tggttcaaga
 1381 aacagcaatg ttgctcatc ttcattcttct agaggagtga agaagggacc aagatcgag
 1441 tacgagagga agcttggtga gtttttcaag caccaaactc agtctgatgc ttctccggtt
 25 1501 tatggagacg ggttcagggc tgggaggtta gcggttaaca agtatggttt gccaaaaaca
 1561 cttgagcata tacagatgac cggtagattc ccggtttaa

SEQ ID NO:16 BrDA1a

30 1 atggggttggg tgaccaaatt ttttagaggt tcaaccaca aaatctcgga agggcaatac
 61 cacagcaaac ccgcgaggga gacgatatgg aatggaccct ctaattccgc agttgtgacg
 121 gatgtcccgat cagaatttga caatgaagat atcgctcgtg ctatatcact ctctctatta
 181 gaggaggaac aaagaaaggc aaaggcaata gaaaaggaca tgcatttggg ggaaggatga
 241 caacttgcaa gagctatcca ggaaagtttg aatgttgaat cgccctcctg tgctcgtgaa
 301 aatggcaacg ccaatgggtg caatatgtat caaccactgc catttatgtt ttcttctgga
 35 361 ttcaggactt gtgcccggatg tcacagttag atttggtcatg ggcgtttcct tagttgcatg
 421 ggagctgttt ggcatccaga atgttttcgc tgtcatgctt gtaatcaacc aatatatgac
 481 tatgagttct ccatgtcggg aaaccatcca taccataaaa catgctacaa ggagcgcttt
 541 caccctaaat gtgatgtctg caagcaattt attcctacaa atatgaatgg cctgattgaa
 601 tatagagcac atccttttctg gttacaaaaa tactgtccat cacatgaggt ggacggtact
 40 661 ccaagatgct gtagttgtga aagaatggag ccaagggaat caagatatgt attgctggac
 721 gatggctcga aactctgcct ggagtgcctt gattctgcag ttatggatac gagcgagtgc
 781 caacctcttt atcttgaat acaggaattt tatgaaggcc taaatatgaa agtggaaacaa
 841 caagttccct tgcttcttgt agaaagacag gctttaaatg aagccatgga aggagagaag
 901 actggtcacc accatcttcc agaaacaaga ggtttatgct tatcagaaga gcaaactgtc
 45 961 agcacgatat tgaggagacc aagaatggct ggaaataaag ttatggaaat gataacggag
 1021 ccatataggt tgactcgctg atgtgaagtg actgcaattc tcattcttta tggctctcca
 1081 agattgttga caggttcaat tttagctcat gagatgatgc atgcgtgggt gcgacttaaa
 1141 ggatatcgca cacttagtcc agacgtagaa gagggcatat gccaaagtct tgctcatatg
 1201 tggattgagt cagagatcat tgcaggatca ggcagtaatg gtgcttcaac gtcttcatcc
 50 1261 tcatcagcat ccacatcatc gaaaaggggg ggaagatctc agtttgagcg aaagcttggg
 1321 gatttttttca agcaccaaat tgagtcagat acctcaatgg cctatggcga tgggttttaga
 1381 gctggcaacc gagctgttct tcagtatggg cttaaagcgca cccttgagca tatccggtta
 1441 acagggactt tcccattttg a

55 SEQ ID NO:17 OsDA1

Sequence Alignments

A. amino acid (SEQ ID NO: 1) and nucleotide (SEQ ID NO: 2) sequences of DA1 and mutation sites of *dal-1*, *sod1-1*, *sod1-2* and *sod1-3*.

The domains predicted by using SMART software are shown.

5

B. Alignment of UIM motifs among different UIM motif- containing proteins. UIM motifs were predicted by using SMART software. The predicted UIM1 (E-value, 6.39e-02) and UIM2 (E-value, 7.34e-02) sequences are shown in A.

10

C. Alignment of LIM domains among LIM domain- containing proteins. In the LIM domain, there are seven conserved cysteine residues and one conserved histidine. The arrangement followed by these conserved residues is C-x(2)-C-x(16,23)-H-x(2)-[CH]-x(2)-C-x(2)-C-x(16,21)-C-x(2,3)-[CHD]. The LIM domain (E-value, 3.05e-10) was predicted by using SMART software and is shown in A.

15

D. Alignment of DA1 and DA1-related proteins in *Arabidopsis*. The conserved regions among DA1 and DARS are in their C-terminal regions. The *dal-1* allele has a single nucleotide G to A transition in gene At1g19270 and is predicted to cause an arginine (R) to lysine change (K) in a conserved amino acid at position 358. An asterisk indicates identical amino acid residues in the alignment. A colon indicates conserved substitutions in the alignment and a period indicates semi-conserved substitutions in the alignment.

20

25

E: Amino acid alignments of DA1-like proteins. Full length amino acid sequences of DA1-like proteins from *Physcomitrella patens* (Pp), *Selaginella moellendorffii* (Sm), *Brassica rapa* (Br), *Arabidopsis thaliana* (At), *Brachypodium distachyon* (Bd) and *Oryza sativa* (Os) were aligned with default setting ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>), and edited display settings in VectorNTi. The red arrow shows the mutation in *dal-1* allele. DAL stands for DA1-Like.

30

A cggtggggaacggttttttctctggaa
gaagaagaagaagagctcaacaagctcaacgacccaaaaaacttcggacacgaagactttt
taattcatttctcctctttttgtttttttcggtccaaaaatattcgatactctcgatctctt
cttcgtgatccctcattaaataaaaaatacgaatttttattctttttttgtgagtgacccaaa
ttttttgactttggattagcgttagaattcaagcacattctgggttttattcgtgtatgagt
agacattgattttgtcaaagttgcattcttttatataaaagaagtttaatttcctttttt
cttttcttttctctttttttttttttcccccattgttatagattcttcccccatttttga
agaaaggagagaactaaagagtcctttttgagattcttttgccttcccttgcttgatt
agatcattttttgtgattctggattttgtgggggtttcgtgaagcttattgggatcttatc
tgattcaggattttctcaaggctgcattggcgtatgagcagatagttttatttaggcatt
atgggttggttttaacaagatcttttaaggcttaaccaaaggctccgggttggaataat
M G W F N K I F K G S N Q R L R V G N N
aagcacaatcacaatgtttattacgataattatccgactgcttcacatgatgatgagcct
K H N H N V Y Y D N Y P T A S H D D E P
agtgcggcggtacagatgctgataatgatgaacctcatcactcaggaacctctaca
S A A A D T D A D N D E P H H T Q E P S T
tctgagataatcatcgaatgaccaggaaatgaagacatagaccgtgcaattgcatgtg
S E D N T S N D Q E N E D I D R A I A L **UII**

sod1-1 (a) G/E
tcgcttttagaagaagaatcaagaacagagacaagtataagcgggaaataactcgatgccgggtg
S L L E E N Q E Q T S I S G K Y S M P V
gatgaagatgagcaacttgctagagccctacaagaagtatggtagttgggaattcacc
D E D E Q L A R A L Q E S M V V G N S P **UII**
cgtcacaagaatggaagtacatagataatgggaatgcataatggagctggagatttat
R H K S G S T Y D N G N A Y G A G D L Y
gggaatggacatatgtatggaggaggaaatgtatatgcaaatggagatatttattatcca
G N G H M Y G G G N V Y A N G D I Y Y P
agacattattcttttcaaattggatttccaggattttgtgctggctgtaatatggagattggc
R P I T F Q M D F R I C A G C N M E I G
catggaagatttctgaatttgccttaattcactatggcatccagaatgttttcgatgttat
H G R F L N C L N S L W H P E C F R C Y **LI**
ggctgcagtcagccgatttctgagtagcaggttttcaacatcagggaactacccttttcac
G C S Q P I S E Y E F S T S G N Y P F H
aaggcttggtacaggagagatatcatcctaaatgtgatgtctgcagccactttatacca
K A C Y R E R Y H P K C D V C S H F I P
acaaatcatgctggtcttattgaatatagggcacatcctttttgggttcagaagtattgt
T N H A G L I E Y R A H P F W V Q K Y C
ccttctcagcaacacgatgctacccccgagatgtttgcagttgtgaaagaatggagccacgg
P S H E H D A T P R C C S C E R M E P R
aatcagagatatgttgaaacttaacgatggacggaaactttgccttgagtgtttggactcg
N T R Y V L N D G R K L C L E C L D S
gcggtcatggacaccatgcaatgccaacctctgtacttgcaaatacaaaatttctatgaa
A V M D T M Q C Q P L Y L Q I Q N F Y E
ggactcaacatgaaggtagagcaggaagttccactcctcttggttgagagacaagcatt
G L N M K V E Q E V P L L L V E R Q A L

dal-1 (a) (R/K)
aacgaagccagagaaggtgaaaagaatggctcactatcacatgccagaacaagaggactc
N E A R E G E K N G H Y H M P E T R G L
(t) (L/F) **sod1-2**
tgcctttcagaagaacaaactgttagtactgtaagaaagcgatcaaagcatggcacagga
C L S E E Q T V S T V R K R S K H G T G
aatgggcccgggaatattacagaaccttacaagttaacacggcaatgtgaagttaccgcc
K W A G N I T E P Y K L T R Q C E V T A
attctcatcttatttcgggctccctaggttacttactggttcgattctagctcatgagatg
I L I L F G L P R L L T G S I L A H E M

sod1-3 (T) (Q/.)
atgcatgcgtggatgaggctcaaaggattccgaacactgagccaagatggttgaagaaggt
M H A W M R L K G F R T L S Q D V E E G
atatgtcaagtgatggctcataaatgggttagatgctgagtttagctgctggttcaacaaat
I C Q V M A H K W L D A E L A A G S T N
agcaatgctgcatcatcctcctcttctcaaggactgaaaaagggaccgagatctcag
S N A A S S S S S S S Q G L K K G P R S Q
tacgagagaaagcttggtgagtttttcaagcaccaaatcgagtctgatgcttctccggtt
Y E R K L G E F F K H Q I E S D A S P V
tatggagacgggttcagagctgggaggttagctgttcacaagtacggtttgcgaaaaaca
Y G D G F R A G R L A V H K Y G L R K T
Cttgagcatatacagatgaccggtagattcccggtttaagaacccaaatggacaaggtct
L E H I Q M T G R F P V -
tctactttatttataggatccttggttagattcctccttatatgctctaattcttttggtgg
aaaatgtactctcgaccatattcttattgtagtctcattcagatgattctttgtattcctc
tgttaaaatccatcagaatcagattcagtggttttctttgtt

Alignment B

UIM1

DA1	QENEDIDRA	AL	SLLEENQ	E
Q9LM05/295-314	DDTALHQQAT	AM	SMAQAAQ	A
O74423/258-277	DSEAEHOKAT	QL	SKEEDEA	R
VP27_YEAST/258	DEEELIRKAT	EL	SLKESRN	S
Q05785/175-194	SYQDDHEKAL	EE	SRITAEQ	D
Q9P2G1/976-995	EDDPNILLAT	QL	SLQESGL	A
AAK61871/105-1	EEEEELNRKAT	AE	SLNSCRP	S
16741231/229-2	DEDLQHQLAL	SL	SRQEHEK	G
Q9XTL2/173-192	QEEDDIAKAT	EL	SLKENKG	S
2623826/295-31	SEEDQIAYAL	RM	SLQOMGE	E
Q9FJX8/64-84	HEAEQMDLAT	QE	SRQEEEE	E
Q9C9U5/139-158	EEENQIQAL	EL	SAREDPE	A
YMI8_YEAST/651	NVDEDTQLAT	AL	SLSEIN-	-
Q9FJX8/119-138	EEDELHARTL	EE	SLKENNR	R
AAK61871/80-99	TEEEQFALAL	KM	SEQEARE	V
Q20187/2-21	SDDEEHQLAT	EL	SKKTFKD	E
Q9HCH8/758-777	REEQEHQQAL	AQ	SLQEQEA	W
Q9V8R1/660-679	YVDPDILAMAM	RL	SQQEORK	F
O81340/323-342	GDDQDILQAL	QL	SVQDSAK	E
Q9TOE1/138-157	IEEEMIRAAAT	EA	SKKEAEG	S
O74749/164-183	REEEELQYDAL	AL	SLSESTA	Q
O35815/224-243	DDEDDIQRAL	AM	SRQEIDM	E
O82143/323-342	VDDQDIALAL	QM	SVQDAGG	S
Q9HFR1/208-227	NMDPEILAMAT	RM	SLQEAQA	Q
Q9FJX8/146-165	NKDEQIALIV	QE	SLNMEEY	P
ENSG0000013275	EDDDLIQFAL	QQ	SLLEAGT	E
YMI8_YEAST/517	ENDIQERIAL	LE	SQEAQAR	N
Q9MA77/5-24	QEDEDIKLAL	KM	SMQYNPP	E
Q9V8R1/510-529	DEDDMHQYAT	EQ	SLVETSG	A
AAH11090/250-2	SEDEDILQALAM	AY	SLSEMEA	A
O13821/260-279	NEDEDIKRAT	EL	SLKEMPO	S
O13821/304-323	DEDEEHKRAAT	AI	SLEEAQK	S
YMI8_YEAST/583	EDDEEFLRAT	RQ	SRVEDER	R
EP15_MOUSE/878	QEQEDIELAT	AL	SKSEISE	A
O35815/329-348	SEEDVIRATV	TV	SLTAKD	S
PSD4_ARATH/282	EDSALIDQAT	AM	SVGDVNM	S
Q9FJX8/38-57	QHEADIQKAK	QR	SLATHEA	E
VP27_YEAST/301	EEDPDHKAAT	QE	SLREAAE	A
O74423/230-249	ENDPEHQRVI	EE	SKRQAAE	D
O82143/291-310	DDAQLHQQAL	AM	SMEEGSS	G
Q9LG27/1615-16	QEDDEHQAAL	AL	SLGNSSE	T
Q9HA18/233-252	GDDLRLQMAAT	EE	SKRETGG	K
O23197/65-84	FDKEEIECAT	AL	SLSEQEH	V
O45266/382-401	SDSIMHKYAT	EL	SLDSKE	D
Q9H3M9/215-234	QDEEDFQRAAL	EL	SRQETNR	E
YHA2_YEAST/162	SDDEEHQKAT	KM	SLFEYEK	Q
YMI8_YEAST/547	DEDEQHRRAL	EE	SOLIYET	Q
2623826/262-28	SEEAMHQQAT	AM	SMQMNNT	E
Q9HCH8/780-799	KEDDDHKRAT	EL	SLQEFNN	S
Q12518/165-184	ENDDDHQRAT	SA	SRLTAAE	D
O75886/165-184	KEDEDIAKAT	EL	SLQEOKQ	Q
EP15_MOUSE/852	SEEDMIEWAK	RE	SEREEEQ	R
O23197/110-129	DEDEEYMRAQ	LE	AAEEEEER	R
Q9HCH8/656-675	SEEELHAAVL	EL	SKRDASP	S
Q9TOE1/170-189	EDDDDIATAV	TM	SLKSAEE	E
Q03291/171-190	QDDENLRMAAT	LE	SLQELNT	N
PSD4_DROME/212	NEDPEHALAL	RV	SMEEQRO	R
Q9H3M9/235-252	REDEHERSTV	EL	SMQGSSG	N
Q9VS85/226-245	SDDVRLQLAL	SQ	SEQDFKD	P

Q05785/206-225	DEDPDFQAA	QL.SKEEEEEL	K
Q13027/227-246	GDDLRLQMA	EE.SRKGAPS	K
Q88321/285-304	TEEEQIAYAM	QM.SIQGAEF	G
Q9LM05/334-353	GDDQDLAYAL	QM.SMQQAAA	A
Q9V8R1/685-701	QEDEMIEQAL	KL.SLQEH--	-
CE17317 B0205-	TEEQQLLEWAL	RL.SMQENAP	A
Q9MA26/374-393	EEEEELQRAL	AA.SLEDNNM	K
Q17796/291-310	KEEEDLALAL	AI.SQSEAEA	K
Q9DOW4/197-216	SEDEALQRAL	EL.SLAEAKP	Q
Q9DOW4/221-240	QEEDDLALAL	AL.SASEAEY	Q
Q15286/347-366	SEEDMLQAA	TM.SLETVRN	D
Q45266/775-794	IENKMIKIAM	KE.SLLGSED	G
Q9FJX8/244-263	DEDEQLAKAV	EE.SLKGKGQ	I
Q9HFR1/347-366	DEEAAIARAI	AM.SLEGQEE	G
UBPP_HUMAN/97-	DDKDDLQRAI	AL.SLAESNR	A
CE06353 T04C10	SDDARIQMA	EE.SQKADR	L
AAH11090/207-2	GVPDDLALGL	EL.SRREQQP	S
Q45266/332-351	FDNIMLEAAI	KQ.SLLDLKG	D
Q9LM99/105-124	DEDEQLARAI	QE.SMVVGNS	P
Q9FDZ8/140-159	EDDDDLDKAI	AL.SLQGSVA	G
Q9LM99/69-88	QENEDIDRAI	AL.SLLEENQ	E
PSD4_ARATH/305	DEDQDLALAL	QM.SMSGES	S
Q35815/244-263	DEEADLRRAI	QL.SMQGSSR	G
ENSG00000132_1	RFEEQLRIAI	EL.SSREQEE	R
Q81340/291-310	DENALLQQA	AM.SMDEPAS	T
Q9HFR1/321-340	EEDEELKRAI	AM.SRGEAGD	I
Q9ZPH8/231-250	REDEDIARAI	SM.SLEAMSY	L
PSD4_DROME/276	TEEAMLIQRAI	AL.STETPED	N
Q9VTK7/621-640	NESEMLOQA	QM.STRDYME	D
Q9NWK2/209-228	EEELQLQLAI	AM.SREEAEK	P
Consensus/60%	p---pLpbA1	pb.Sbp-.pp	p

UIM2

DA1	DEDEQLARAI	QE.SMVVGNS	P
Q9LM05/295-314	DDTALLQQA	AM.SMAQAAQ	A
Q9HA18/233-252	GDDLRLQMA	EE.SKRETGG	K
Q9V8R1/685-701	QEDEMIEQAL	KL.SLQEH--	-
ENSG0000013275	EDDDLLQQA	QQ.SLLEAGT	E
CE17317 B0205-	TEEQQLLEWAL	RL.SMQENAP	A
YMI8_YEAST/517	ENDIQLRIAI	LE.SQEAQAR	N
Q9MA77/5-24	QEDEDLKLAI	KM.SMQYNPP	E
Q74423/258-277	DSEAELOKAI	QL.SKEEDEA	R
VP27_YEAST/258	DEEELIRKAI	EL.SLKESRN	S
Q9MA26/374-393	EEEEELQRAL	AA.SLEDNNM	K
Q9V8R1/510-529	DEDDMLQYAI	EQ.SLVETSG	A
Q05785/175-194	SYQDDLEKAI	EE.SRITAQE	D
Q9P2G1/976-995	EDDPNILLAI	QL.SLQESGL	A
Q17796/291-310	KEEEDLALAL	AI.SQSEAEA	K
Q23197/65-84	FDKEEIECAI	AL.SLSEQEH	V
AAK61871/105-1	EEEEELIRKAI	AE.SLNSCRP	S
Q9DOW4/197-216	SEDEALQRAL	EL.SLAEAKP	Q
AAH11090/250-2	SEDEDLQLAM	AY.SLSEMEA	A
Q9DOW4/221-240	QEEDDLALAL	AL.SASEAEY	Q
Q15286/347-366	SEEDMLQAA	TM.SLETVRN	D
Q16741231/229-2	DEDLQLQLAI	SL.SRQEHK	G
Q45266/775-794	IENKMIKIAM	KE.SLLGSED	G
Q45266/382-401	SDSIMIKYAI	EL.SLLDSKE	D
Q13821/260-279	NEDEDIKRAI	EL.SLKEMPQ	S
Q9H3M9/215-234	QDEEDFORAI	EL.SRQETNR	E
Q9FJX8/244-263	DEDEQLAKAV	EE.SLKGKGQ	I
Q9HFR1/347-366	DEEAAIARAI	AM.SLEGQEE	G
UBPP_HUMAN/97-	DDKDDLQRAI	AL.SLAESNR	A
CE06353 T04C10	SDDARIQMA	EE.SQKADR	L

YHA2_YEAST/162	SDDEELQKAL	KM.	SLFEYEK	Q
AAH11090/207-2	GVPDDLALGL	EL.	SRREQQP	S
YMI8_YEAST/547	DEDEQLRRAL	EE.	SOLIJET	Q
O45266/332-351	FDNIMLEAAI	KQ.	SLDLKG	D
Q9XTL2/173-192	QEEDDIAKAI	EL.	SLKENKG	S
Q9LM99/105-124	DEDEQLARAL	QE.	SMVVGNS	P
Q9FDZ8/140-159	EDDDDLDKAI	AL.	SLQGSVA	G
2623826/262-28	SEEAMIQOAL	AM.	SMQMNNT	E
2623826/295-31	SEEDQIAYAL	RM.	SLQQMGE	E
O13821/304-323	DEDEELKRAI	AI.	SLEEAQK	S
Q9HCH8/780-799	KEDDDLKRAT	EL.	SLQEFNN	S
Q9FJX8/64-84	HEAEQLDLAI	QEFSRQEEEE	E	
Q9C9U5/139-158	EEENQIOLAL	EL.	SAREDP	A
Q12518/165-184	ENDDDLQRAI	SA.	SRLTAE	D
PSD4_ARATH/305	DEDQDLALAL	QM.	SMSGES	S
Q9LM99/69-88	QENEDIDRAI	AL.	SLLEENQ	E
O75886/165-184	KEDEDIAKAI	EL.	SLQEQKQ	Q
YMI8_YEAST/651	NVDEDLQALAI	AL.	SLSEIN-	-
EP15_MOUSE/852	SEEDMIEWAK	RE.	SEREEEQ	R
Q9FJX8/119-138	EEDELARTI	EE.	SLKENNR	R
O23197/110-129	DEDEEYMRQ	LE.	AAEEER	R
Q9HCH8/656-675	SEEELAAVIL	EL.	SKRDASP	S
O35815/244-263	DEEADLRRAL	QL.	SMQSSR	G
YMI8_YEAST/583	EDDEEFLRAI	RQ.	SRVEDER	R
EP15_MOUSE/878	QEQEDIELAI	AL.	SKSEISE	A
Q20187/2-21	SDDEELQALAI	EL.	SKKTFKD	E
AAK61871/80-99	TEEEQFALAI	KM.	SEQEARE	V
Q9HCH8/758-777	REEQELQOAL	AQ.	SLQEQEA	W
ENSG00000132_1	RFEEQLRLAI	EL.	SSREQEE	R
Q9V8R1/660-679	YVDPDIAMAM	RL.	SOQEQRK	F
Q9TOE1/170-189	EDDDDIATAV	TM.	SLKSAEE	E
O35815/329-348	SEEDVLRATV	TV.	SLETAKD	S
Q03291/171-190	QDDENLRMAI	LE.	SLQELNT	N
O81340/291-310	DENALLQOAL	AM.	SMDEPAS	T
PSD4_DROME/212	NEDPELALAI	RV.	SMEEQRQ	R
Q9HFR1/321-340	EEDEELKRAI	AM.	SRGEGGD	I
O81340/323-342	GDDQDLQALAI	QL.	SVQDSAK	E
Q9ZPH8/231-250	REDEDIARAI	SM.	SLEAMSY	L
PSD4_ARATH/282	EDSALLDOAI	AM.	SVGDVNM	S
Q9TOE1/138-157	IEEEMIRAI	EA.	SKKEAEG	S
O35815/224-243	DDEDDLQRAI	AM.	SRQEIDM	E
O74749/164-183	REEEELQYAI	AL.	SLSESTA	Q
Q9FJX8/38-57	QHEADIQKAI	QR.	SLATHEA	E
PSD4_DROME/276	TEEAMLQRAI	AL.	STETPED	N
O82143/323-342	VDDQDLALAI	QM.	SVQDAGG	S
Q9VTK7/621-640	NESEMLOQAI	QM.	STRDYME	D
Q9HFR1/208-227	NMDPELAMAI	RM.	SLQEAQA	Q
VP27_YEAST/301	EEDPDLKAI	QE.	SLREAEE	A
O74423/230-249	ENDPELQRAI	EE.	SKRQAE	D
Q9H3M9/235-252	REDEHLRSTI	EL.	SMQSSG	N
Q9VS85/226-245	SDDVRLQALAI	SQ.	SEQDFKD	P
Q9NWK2/209-228	EEELQLQALAI	AM.	SREEAEK	P
Q05785/206-225	DEDPDFQAI	QL.	SKEEEL	K
O82143/291-310	DDAQLLQOAI	AM.	SMEEGSS	G
O88321/285-304	TEEEQIAYAI	QM.	SLQGAEF	G
Q9LG27/1615-16	QEDDEIAQAI	AL.	SLGNSSE	T
O13027/227-246	GDDLRLQMAI	EE.	SRKGAPS	K
Q9FJX8/146-165	NKDEQLALIV	QE.	SLNMEY	P
Q9LM05/334-353	GDDQDIAYAI	QM.	SMQAAA	A
Consensus/60%	p---pLpbAl	pb.	Sbp-spp	p

Alignment C

LIM

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DA1
CEF25H5_6
LIM3-XENLA
DDU83086+1
PINC=HUMAN
CEU73546-1
MMU79776_1
CEF28F5_2
H3295381-1
CLP-HUMAN
CEU72211-1
H36011610_1
H3281321_1
H3U49957_1
H3MLN50_1
CEB0496_8
H3ENIGMA-1
LI11-CAEEL-2
Y2H2-CAEEL
M2SEQUENC=1
CRIP_RAT
RHM1_HUMAN-1
AV07024+1
SCRG1-1
CET11B7_5
Y2H2-CAEEL
CEU09B9-2
TRI6-HUMAN
TES2_MOUSE
H3DRAL-1
TES2-MOUSE
CEU0706_2
Y2H2-CAEEL
H3ENIGMA+1
H3295381+1
HM14-CAEEL
LRG1+YEAST
AFTE_DROME-1
NFLIMDO/9
H36011610-1
LX1_MESAU
LX1_MOUSE
H36011610+1
CEC260/75
YF96-CAEEL
H3ENIGMA_1
MMU79776+1
CEF25H5_5
OC15158-1
DMMLP84B-1
RN48247-1
YF96-CAEEL
MEC3-CAEEL
CEK03E6_4
CEC09G9+8
DR09403_1
CEZC64_4
LIK2_HUMAN
CEU0706_2+1
PINC_HUMAN
CEB0496+8
CG22818_1
CEZK381_5
CEC09G9-8
MMU46687_1
ZYX_CHICK
LIM1-XENLA-2
SCI9481-16
Y2H2-CAEEL+1
PINC_HUMAN+1
DDU83086-1
PAXI_HUMAN
TSF3-HELAN
H3281321-1
AFAP_1

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HSDRAL_1	TCEECGKPIG	C...DCKDLS	YK.DRHN	E..	ACHGCS..O	GRNSTVD...	.KPEAAKEDQ	ELCTDC...
CEZK622_2	PCFRCKRPIY	FN...DKMGEL	KD.GSM	K..	GCHKOW..I	GTRISLK(4)	)NRNDNTDLE	VHCAGH...
HMI4-CAEEL	KCSSNEGIV	P...DHVVRK	AS.NBVE	V..	ECBCCF..I	GKRSJETGE	EFYLIADDA	EVCKDD...
LIM1_XENLA-1	HCAGGERPIL	D...RPLIN	VL.DRAA	V..	KCVUCC..E	GKCNITE...	..KCFPSREGK	ELCKND...
LIK2-CHICK	SCHGSLIMT	G....FVMV	AG.EYKE	P..	ECTACM..S	GKVIEDG...	DTYALVQHS	ELCGKC...
HS295361_1	TCVEGRKPIG	A...DSKEFH	YK.NRFA	D..	TCRCA..K	GLHPLAN...	..ETEVAKDNK	ELCNKC...
LH2_RAT	LCAGCGKIS	D...RYMIL	AV.DKON	M..	RCLKCC..E	GKLNIESE...	..LTCPSKDG	ELCKED...
CEF25H5-6	RCSMKKVIIT	A....GGT	YK.NEFA	R..	ECBCCT..N	GNSUAG...	..ORFISKDEK	ELCANC...
MMSEQUENC+1	LCGSNKPDA	G....QVMT	AL.GRAA	P..	EBHICS..G	GSTTIGG...	..SSHFEDGA	ELCPEC...
MMSEQUENC-1	RCGECNQPIR	H....KMT	AL.GTHA	P..	EBECGV..S	GCEPTGE...	..EGHEREGR	ELCRRD...
PAXI-HUMAN	KCGGECARAT	E....NYES	AL.NTIA	P..	ECFVCR..E	GTPPFVN...	..GSHFEDGO	ELCEVH...
MEC3_CAEEL	KCNCCNEQIV	D...RYTYR	MD.NRSE	E..	KCVKCT..I	GESHAE...	..KCFWKNGR	ELCSQH...
OV14530+1	KCSNCKPIIT	E....KILR	AT.GGA	P..	DCEIOT..V	GKKODGV...	..PFTVDSANQ	ELCVVC...
LIM3_XENLA	QCAGCNQIV	D...RPLIK	VL.DRHN	S..	KCLKON..D	QIQIAE...	..KCFSRGDS	ELCKDD...
CEF09B9_2	TCAACDQALH	S....QVIL	AL.GLSA	V..	YCHKCS..E	CSAVIHG...	..FMSHGGK	ELCLRD...
HSDRAL+1	KCGCKKTIIM	P...GTRKME	YK.GSSA	E..	TCCHCH..R	COOPIGT...	..KSHIPKDNQ	ELCVPC...
CEF07C6=2	NCNKKQKHVD	N...SDIIT	YQ.ENPY	A..	YEHKCT..T	GKKVIES...	..DARTIKDD	ELCPRCFDE
RN46247_1	MCAGCNQAIR	G....PEEV	AL.GKSA	P..	EBENCA..H	GKNTMAY...	..ICHVEEKGA	ELCELC...
RHM1-HUMAN-2	MCAGCSKLIIP	A...FEMVMR	AR.DNVE	L..	DCHACQ..L	GNRFVCG...	..DKHFLKNM	ELCQMD...
YK70-YEAST	ICKVCSNFI	G....EOLN	DK.VERE	V..	DCLNCF..L	GKTAITN...	..DYHIFNGEI	ELCGNH...
CEF25H5+6	RCDCGNEIFR	A...GMKIME	YK.GKON	D..	KCHCOA..H	GKLAIGT...	..KSHIPKDD	ELCGPC...
LMX1-MESAU	KCGGCFEADA	P...NEFVMR	AO.KSV	L..	SCHCCC..V	CEROIQG...	..DEHVLKEGO	ELCKGD...
CYSR_CHICK	KCGVCKKAVI	F....AEELQ	CE.GSSA	K..	SCHICM..V	GKKNIDS...	..TTVAVHGE	ELCKSC...
CEF07C6-2	VGFDCKKVID	PQ..VEQSEF	TM.NKHA	T..	DHERCA..T	GARPFFG...	..HEHYEKNGK	ELCRRD...
CEZC247_3	ECACAAQPII	D...RYVFT	VL.GKON	Q..	SCLRCC..D	GRAPMSM...	..TCFSRDGL	ELCKTD...
DMMLP84B_1	KCPRCCKSVI	A...AEERL	AR.DNVE	L..	NCHKCG..M	GKNSIDS...	..TNCHEHERE	ELCKTC...
LRG1-YEAST	ICARCNKLVIT	P(4)TKITHK	AL.GKY	E..	SCHTCQ..D	GOKPKPK(4)	)QVDKTSESI	ELCQYD...
APTE-DROME-2	RCSRECLASTS	S...NELVMR	AR.NLVE	V..	NCHCOT..V	GHTHFKG...	..DOGGIIDL	ELCORTH...
CEB0496-8	RCAGCDELIF	A...NEVIF	AE.EKSA	F..	DHACAY..K	GDFRIGG...	..SRMIRDEN	ELGLDC...
OV14530_1	KCCRCGEGIT	N...IREGCT	AI.GEMA	V..	ACHTON..E	GKNCIAG...	..GSHYVNDGK	ELCEDD...
YK70_YEAST	PCRCAGLEVI	GKRMFSKKEN	EL.SGON	R..	ECBKCI..E	GKIKFNKH...	..VPCYILGDE	ELCQKH...
PINC-HUMAN	ICGACRRPIE	G....RYVIN	AM.GKON	V..	EBHVOA..K	GCKPFLG...	..HRHYERKGL	ELCETH...
HS6011610=1	RCAGCTKTIT	A....GGIT	YR.DLEA	P..	KCLVOT..G	GOTPWOG...	..TTSPPGMKN	ELCVAC...
DMMLP84B=1	CCPRCGFAMV	A...AEQMI	SK.TRIA	K..	RCHYCS..D	GKRSIDST...	..NLNDGPDGD	ELCRAC...
CEZC64-4	KGASCDRTIQ	A...IDWVRR	AR.NVVE	L..	ACHSON..Q	GKRCSTG...	..EBHALQEGN	ELCKQH...
HS2457/86	ACASGQSQIP	A...SELVMR	AO.GNVE	L..	KCHTCS..T	GRNRVPG...	..DRHYINGS	ELCEHD...
RHM2-HUMAN-2	LCASCDKRI	A...YEMVMR	VK.DKVE	L..	ECBKCA..A	GOKHFCVG...	..DRMLINS	ELCEQD...
CEU72211_1	QCCLCTFAMV	D...KELSV	VD.GKY	N..	KCLRCQ..M	GDIFFEYS...	..DKCYVRDGV	ELCRAD...
RIL_RAT	ECTRCGHGIV	G....TIVK	AR.DKIL	P..	ECBKCS..D	GGLNKQ...	..RCYFFLDER	ELCENH...
LRG1_YEAST	SCAGCNKYHO	E....ECHO	FY.EHRA	I..	ACHTCS..S	GKKNINPR(4)	)PTENKEKKK	ELCSHC...
CEU73946_1	VCAGCRLEIS	D...RYPLRV	NP.NLEF	A..	DCLKOV..O	GSRFDEN...	..QTAFFVKNGO	ELCRRD...
OTIS11LMRD_1	MOVCGSQNH	D...QYILRV	AP.DLEA	A..	ACHKOA..E	GSOYDET...	..CTCFVRDGK	ELCKRD...
SPAC29A4_11	VCFRGQAFQ	R...REIPE	FG.GHMA	K..	DCECCT..K	GDKGLEHSD	QMLVQTS	ELCGR...
HS295361=1	HCVKCNKAIT	S....CGIT	YQ.DQFA	A..	DCEVCV..T	GSKKIAG...	..ARHTAVEDO	ELCVDC...
CEF42G4_5	KCTACNRAS	D....KILR	AC.GGVV	V..	NCHVCF..S	GKKSIDGI...	..PFTLDKNN	ELCVPC...
CEK03E6-4	LCSGCGCLIK	D(5)LDKSN	VM.EDSA	E..	SCLRCS..C	GQLSISF...	..KKCFSRHGN	ELCEHD...
CEF42G4-5	RCALCSKPIV	P(5)ESVRIV	AM.DKSE	V..	DGYKCE..D	GEMQLSSK(8)	)FLITVIADS	ELCRKV...
DDU83086_1	NCKRCKLEIF	G...NILEN	HL.QETI	P..	ECBKCS..N	GESSIVDP...	..YFTEPSTNK	ELCAKC...
CEC09G9_8	RCNKKSKPII	S....DCIN	AL.QKKA	P..	TCHTCA..H	GOKPFGN...	..SAHYLEQGL	ELCEOD...
PAXI-HUMAN	LCSCGCKPII	G....RCIT	AM.AKKE	P..	EBHVOA..F	GLKQINK...	..GTEKEQNDK	ELCQNC...
CEF09B9+2	KCYECEKPIA	G....KVIO	AG.GYKE	P..	TCARCS..R	GSHFGDG...	..EEMYMGGDE	ELHPS...
CEF07C6+2	VCAGKNEFVI	G....QVH	SS.NNSE	L..	ACHTCD..E	GNVHINS...	..QIAYRYQGT	ELCFLC...
YP96_CAEEL	KCGGCHRAIT	D....RCUS	VM.NKIE	I..	ECBTCA..E	GNOPFGE...	..DCHHEKNGO	ELCKRD...
SCL9481_16	LCVRCKNSIA	S...SGVYE	LE.SKKA	D..	CTCTCY..K	GDKKINAD...	..SDFVLIDIGT	ELCYDC...
TES2+MOUSE	VCQGCNNAID	P...EVORIT	YN.NFSA	AS	TECHLCS..C	GSKQIEG...	..OKEMFVEGM	ELCSVE...
Consensus/60t	pCs.CscsIh	s....bhip	tb.sp.aH...	pCfPcs..p	CppsLss...	p.ab.pcsp	baCpps...	

Explanation of colour codes used by CHROMA

Group name	Amino acids	Displayed as
Default	X	.
Single	X	-
Alanine	A	A
Cysteine	C	C
Aspartic Acid	D	D
Glutamic Acid	E	E
Phenylalanine	F	F
Glycine	G	G
Histidine	H	H
Isoleucine	I	I
Lysine	K	K
Leucine	L	L
Methionine	M	M
Asparagine	N	N
Proline	P	P
Glutamine	Q	Q
Arginine	R	R
Serine	S	S
Threonine	T	T
Valine	V	V
Tryptophan	W	W
Tyrosine	Y	Y
Negative	D,E	-
Ser/Thr	S,T	*
Aliphatic	I,L,V	I
Positive	H,K,R	+
Tiny	A,G,S	t
Aromatic	F,H,W,Y	a
Charged	D,E,H,K,R	c
Small	A,C,D,G,N,P,S,T,V	s
Polar	C,D,E,H,K,N,Q,R,S,T	p
Big	E,F,H,I,K,L,M,Q,R,W,Y	b
Hydrophobic	A,C,F,G,H,I,L,M,T,V,W,Y	h

Alignment D

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AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 MEPPAARVTPSIKADCSHVNIIICEETVLHSLVSHLSAALRREGISVFVDACGLQETKFF 60

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 SIKONQPLTDGARVLVVVISDEVEFYDFWFPKFLKVIQGWQNNNGHVVPVIFYGVDSLTRY 120

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 YGILSNNVLTDSSELVEEIVRDVYGKLYPAERVGIIYARLLEIEKLLYKQHRDIRSIGIWM 180

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 PGIGKTLAKAVFNHMTDIDASCFTENFDEAFHKEGLHRLMERIGKILKDEFDISSY 240

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 IMRPTLHRDELYDKRIILVVLDDVRDSLAAESFLKRLDWFGSGSLIIEITSVDKQVFAFCQI 300

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 NQIYTVQGLNVHEALQLFSQSVFGINEPEQNDRKLSMKVIDYVNGNPLALSIYGRELMGX 360

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 KSEMETAFFELKHCPPLKIQDVLKNAYSALSQNEKNIVLDIAFFFKGETVNYVMQLLEES 420

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----

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AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 HYFPRLAIDVLVDKCVLTISENTVQMNLIQDTCQEIFNGEIECTCTRMWEPSTRIRYILEY 480

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 DELEGSGETKAMPKSGLVAEHIESIFLDTSNVKFDVKHDAFKNMFNLKFLKIYNSSCKYI 540

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 SGLNFPKGLDSLPEYELRLHWHENYPLQSLPQDFDFGHLVKLSMPYSQLHKLGRVKDLVM 600

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 LKRLILSHSLQLVECDILIYAQNIELIDLQGGCTGLQRFDPDTSQLQNLRVVNLSGCTEIKC 660

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 FSGVPPNIEELHLOGTRIREIPIFNATHPPKVKLDRKKLWNLLENFSDVEHIDLECVTNL 720

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 ATVTSNNEVMGKLVCLNMKYCSNLRGLPDMVSLESKAVLYLSGCSELEKIMGFPRNLKEL 780

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----
 AT5G66630 -----
 AT5G66610 -----
 AT5G66640 -----
 AT5G17890 YVGGTAIRELPQLPNSLEFLNAHGCKHLKSINLDFEQLPRHFIFSNCYRFSSQVIAEFVE 840

AT1G19270 -----
 AT4G36860 -----
 AT2G39830 -----
 AT5G66620 -----MASDYYSDDDEGFGE 15
 AT5G66630 -----MPISDVASLVGGAAL 15
 AT5G66610 -----MWCLS---CFKP 9

AT5G66640 -----
 AT5G17890 KGLVASLARAQEEELIKAPEVIICIPMDTRQRSSFRLOAGRNAMTDLVPWMQKPISGFSM 900

AT1G19270 -----MGWFNKIFKGSNQRLRVGNKHNHNVYDNYPTASHDDDEPSAAD 44
 AT4G36860 -----DDDDDEDEDEEYMRAQ 16
 AT2G39830 -----
 AT5G66620 KVGGLIGCKDRFEATIIHVIEVSOHEADIOKAKORSLATHEAEKLDLATHEAEQLDLAIQE 75
 AT5G66630 GAPLSEIFKLVIEEAKKVKDFKPLSODLASTMERLVPIFNEIDMMQGSNRGTSELKVL 75
 AT5G66610 STKHDPSEDREFEETNIVTIGISLYEDVILRQR-----SEADQIEWAIQD 54
 AT5G66640 -----
 AT5G17890 SVVVSFQDDYHNDVGLRIRCVGINKTWNQDRIVERFFQCNAPTEAPKVVADHIFVLYD 960

AT1G19270 TDAADNDEPHHTQEPSTSEDNTS-NDQENED----- 73
 AT4G36860 LEAAEEEEERRVAQAQIEEEEKRRAEAQLEE----- 46
 AT2G39830 -----NMVFLPPSSLDLDRSRGARDKEE----- 23
 AT5G66620 FSRQEEEEERRTRELENDQAIVLQHEERERLIN--KKTALDEDEDELLARTILESLK 133
 AT5G66630 ETHERAGEMVHKCSRIQWYSIAKKALYTREIKAINQDFLKFCQIELQLIQRNQLQYMRS 135
 AT5G66610 SFNPQE---TSRCRQREDDQIARGLOVVEETELD---KSVVDEE----- 93
 AT5G66640 -----
 AT5G17890 TKMHPDSSEINHISMWAHEVKKFEFHIVSGENNPGLGASCKVTECGVEVITAAITGDTSVSGI 1020

AT1G19270 -----IDRAIALS 81
 AT4G36860 -----TEKLLAKA 54
 AT2G39830 -----LDRSISLS 31
 AT5G66620 ENNRKMEFEQVVKDEQLALIVQESLNMEEYPIRLEEYKSISRRAPLDVDEQFAKAVKES 193
 AT5G66630 MGMAVSSTKADLLSDIGNEFSKLCLVAQPEVVTKFWLKRPLMLKMLFEDGVTVVVSA 195
 AT5G66610 -----DQQLSKIVEES 104
 AT5G66640 -----
 AT5G17890 IRESETITIIIEKEDIIIDEEDTPLLRSKPEETNRSRSSSELQKLSSTSSKPKNLSRSRR 1080

AT1G19270 LLEENQEQTISISGKYSMPVDEDEQLARALQES----- 113
 AT4G36860 RLEEEEMRRSK-----AQLEEDELLAKALQES----- 81
 AT2G39830 LADNTRKPHGYG---WSMNNRDFPRPFHGG----- 59
 AT5G66620 LKNKKGK---KQFEDEQVKKDEQLALIVQES----- 221
 AT5G66630 PYALGKTLVTKLCHDADVKEKFKQIFFISVSKFPNVLIGHKLEHIGCKANEYENDLD 255
 AT5G66610 LKEKGKS---KQFEDDQVENDEQQALMVQES----- 132
 AT5G66640 -----MVRKRQEEDEKIEIERVKEES----- 22
 AT5G17890 TTALAEALAEALKEREKLEDTRLOLALIESKK----- 1113

AT1G19270 --MVVGNsprhKSGSTYDNGNAYGAGDLYGNCHMY-----G 147
 AT4G36860 --MNVGSPPR----- 89
 AT2G39830 --LNPSSFP----- 67
 AT5G66620 --LNMVESPPRLEENNNISTRAP---VDEDE----- 247
 AT5G66630 AMLYIQQLKQLGRNGSILVLDDVWAEES----- 286
 AT5G66610 --LYMVLSAQLEEDKNISTIPP---LNEDA----- 159
 AT5G66640 --LKLAKQAEKRRLEESKEQKRIQVDDD----- 50
 AT5G17890 --IKKIKQADERDQIKHADEREQKHSKDHEEEIESNEKEERRHSKDYVIEELVLKKGK 1171

AT1G19270 GGNVYANGDIYPRPIT-----FOM 167
 AT4G36860 ----YDPGNILQYPFL-----IPS 105
 AT2G39830 ----PYEPSYQ-----YRR 77
 AT5G66620 QLAKAVEESLKGKGQIK-----QSKDEVEGDGML---LELNP 281
 AT5G66630 LLQKFLIQLPDYKILVTSRFEFTSFGPTFHLKPLIDVEVECRDEIEENEKLP---EVNP 342
 AT5G66610 QLQKVIWESAKGKGQIE-----HFKDPVEEDGNLPRVDLNVNH 196
 AT5G66640 ---QLAKTISKDKGQIN-----HSKDVVEE-----DVNP 76
 AT5G17890 RKKQLDDDKADEKEQIK-----HSKDVVEE-----EVNP 1200

AT1G19270 DFRICAGCNMEIGHGRFLNCLNSLWHPECFRYGCSPPISEYEFSTSGNYPFHKACYPER 227
 AT4G36860 SHRICVGCQAEIGHGRFLSCMGVWHPECFCNACDKPIIDYEFMSGNRPVHKLCYKEQ 165
 AT2G39830 RQRICGGCNSDIGSGNYLGCMTFFHPECFRCHSCGYAITEHEFSLSGTKPYHKLCFKEL 137
 AT5G66620 PPSLCGGCNSFAVEHGGSVNIGVLWHPGCFCCRACHKPIAIHDHNVNSNRGKFKHSCY 341
 AT5G66630 PLSMCGGCNSAVKHESVNLGLVWHPGCFCCRSCDKPIAHELENHVSNSRGKFKHSCY 402
 AT5G66610 PHSICDGCKSAIEYGRSVHALGVNWHPECFCRCRYCDKPIAMH----- 238
 AT5G66640 PPS-SIDGKSEIGDGTSVN-----PRCLCFHCHRPVFMHEILKK-GKFHIDCYKEYY 127
 AT5G17890 PLSKCKDKCSAIEDGISINAYGSVWHQCFCCLCRCREPIAMNEISDLRGMYHKPCYKELR 1260

AT1G19270 YHPKCDVCSHFIPITNHAAGLIEYRAHPFWQKYCPSHEHDATPRCCSCERMEPRNTRYVEL 287
 AT4G36860 HHFKCDVCHNFIPITNPAGLIEYRAHPFWQKYCPSHERDGTFRCCSCERMEPKDTKYLIL 225
 AT2G39830 THFKCEVCHHFIPITNDAGLIEYRCHFPFWQKYCPSHEYDKTARCCSCERLESWDVRYTYL 197
 AT5G66620 -ERYCYVCKEKK-----MKTYNHHPFWEEERYCPVHEADGTPKCCSCERLEPRESNYVML 394
 AT5G66630 -ERYCYVCKEKK-----MKTYNHHPFWEEERYCPVHEADGTPKCCSCERLEPRGTYKYL 455
 AT5G66610 -----EYKEHPPFWKEKYCPHEVDGTPKCCSCERLEPWGTYKYL 278
 AT5G66640 RNPNCYVCCQKIPVNAEGIRKFSHEHFWKEKYCPIHDEEDGTAKCCSCERLEPRGTYNYVML 187
 AT5G17890 -HPNCYVCEKKIPRTAEG-L-KYHEHFFWMETYCPSHDGDGTPKCCSCERLEHCGTYVML 1318
 : **** : *** * : * * :*****: * * *

AT1G19270 NDGRKLCLECLDSAVMDTMCCQPLYLQIQNFYEGLNMKVEQEVPLLLVERQALNEAREGE 347
 AT4G36860 DDGRKLCLECLDSAIMDTHCCQPLYLEIREFYEGHMKVEQQIPMLLVERSALNEAMEGE 285
 AT2G39830 EDGRSLCLECMETAITDTGECQPLYHAIRDYEGMYMKLDQQIPMLLVQREALNDAIVGE 257
 AT5G66620 ADGRWLCLECMNSAVMDSDECQPLHDFMDRPFEGHMKIEKEFPFLVEKQALNKAKEE 454
 AT5G66630 SDGRWLCLECGKS-AMDSDECQPLYDFMDRPFESLNMKIEKEFPFLVRKELLNKKE--E 512
 AT5G66610 ADNRLWLCVCKMECAVMDTYECQPLHFEIREFFGSLNMKVEKEFPFLVEKEALKKAQAQ 338
 AT5G66640 GDFRWLCIECMGSAVMDTNEVQPLHFEIREFFEGFLKVDKEFALLVEKQALNKAEEEE 247
 AT5G17890 ADFRWLCRECMDSAIMDSDECQPLHFEIREFFEGHMKIEEEFPVYLVEKNALNKAKEE 1378
 * * * * : * * : **** : : : : : : : : * * : * * *

da1-1 (R/K)

AT1G19270 KNGHYHMPETRGLCLSEEQTVSTVRKRSKH-GTG-KWAGNITEPYKLTRQCEVTAILILF 405
 AT4G36860 KHGHHHLPETRGLCLSEEQTVTTVLRRPRI-GAGYKLIDMITEPCRLIRRCCEVTAILILY 344
 AT2G39830 KNGHYHMPETRGLCLSEEQTVSTVLRPRRL-GAH-RLVGMRTQPPRLTRKCEVTAILVLV 315
 AT5G66620 KIDYQYEVVTRGICLSEEQIVDSVSQRPVR-GPNKLVGMATESQKVTRCEVTAILILY 513
 AT5G66630 KIDNHYEVLIRAYCMSEQKIMTYVSEEPRT-GQNRQLIDMDTEPQGVVHECKVTAILILY 571
 AT5G66610 KIDNQHGVTGICLSEGQIVNSVFKKPTM-GPNGLVSLGTEPQKVVGCEVTAILILY 397
 AT5G66640 KIDYHRAAVTRGLCMSEEQIVPSIIGKPRMGPDNQLITDIVTESQRVS-GFEVTGILIIY 306
 AT5G17890 KIGDQCLMVVRGICLSEEQIVTSVSQVRR-MLNKQILDVTVESQVRVKECEVTAILILY 1437
 * . * : * * : : : . * : : : : : : : : * * : * * *

AT1G19270 GLPRLLTGSILAHMMHAWMLRKGFTLSQDVEEGICQVMAHKWLDLAAGSTNSNAAS 465
 AT4G36860 GLPRLLTGSILAHMMHAWLRNGYPNLRPEVEEGICQVLAMWLESETYAGSTLVDIAS 404
 AT2G39830 GLPRLLTGAILAHMLHGWLPRLNGFRNLNPEVEEGICQVLSYMWLESEVLSDPSTPNLPS 375
 AT5G66620 GLPRLLTGYILAHMMHAYLRLNGHNRNLNNILEEGICQVLGHLWLDSTYATADATADAS 573
 AT5G66630 GLPRLLTGYILAHMMHAWLRNGHNMNLNNILEEGICQVLGHLWLESQTYATADTADAA 631
 AT5G66610 GLPRLLTGYILAHMMHAWLRNGYRNKLLEEGICQVLGHMWLESQTYYS----SSAAA 453
 AT5G66640 GLPRLLTGYILAHMMHAWLRNGYRNKLLEEGICQVLAALGLRWLESQTFASTDAADAAAA 366
 AT5G17890 GLPRLLTGYILAHMMHAYLRLNGYRNLMVLEEGICQVLGYMWLECCQTYVFD----TAT 1493
 ***** : * * : * * : * * : * * : * * : * * : * * : * * : * * : * * : *

AT1G19270 SSSS-----SQGLKKGP-RSQYERKLGEFFKHQIESDASPVYGDGFRAGRLAVH 513
 AT4G36860 SSSSA-----VV---SASSKKEG-RSDFEKKLGEFFKHQIESDSSAYGDGFRQGNQAVL 455
 AT2G39830 TSSVA-----TSSSSSFSNKKGG-KSNVEKKLGEFFKHQIAHDASPAYGGGFRAANAAAC 429
 AT5G66620 SSASS---SSRTPPAASASKKGE-WSDFDKKLVEFCRNQIETDDSPVYGLGFRTVNEMVT 629
 AT5G66630 SASSS---SSRTPPAASASKKGE-WSDFDKKLVEFCRNQIETDESPPVYGLGFRTVNEMVT 687
 AT5G66610 SSASS---SSRTP-AANASKKGA-QSDYEKKLVEFCRDQIETDDSPVYGVGFRKVNQMVS 508
 AT5G66640 VASSSSFSSTAPPAAITSKKSDWSIFEKKLVEFCMNQIETDDSPVYGLGFKQVYEMMV 426
 AT5G17890 IASSS---SSRTPPLSTTSKKVD-PSDFEKLRLVNFCKHQIETDESPPFGDGRKVNKMA 1550
 : : : * * * : : * * : * * : * * : * * : * * : * * : * * : * * : *

AT1G19270 KY--GLRKTLEHIQMTGRFPV---- 532
 AT4G36860 KH--GLRRTLDHIRTGTGTFPKWI-- 476
 AT2G39830 KY--GLRRTLDHIRTGTGTFPL---- 448
 AT5G66620 NS--SLQETLKEILRQR----- 644
 AT5G66630 NS--SLQETLKEILRRR----- 702
 AT5G66610 DS--SLHKILKSIQHWTKPDNL-- 529
 AT5G66640 SNNYNIKDTLKDIVSASNATPDSTV 451
 AT5G17890 SNNHSLKDTLKEIISISKTPQYSKL 1575

Alignment E

[illegible]

	(1601)	1601	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700
AtDA1.pro (378)	GTGK	WAG-	NITE	HYRL	TROGEV	TAIL	HYGLPR	-LITG	STSL	AHEM	MHAWMR	-----
AtDA1.pro (387)	GAGY	KIDM	ITEP	CR	TR	RE	EV	TAIL	HYGLPR	-LITG	STSL	AHEM
AtDA2.pro (367)	LGAR	HY	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR	-LITG
AtDA3.pro (277)	GPDN	Q	IT	DI	V	T	S	OR	V	G	F	E
AtDA4.pro (1447)	MLNK	Q	IT	DI	V	T	S	OR	V	G	F	E
AtDA5.pro (543)	GQNK	Q	IT	DI	V	T	S	OR	V	G	F	E
AtDA6.pro (485)	GFNN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
AtDA7.pro (400)	GFNG	E	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
BrDA1a.pro (378)	GTGN	WAGN	MITE	HYRL	TROGEV	TAIL	HYGLPR	-LITG	STSL	AHEM	MHAWMR	-----
BrDA1b.pro (359)	-KGN	WAGN	MITE	HYRL	TROGEV	TAIL	HYGLPR	-LITG	STSL	AHEM	MHAWMR	-----
BrDA1.pro (429)	GAGY	KIDM	ITEP	CR	TR	RE	EV	TAIL	HYGLPR	-LITG	STSL	AHEM
BrDA2.pro (352)	LGAR	HY	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR	-LITG
BrDA3-7.pro (351)	GFNN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
BdDA1.pro (355)	MTGN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
BdDA2.pro (364)	MAGN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
BdDA3.pro (346)	IGGN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
OsDA1.pro (329)	MAGN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
OsDA2.pro (338)	IGAN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
OsDA3.pro (356)	GFNN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
OsDA4.pro (982)	GFNN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA1.pro (247)	KSRN	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA2.pro (219)	SFPL	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA3.pro (319)	VFSR	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA4.pro (80)	GEYD	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA6.pro (262)	DEYD	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA5.pro (234)	SRFQ	K	Y	VG	MR	PO	RE	TR	KE	EV	TAIL	HYGLPR
PpDA7.pro (220)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
PpDA8.pro (278)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
SmDA1.pro (336)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
SmDA2.pro (305)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

	(1701)	1701	1710	1720	1730	1740	1750	1760	1770	1780		
AtDA1.pro (471)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----		
AtDA1.pro (480)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----		
AtDA2.pro (462)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----		
AtDA3.pro (372)	FSST	APPAA	ITSK	SD	DD	IF	FE	K	Q	I	E	D
AtDA4.pro (1539)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
AtDA5.pro (638)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
AtDA6.pro (579)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
AtDA7.pro (493)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BrDA1a.pro (471)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BrDA1b.pro (453)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BrDA1.pro (525)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BrDA2.pro (447)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BrDA3-7.pro (442)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BdDA1.pro (449)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BdDA2.pro (461)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
BdDA3.pro (441)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
OsDA1.pro (423)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
OsDA2.pro (433)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
OsDA3.pro (451)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
OsDA4.pro (1077)	S-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA1.pro (337)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA2.pro (309)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA3.pro (409)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA4.pro (165)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA6.pro (349)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA5.pro (324)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA7.pro (292)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
PpDA8.pro (363)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
SmDA1.pro (411)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
SmDA2.pro (379)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	

Identical

Conservative

Block of similar

Identical
 Conservative
 Block of similar

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of increasing the life-span, organ size and/or seed size of a plant compared to wild type plants comprising;
expressing a nucleic acid encoding a dominant-negative DA polypeptide within cells of said plant,
wherein the dominant-negative DA polypeptide comprises;
an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1; and,
a mutation of R to K at a position equivalent to position 358 of SEQ ID NO: 1; and
wherein the dominant-negative DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation; and
wherein the plants differ from wild type plants only in the expression of said nucleic acid encoding a dominant-negative DA polypeptide.
2. A method according to claim 1 comprising reducing or abolishing expression of a Big Brother (BB) polypeptide within cells of said plant compared to wild type plants which differ only in the expression of said polypeptide,
wherein the Big Brother (BB) polypeptide comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 9 and has E3 ubiquitin ligase activity.
3. A method of producing a plant with increased life-span, organ size and/or seed size compared to wild type plants comprising:
incorporating a heterologous nucleic acid which encodes a dominant-negative DA polypeptide into a plant cell by means of transformation, and;
regenerating the plant from one or more transformed cells,
wherein the dominant-negative DA polypeptide comprises;
an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1 and

a mutation of R to K at a position equivalent to position 358 of SEQ ID NO: 1; and

wherein the dominant-negative DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation; and

wherein the plant differs from wild type plants only in that it comprises said heterologous nucleic acid encoding a dominant-negative DA polypeptide.

4. A method according to claim 3 further comprising incorporating a heterologous nucleic acid which expresses a suppressor nucleic acid which reduces expression of a Big Brother (BB) polypeptide compared to wild type plants into said plant cell by means of transformation,

wherein the Big Brother (BB) polypeptide comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 9 and has E3 ubiquitin ligase activity.

5. A method according to any one of claims 1 to 4 wherein the nucleic acid encoding the dominant negative DA polypeptide and/or the suppressor nucleic acid is operably linked to a heterologous promoter.

6. A method according to claim 5 wherein the promoter is a tissue-specific promoter.

7. A method according to claim 5 wherein the promoter is an inducible promoter.

8. A method according to any one of claims 5 to 7 wherein the nucleic acid encoding the dominant negative DA polypeptide and/or the suppressor nucleic acid is comprised in one or more vectors.

9. A method according to any one of claims 1 to 8 wherein the plant is *Lithospermum erythrorhizon*.

10. A method according to any one of claims 1 to 8 wherein the plant is *Taxus* spp.

11. A method according to any one of claims 1 to 8 wherein the plant is an agricultural plant selected from the group consisting of tobacco, cucurbits, carrot, vegetable brassica, melons, capsicums, grape vines, lettuce, strawberry, oilseed brassica, sugar beet, wheat, barley, maize, rice, soyabeans, peas, sorghum, sunflower, tomato, potato, pepper, chrysanthemum, carnation, linseed, hemp and rye.

12. A plant cell expressing a heterologous nucleic acid encoding a dominant-negative DA polypeptide within its cells,
wherein the dominant-negative DA polypeptide comprises;

an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1 and

a mutation of R to K at a position equivalent to position 358 of SEQ ID NO: 1;

wherein the dominant-negative DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation.

13. A plant cell according to claim 12 wherein the plant cell has reduced or abolished expression of a Big Brother (BB) polypeptide compared to wild type plants which differ only in the expression of said polypeptide,

wherein the Big Brother (BB) polypeptide comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 9.

14. A method of increasing the life-span, organ size and/or seed size of a plant comprising;

reducing or abolishing the expression compared to wild type plants of two or more nucleic acids encoding DA polypeptides in one or more cells of the plant,

wherein said DA polypeptides comprise an amino acid sequence having at least 80% sequence identity to the full length of SEQ ID NO: 1;

wherein the DA polypeptides have the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation; and

wherein the plants differ from wild type plants only in the expression of said two or more nucleic acids encoding DA polypeptides.

15. A method of producing a plant with increased life-span, organ size and/or seed size compared to wild type plants comprising:

reducing or abolishing the expression of two or more nucleic acids encoding DA polypeptides in a plant cell compared to wild type plants, and;

regenerating the plant from the plant cell,

wherein said DA polypeptides comprise an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1 and have the same activity as the polypeptide of SEQ ID NO: 1,

wherein expression is reduced or abolished by mutating the two or more nucleic acid sequences in the plant cell and regenerating the plant from the mutated cell or

wherein expression is reduced or abolished by expressing two or more heterologous nucleic acids which suppress expression of said two or more nucleic acids in the plant cell; and

wherein the plants differ from wild type plants only in the expression of said two or more nucleic acids encoding DA polypeptides.

16. A method according to claim 14 or claim 15 wherein one or more DA polypeptides comprise an R residue at a position equivalent to position 358 of SEQ ID NO: 1.

17. A method of reducing the life-span, organ size and/or seed size of a plant compared to wild type plants comprising;

expressing a heterologous nucleic acid encoding a DA polypeptide within cells of said plant;

wherein the DA polypeptide comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1 and an R residue at a position equivalent to position 358 of SEQ ID NO: 1;

wherein the DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1; and

wherein the plants differ from wild type plants only in the expression of said heterologous nucleic acid encoding a DA polypeptide.

18. An isolated DA polypeptide comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO: 1; and

a mutation of R to K at a position equivalent to position 358 in SEQ ID NO:1;

wherein the DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation.

19. A plant cell comprising the DA polypeptide according to claim 18.

20. An isolated nucleic acid encoding a DA polypeptide according to claim 18.

21. A vector comprising the nucleic acid according to claim 20 operatively linked to a promoter.

22. The vector according to claim 21 further comprising nucleic acid sequences encoding at least one *eod* sequence,

wherein the *eod* sequence comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 9 with a mutation at a position corresponding to position 44 of SEQ ID NO:9;

wherein the *eod* sequence has the same activity as the polypeptide of SEQ ID NO: 9 with an A44T mutation.

23. A method of prolonging the growth period in a plant compared to wild type plants which comprises expressing *DA1^{R358K}* within said plant,

wherein the *DA1^{R358K}* comprises an amino acid sequence having at least 60% sequence identity to the full length of SEQ ID NO: 1, and an R to K mutation at a position equivalent to position 358 of SEQ ID NO: 1;

wherein the *DA1^{R358K}* has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation; and

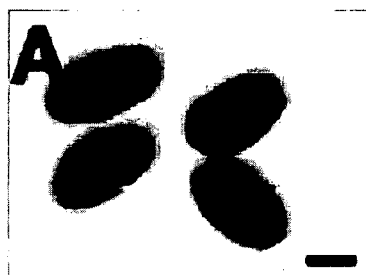
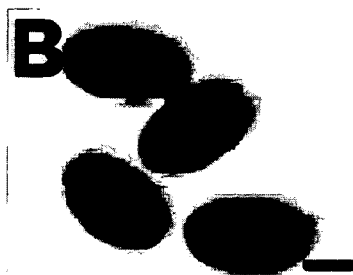
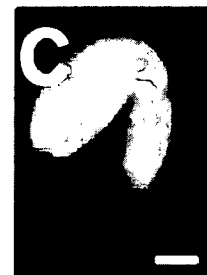
wherein the plants differ from wild type plants only in the expression of *DA1^{R358K}*.

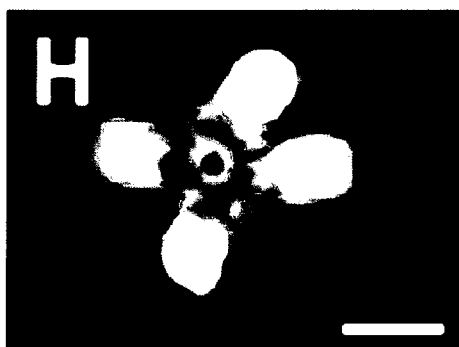
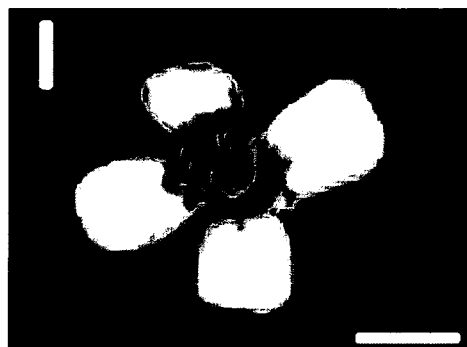
24. A method according to any one of claims 1 to 11 and 14 to 17 wherein the dominant-negative DA polypeptide comprises an amino acid sequence having at least 80% identity to the full length of one or more of SEQ ID NOS: 1 and 301 to 330; wherein the DA polypeptide has the same activity as the polypeptide of SEQ ID NO: 1 with an R358K mutation.

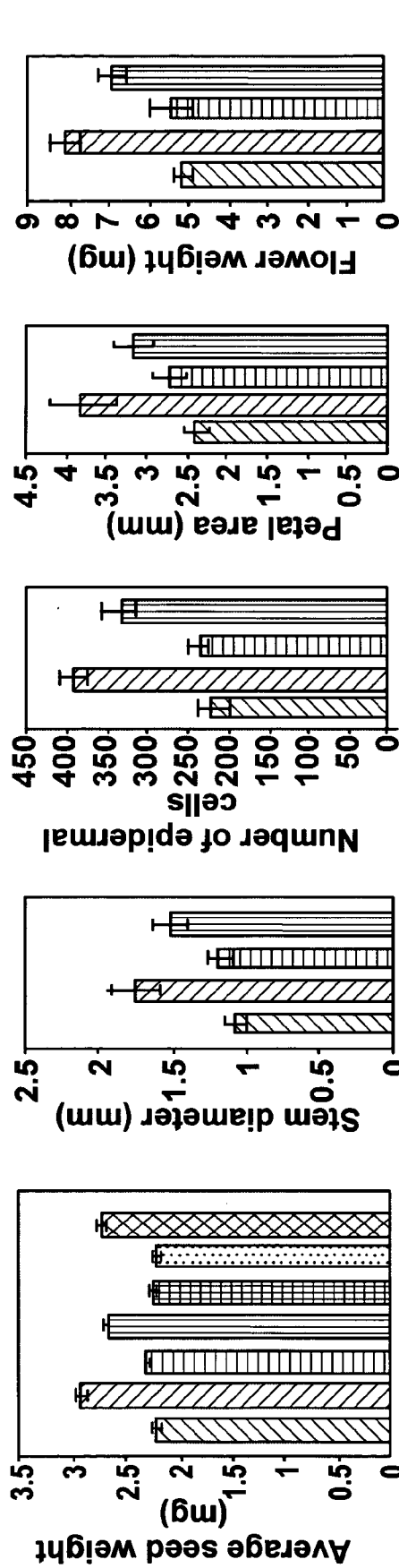
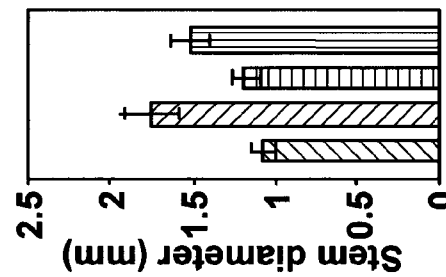
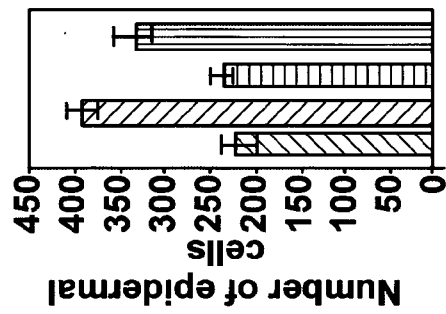
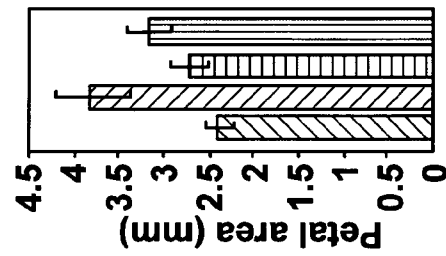
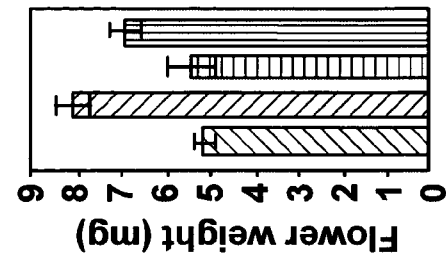
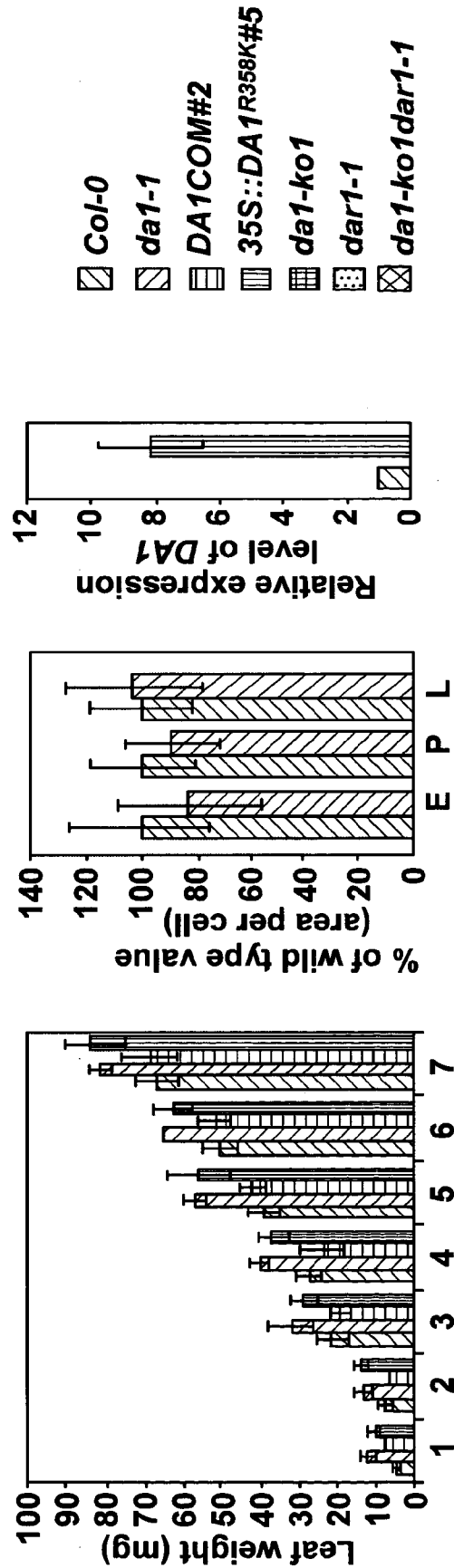
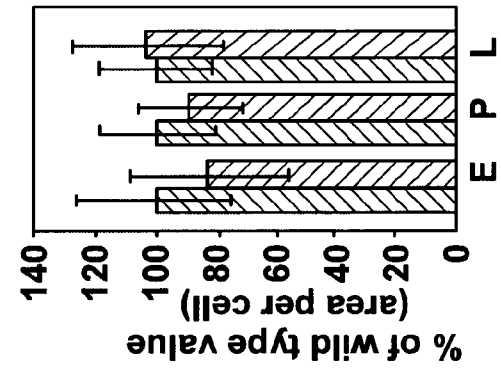
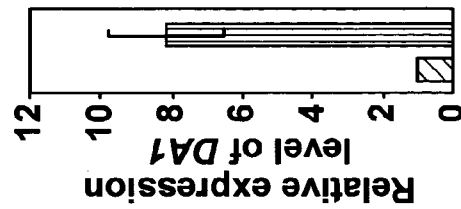
25. A method according to any one of claims 1 to 11 and 14 to 17 wherein the dominant-negative DA polypeptide comprises the amino acid sequence of one of SEQ ID NOS: 1 and 301 to 330.

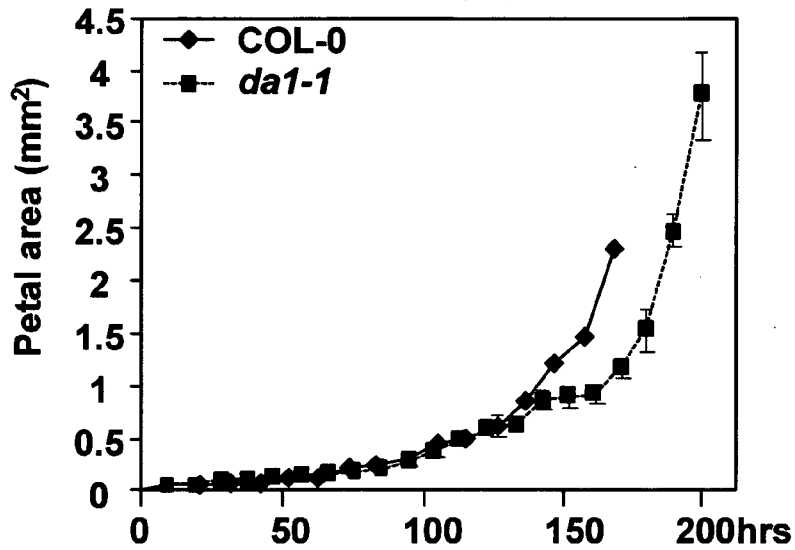
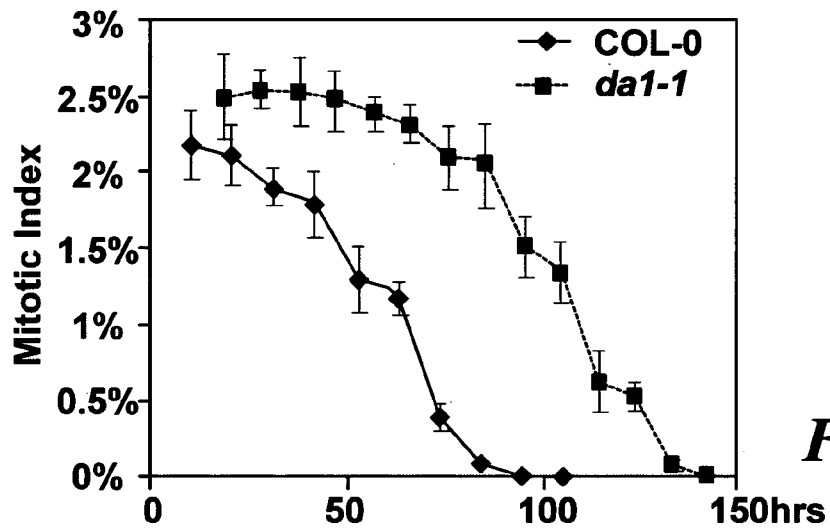
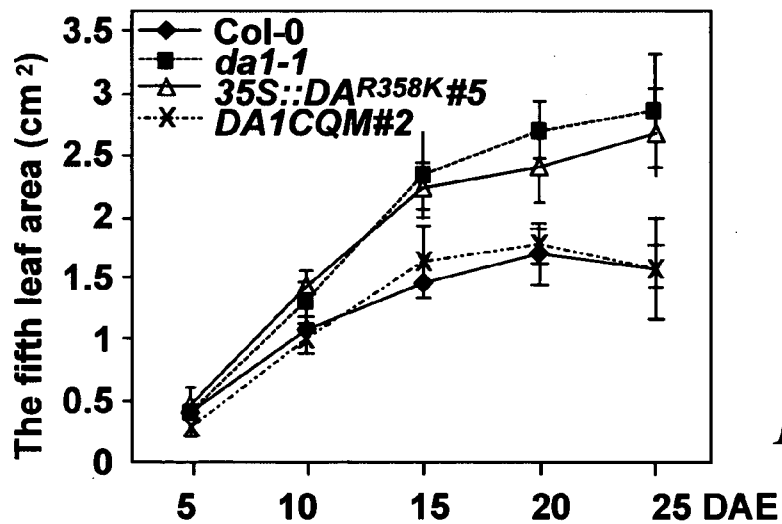
26. A plant cell according to claim 12 or claim 13 wherein the dominant-negative DA polypeptide comprises an amino acid sequence having at least 80% identity to the full length of one or more of SEQ ID NOS: 1 and 301 to 330.

27. A plant cell according to claim 12 or claim 13 wherein the dominant-negative DA polypeptide comprises the amino acid sequence of one of SEQ ID NOS: 1 and 301 to 330.

*Fig. 1A**Fig. 1B**Fig. 1C**Fig. 1D**Fig. 1E**Fig. 1F**Fig. 1G*

*Fig. 1H**Fig. 1I**Fig. 1J**Fig. 1K*

*Fig. 1L**Fig. 1M**Fig. 1N**Fig. 1O**Fig. 1P**Fig. 1Q**Fig. 1R**Fig. 1S*

*Fig. 2A**Fig. 2B**Fig. 2C*

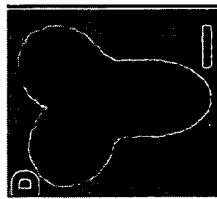
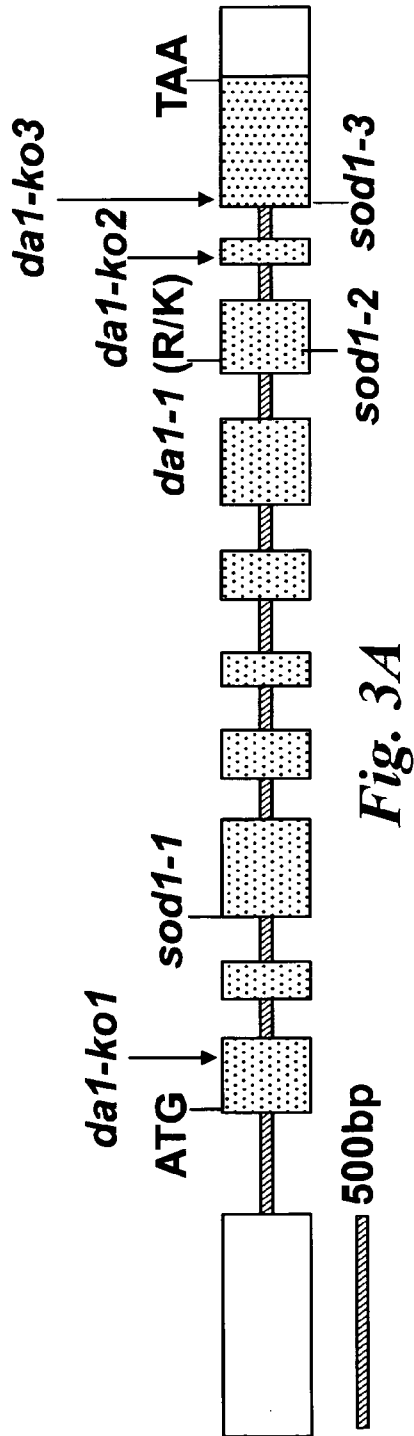


Fig. 3D



Fig. 3C



Fig. 3B

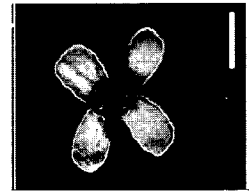


Fig. 3H



Fig. 3G



Fig. 3F



Fig. 3E

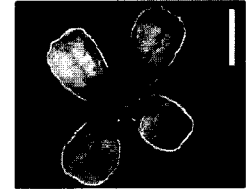


Fig. 3I

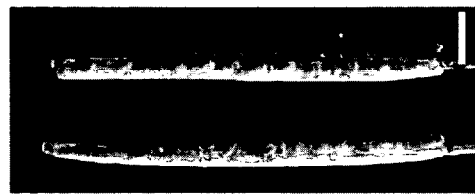


Fig. 3J

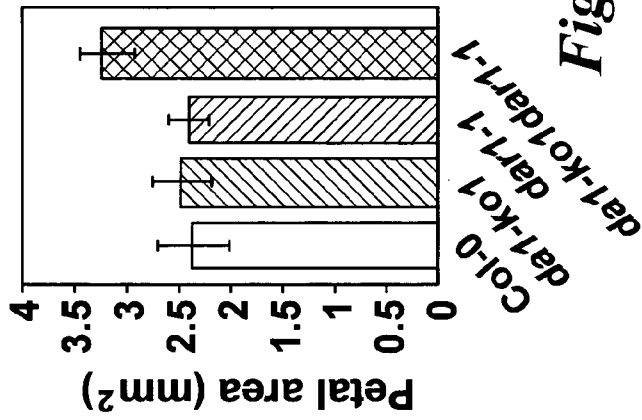


Fig. 3K

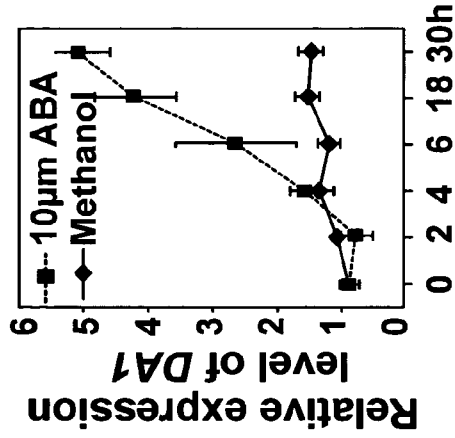


Fig. 3L



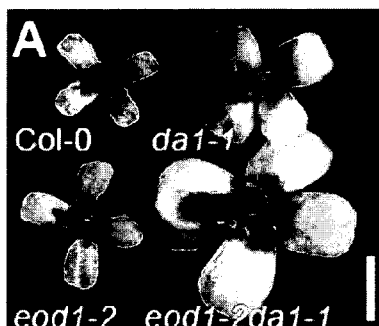
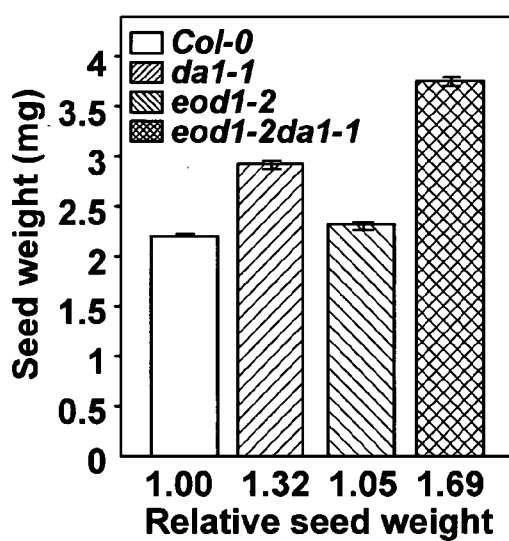
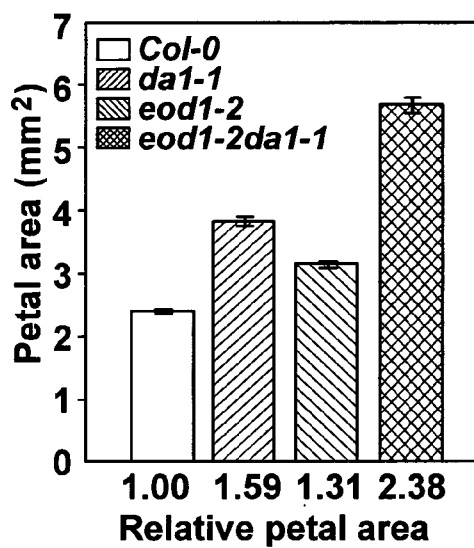
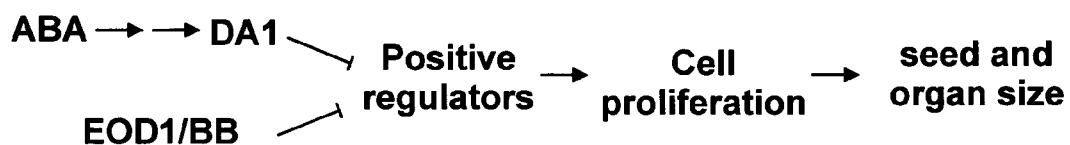
Fig. 3M



Fig. 3N



Fig. 3O

*Fig. 4A**Fig. 4B**Fig. 4C**Fig. 4D**Fig. 4E*

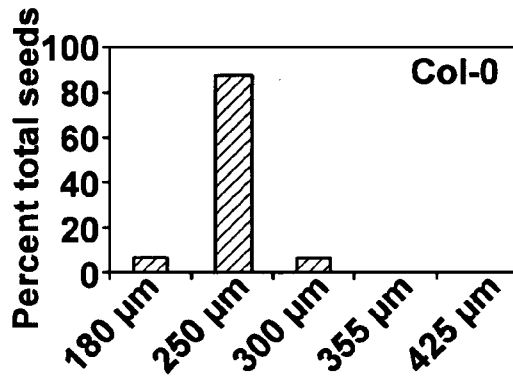
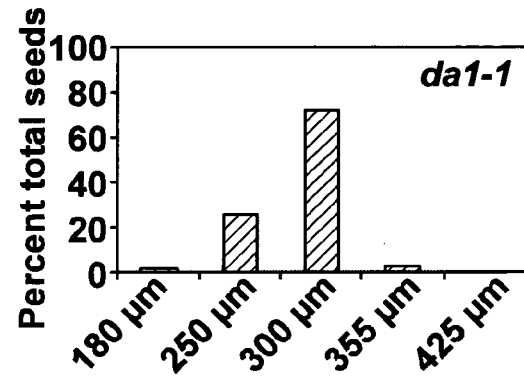
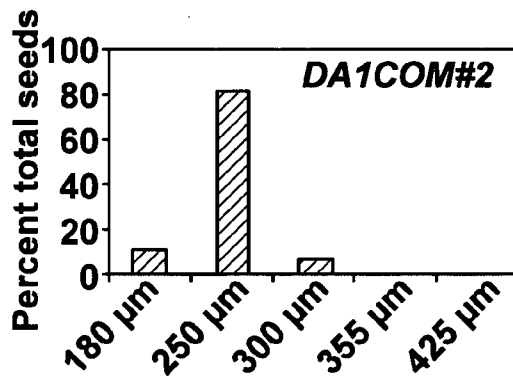
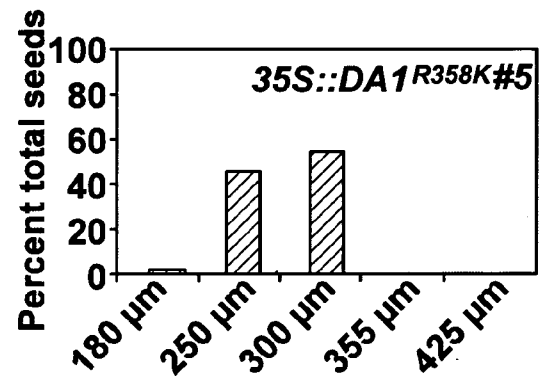
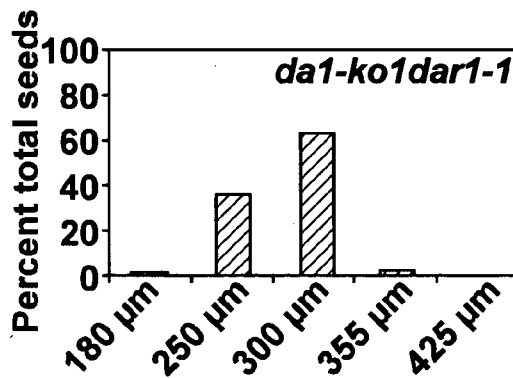
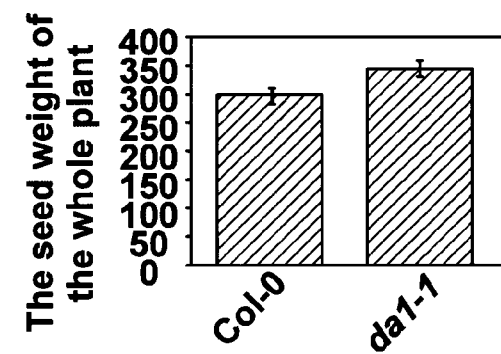
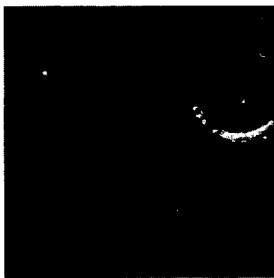
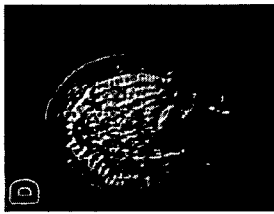
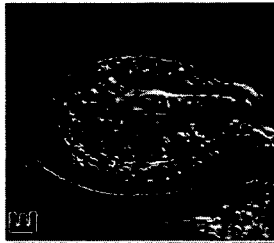
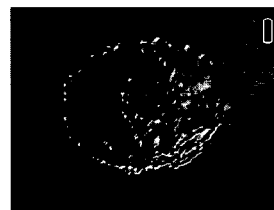
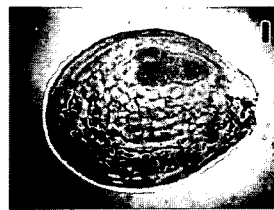
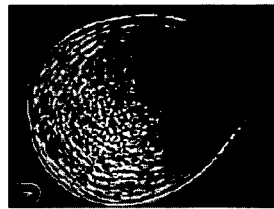
*Fig. 5A**Fig. 5B**Fig. 5C**Fig. 5D**Fig. 5E**Fig. 5F*

*Fig. 6A**Fig. 6B**Fig. 6C**Fig. 6D**Fig. 6E**Fig. 6F**Fig. 6G**Fig. 6H**Fig. 6I**Fig. 6J**Fig. 6K**Fig. 6L*

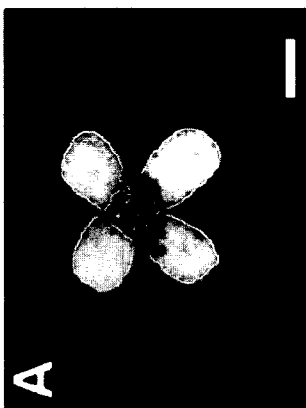


Fig. 7A



Fig. 7B

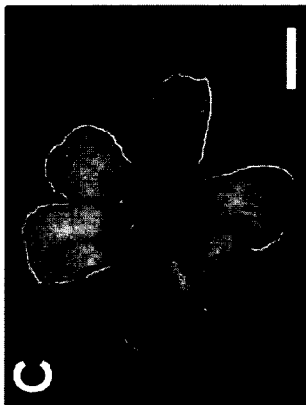


Fig. 7C



Fig. 7D

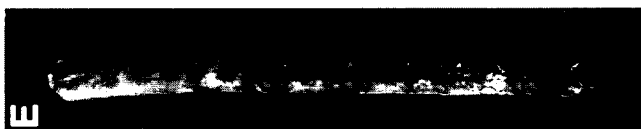


Fig. 7E



Fig. 7F

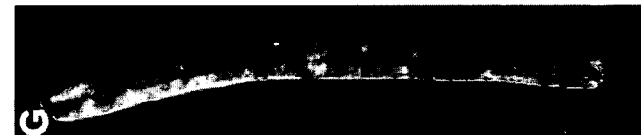
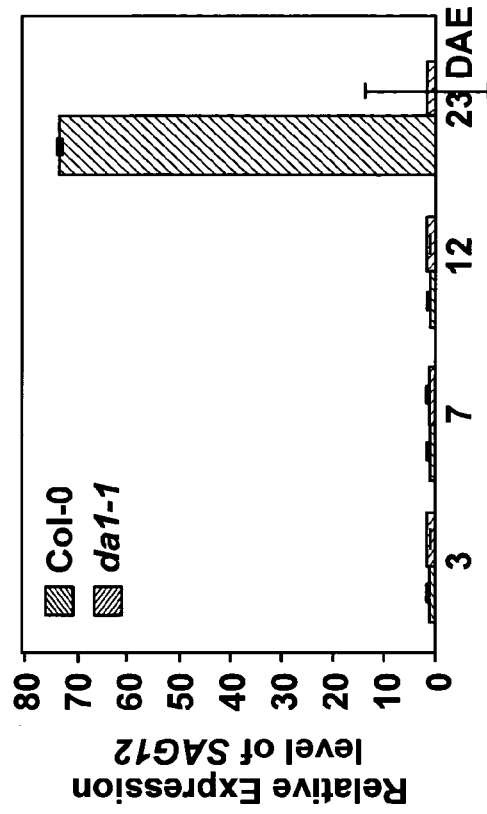
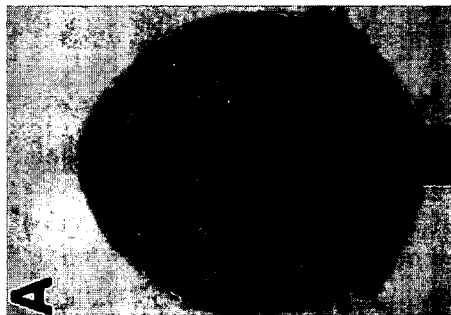


Fig. 7G

*Fig. 8C**Fig. 8B**Fig. 8A*

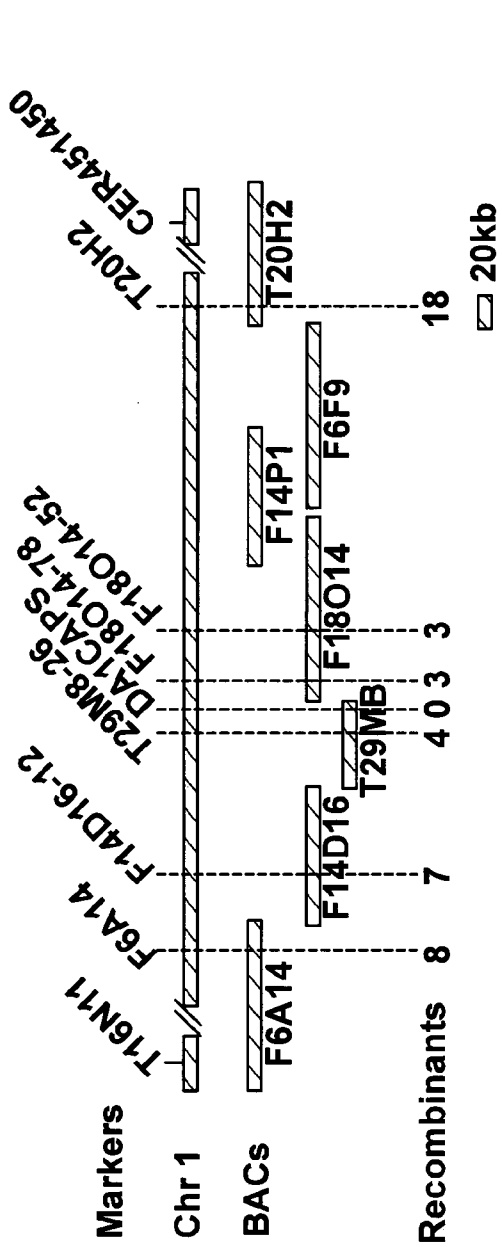


Fig. 9A

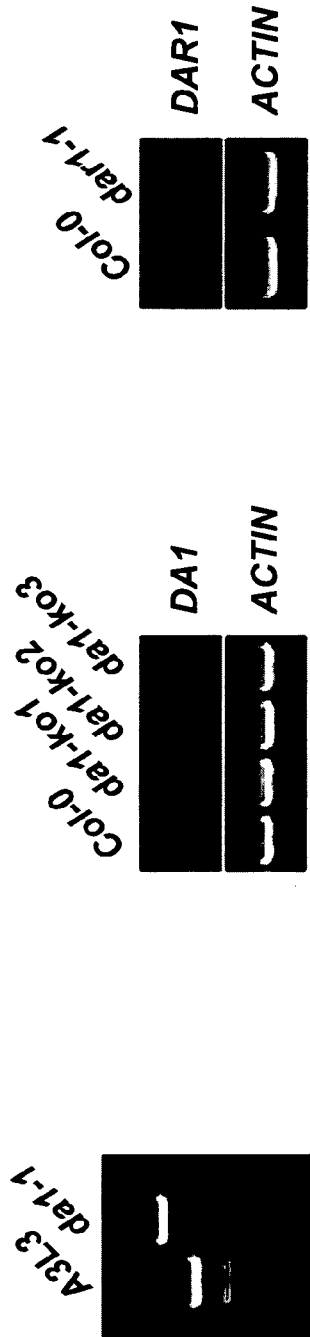
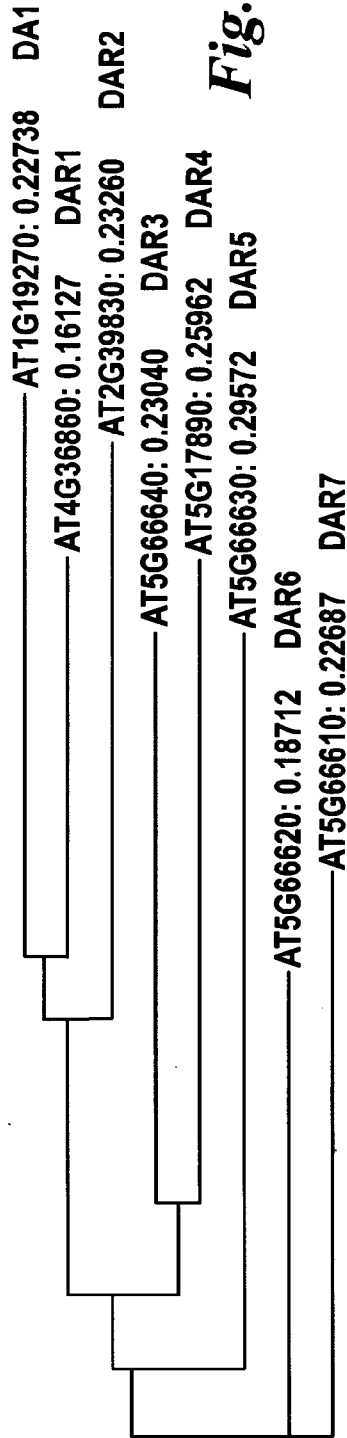
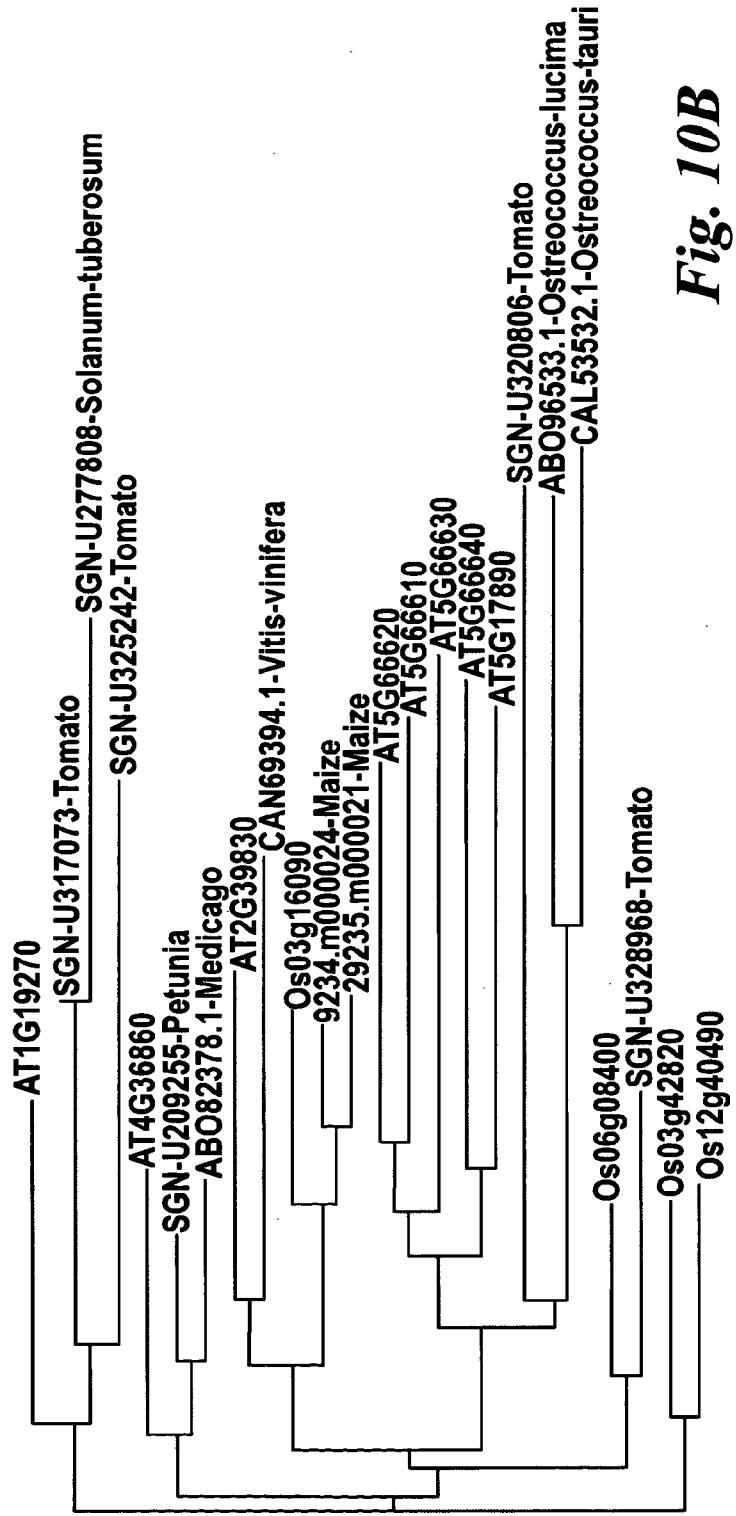
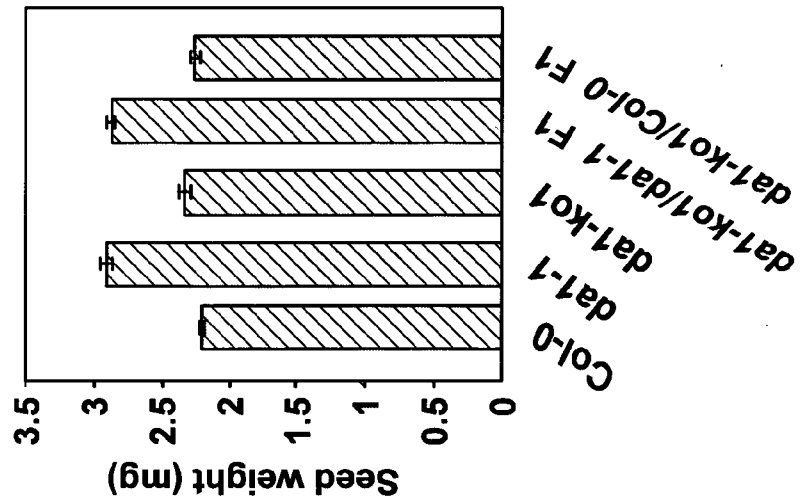
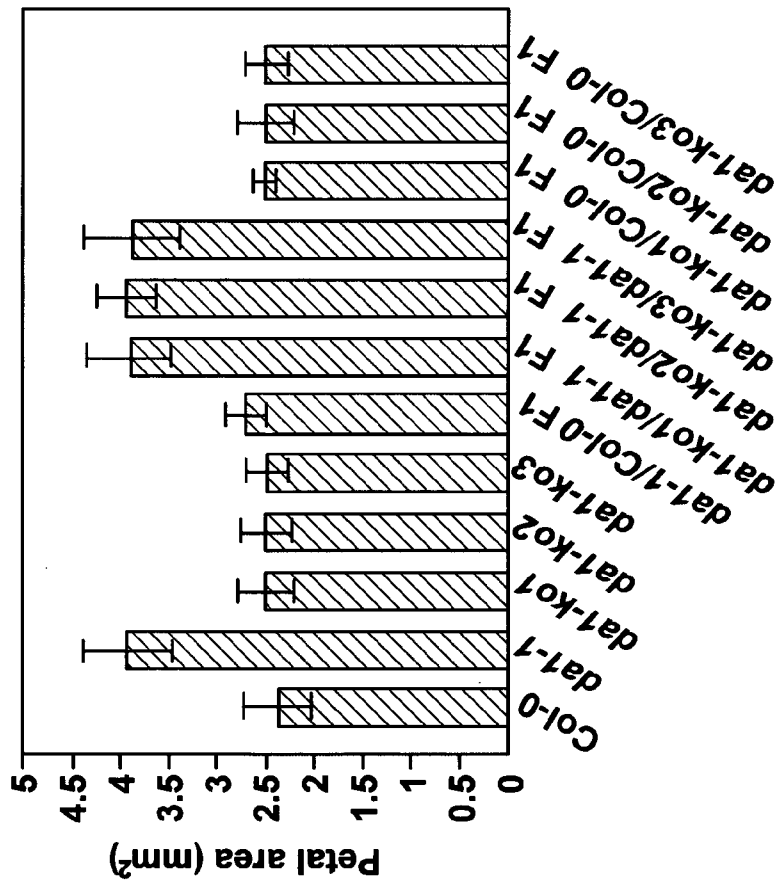


Fig. 9B

Fig. 9C

Fig. 9D

**Fig. 10A****Fig. 10B**

*Fig. 11B**Fig. 11A*

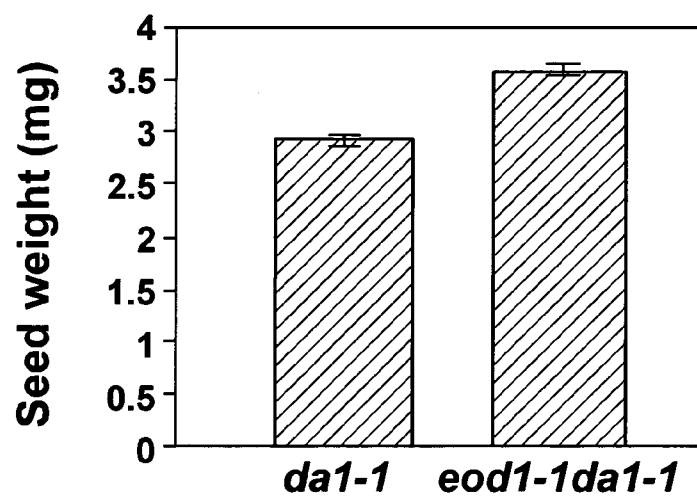
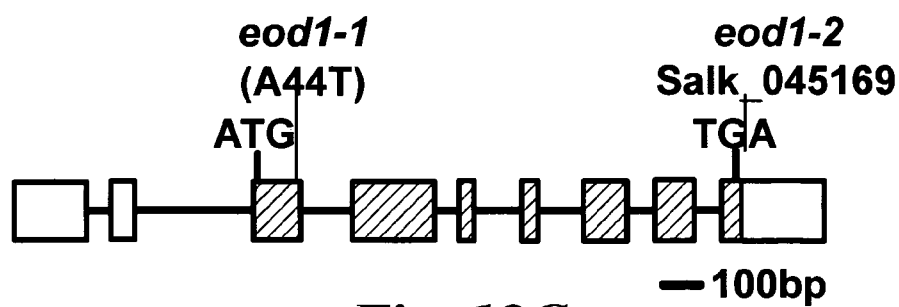
*Fig. 12A**Fig. 12B**Fig. 12C*



Fig. 12D

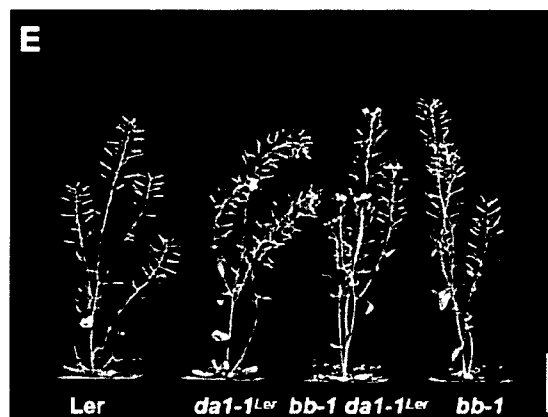


Fig. 12E

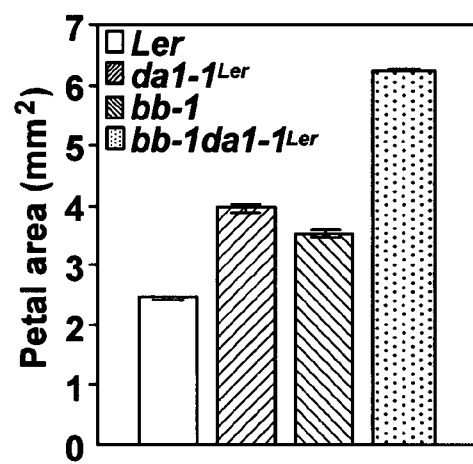
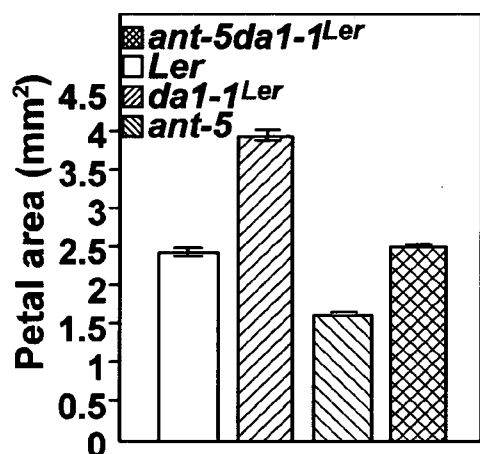
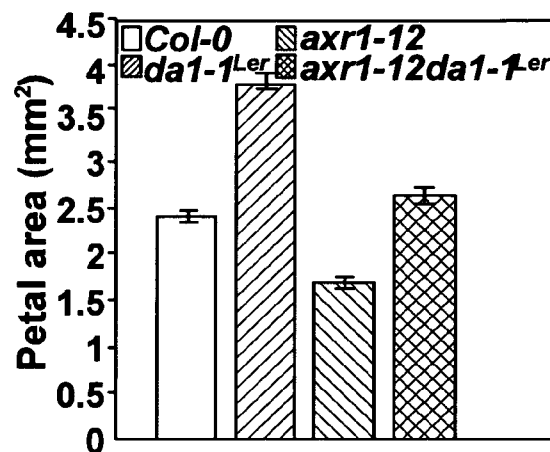
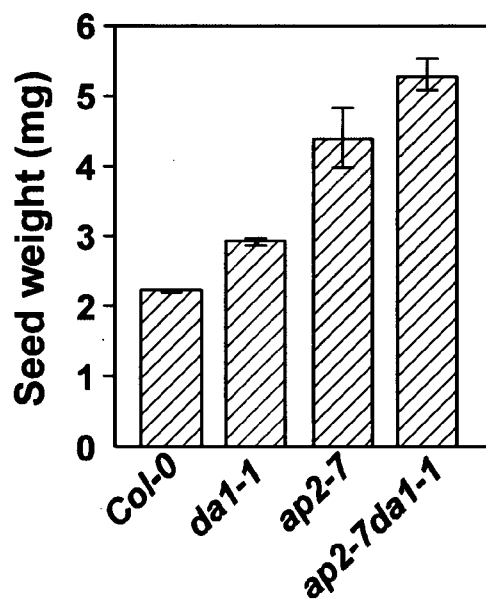
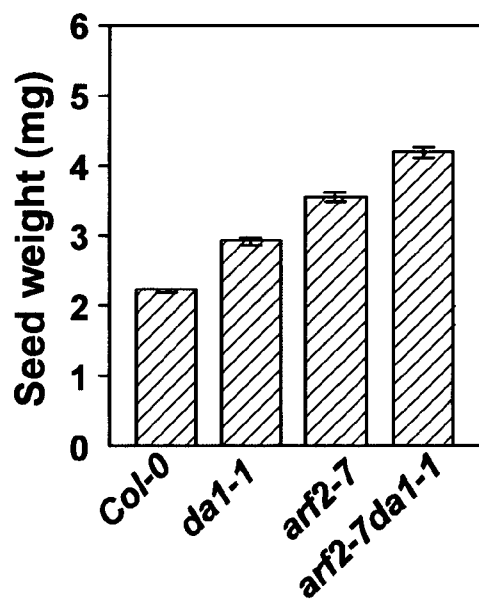
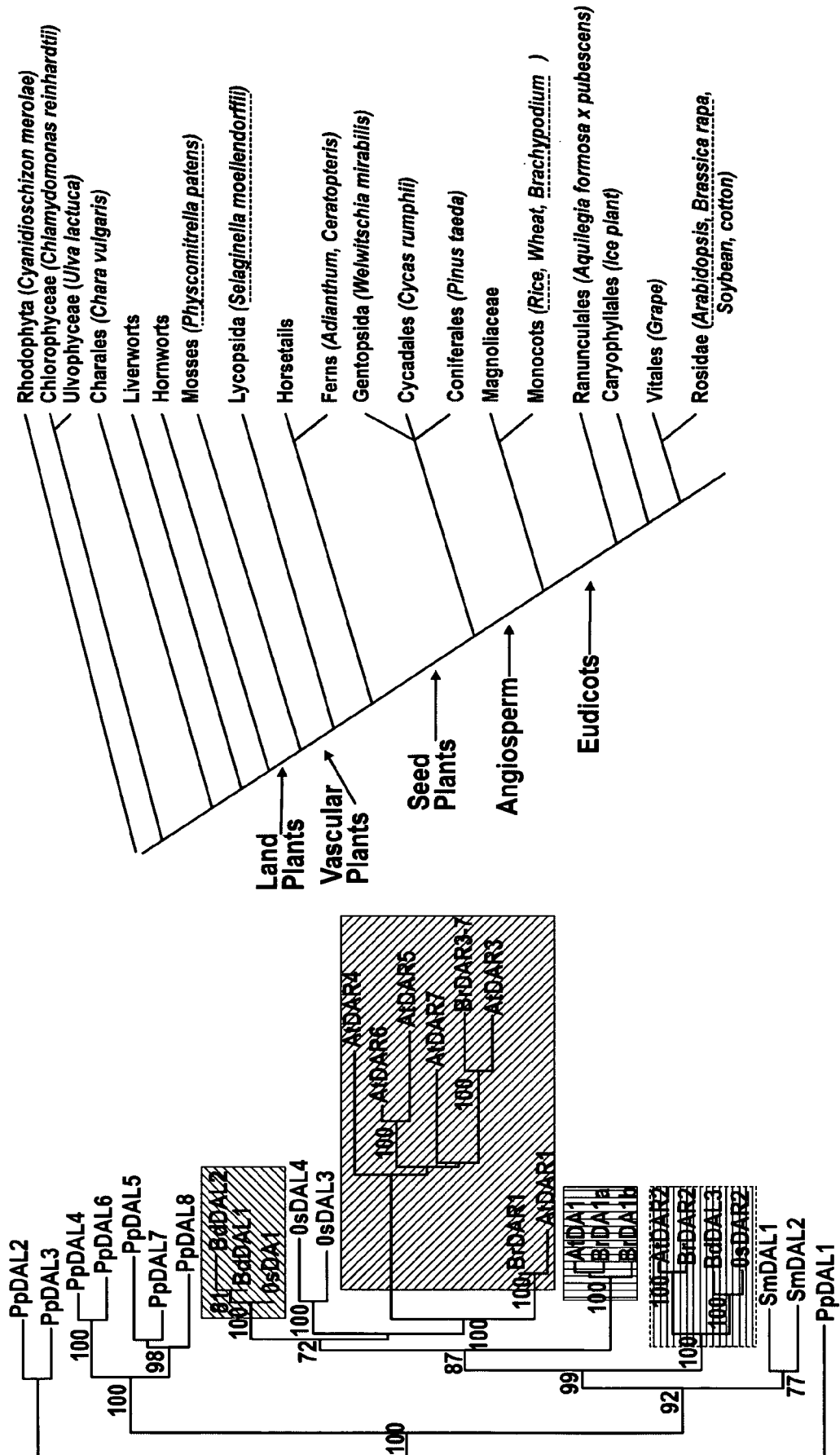
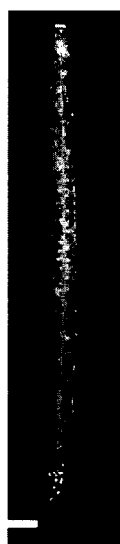
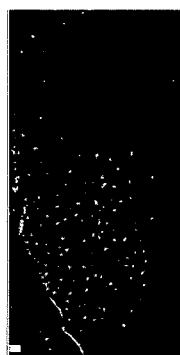
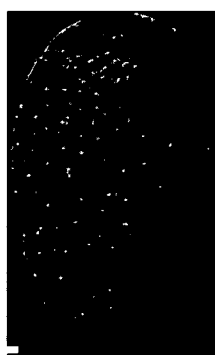
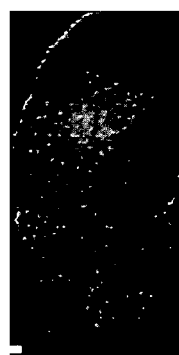
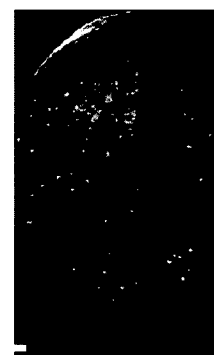
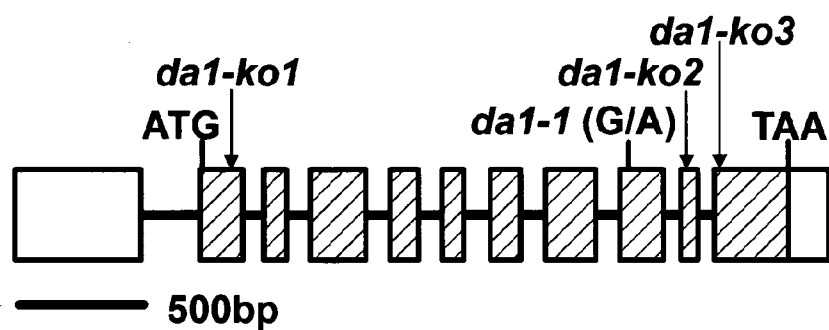


Fig. 12F

**Fig. 13A****Fig. 13B****Fig. 13C****Fig. 13D**



*Fig. 15A**Fig. 15B**Fig. 15C**Fig. 15D**Fig. 15E**Fig. 15F**Fig. 15G**Fig. 15H**Fig. 15I*