

(19) **DANMARK**

(10) **DK/EP 2201121 T3**



(12)

Oversættelse af europæisk patentskrift

Patent- og
Varemærkestyrelsen

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- (51) Int.Cl.: **C 12 N 15/82 (2006.01)** **A 01 H 5/00 (2006.01)** **C 07 K 14/405 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2017-02-27**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2016-11-30**
- (86) Europæisk ansøgning nr.: **08806580.0**
- (86) Europæisk indleveringsdag: **2008-10-10**
- (87) Den europæiske ansøgnings publiceringsdag: **2010-06-30**
- (86) International ansøgning nr.: **GB2008003444**
- (87) Internationalt publikationsnr.: **WO2009047525**
- (30) Prioritet: **2007-10-11 GB 0719919**
- (84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MT NL NO PL PT RO SE SI SK TR**
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- (54) Benævnelse: **FREM GANGSMÅDER TIL STYRING AF PLANTEFRØ- OG -ORGANSTØRRELSE**
- (56) Fremdragne publikationer:
US-A1- 2006 048 240
LI YUNHAI ET AL: "Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana"
GENES & DEVELOPMENT, vol. 22, no. 10, May 2008 (2008-05), pages 1331-1336, XP002512707 ISSN: 0890-9369
DISCH ET AL: "The E3 Ubiquitin Ligase BIG BROTHER Controls Arabidopsis Organ Size in a Dosage-Dependent Manner" **CURRENT BIOLOGY, CURRENT SCIENCE, GB**, vol. 16, no. 3, 7 February 2006 (2006-02-07), pages 272-279, XP005276162 ISSN: 0960-9822 cited in the application

DESCRIPTION

Field of Invention

[0001] This invention relates to methods of controlling the size of the seeds and organs of plants.

Background of Invention

[0002] The size of seeds and organs is an agronomically and ecologically important trait that is under genetic control (Alonso-Blanco, C. 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 (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. Jumbo 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). 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 organ size, however, little is known about the molecular and genetic mechanisms that control final organ and seed size in plants.

[0003] The genetic regulation of seed size has been investigated in plants, including in tomato, soybean, maize, and rice, using quantitative 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. Natl Acad. Sci USA 102, 3123-3128 (2005); Jofuku, K.D. Proc. Natl 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)).

[0004] 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

[0005] 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 *da1-1* allele) is shown herein to act as a dominant negative interfering mutation for DARs or DA1-related proteins. Over-expression of the *da1-1* mutant gene (R358K) in wild type causes an increase in seed and organ size in wild type plants, indicating that the *da1-1* 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 *da1-1*, 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.

[0006] An aspect of the invention provides a method of increasing the life-span, organ size and/or seed size of a plant as set out in claim 1.

[0007] The disclosure provides 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.

[0008] The disclosure also provides an isolated protein (DA1R358K) which interferes with the function of DA1 and DA1-related proteins and an isolated nucleic acid encoding such a protein.

[0009] The disclosure also 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.

[0010] The disclosure also 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

[0011]

Figure 1 shows that *da1-1* has large seeds and organs. (A and B) Dry seeds of Col-0 (A) and *da1-1* (B). (C and D) Mature embryos of Col-0 (C) and *da1-1* (D). (E and F) 9d- old seedlings of Col-0 (E) and *da1-1* (F). *da1-1* has larger cotyledons than WT. (G) The fifth leaves of Col-0 (left) and *da1-1* (right). *da1-1* has larger and rounder leaves compared with wild type Col-0. (H and I) Flowers of Col-0 (H) and *da1-1* (I). (J and K) Siliques of Col-0 (J) and *da1-1* (K). (L) Average seed weight of Col-0, *da1-1*, *da1-ko1*, *dar1-1*, and *da1-ko1dar1-1* 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, *da1-1*, *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 *da1-1*. 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 *da1-1* mutant petals. The largest petals of each series are from opened flowers (stage 14). (B) Mitotic index in WT and *da1-1* mutant petals. Time axis in (B) corresponds to the one in (A). (C) Growth of the fifth leaf of Col-0, *da1-1*, *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 *da1-1*, *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-kol*, *da1-ko2* and *da1-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 *da1-ko1dar1-1* double mutant (I). (J) Siliques of Col-0 (left) and *da1-ko1dar1-1* double mutant (right). (K) Petal area of Col-0, *da1-ko1*, *dar1-1*, *da1-ko1dar1-1* double mutants. The *da1-ko1dar1-1* double mutant displays a *da1-1* 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 10 μ m ABA for 2, 4, 6, 18 and 30 hours. (M and N) Wild type Col-0 and *da1-1* seeds were grown on MS medium with 2 μ m ABA under constant light conditions. The *da1-1* mutant (N) exhibits ABA- insensitive seedling establishment compared with wild type Col-0 (M). (O) 4d- old seedlings of Col-0 (left), *da1-1* (middle) and *DA1COM #2* (right) were transferred to MS medium with 5 μ m ABA for 3 weeks. *da1-1* 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 μ m (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 *da1-1*. (A) Flowers of Col-0, *da1-1*, *eod1-2* and *eod1-2da1-1* double mutants. (B) Soil grown plants of Col-0, *eod1-2*, *eod1-2* and *eod1-2da1-1* double mutants. (C) Average seed weights of Col-0, *da1-1*, *eod1-2* and *eod1-2da1-1* 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, *da1-1*, *eod1-2* and *eod1-2da1-1* 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 *da1-1* has large seeds. Prewieghed batches of wild type Col-0 (A), *da1-1* (B), *DA1COM#2* (C),

35S::DA1^{R358K}#5 (D), and *da1-ko1da1-1* 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 *da1-1* plants. (A to L), Cleared ovules (A,B) and seeds (C to L) of wild type (A, C, E, G, I and K), and *da1-1* (B, D, F, H, J and L) imaged with differential contrast optics. Scale bars: 50 μm (A to L).

Figure 7 shows that *da1-1* plant has large flower with extra petals and deformed silique with extra carpels. (A) Wild type flower. (B and C) *da1-1* flowers with extra petals. (D) Wild type silique, (E to G) *da1-1* siliques with extra carpels. Scale bars: 1mm (A to C), 2mm (D to G).

Figure 8 shows that *da1-1* 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 *da1-1* (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 *da1-1* 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 F18014-52 and co-segregated with CAPS marker DA1CAPS. The number of recombinants identified from F_2 plants is shown. (B) The mutation in *da1-1* 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, *da1-1*, *da1-ko1*, *da1-ko2*, *da1-ko3*, *da1-1/Col-0* F_1 , *da1-ko1/da1-1* F_1 , *da1-ko2/da1-1* F_1 , *da1-ko3/da1-1* F_2 , *da1-ko1/Col-0* F_1 , *da1-ko2/Col-0* F_1 , *da1-ko3/Col-0* F_1 , and *da1-ko1/da1-1* F_1 . Standard deviation values are given ($n>50$). (B) Average seed weight of Col-0, *da1-1*, *da1-ko1*, *da1-ko1/da1-1* F_1 , and *da1-ko1/Col-0* F_1 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 *da1-1* (*EOD1/BB*) synergistically enhance the large seed and organ phenotypes of *da1-1*. (A) The *eod1-1da1-1* double mutant has an increased seed weight compared with *da1-1*. Average seed weight of *da1-1* and *eod1-1da1-1* double mutant is given in mg per 100 seeds. Standard deviation values are shown ($n=5$). Plants were grown under identical conditions. (B) The *eod1-1da1-1* double mutant has larger flower than *da1-1*. (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, *da1-1*, *eod1-2*, and *eod1-2da1-1* plants are shown. The *eod2-1da1-1* plant has a longer growing period than *da1-1*. (E) Eight week old plants of Ler, *da1-1*^{Ler}, *bb-1*, and *bb-1da1-1*^{Ler} plants are shown. The *bb-1da1-1*^{Ler} plant has a longer growing period than *da1-1*^{Ler}. Scale bars: 1mm (B), 5cm (D and E). (F) Petal areas of Ler, *da1-1*^{Ler}, *bb-1*, and *bb-1da1-1*^{Ler} double mutants. Standard deviation values are shown ($n>50$). Mutations in *BB* synergistically enhance the petal size phenotype of *da1-1*, suggesting that *DA1* and *BB* act in parallel pathways.

Figure 13 shows that genetic analysis between *da1-1* and *ant-5*, *axr1-12*, *ap2-7*, and *arf2-7*. (A and B) The petal size phenotype of *ant-5da1-1*^{Ler} and *axr1-12da1-1* double mutant is essentially additive, compared to their parental lines. (C and D) The seed size phenotype of *ap2-7da1-1* and *arf2-7da1-1* 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 *da1-1*. (E-H) Rosette leaves of Col-0, *da1-1*, *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 *da1-1* and T-DNA insertion sites (*da1-ko1*, *da1-ko2* and *da1-ko3*).

Detailed Description of Embodiments of the Invention

[0012] The disclosure provides isolated DA polypeptides encoded by *DA* genes and nucleic acid sequences described herein.

[0013] DA polypeptides include both DA-1 polypeptides and DA-1 related (DAR) polypeptides, and functional homologues thereof, as described herein.

[0014] DA polypeptides, including DA-1 polypeptides and DA-1 related (DAR) polypeptides, possess a characteristic domain structure.

[0015] 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---pLpbA1 pb.Sbp-.pp p (SEQ ID NO: 3)

p---pLpbA1 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;

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;

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

. is absent or is any amino acid, and

- is any amino acid.

[0016] 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).

[0017] A DA polypeptide may comprise an LIM domain. An LIM domain may consist of the sequence of SEQ ID NO: 5;

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

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, 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;

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;

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

- is absent or is any amino acid; and

- is any amino acid.

[0018] 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).

[0019] 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 define the C terminal domain of DA1.

[0020] A DA polypeptide may further comprise R at a position equivalent to position 358 of SEQ ID NO: 1.

[0021] 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.

[0022] 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

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

[0023] A preferred DA polypeptide may further comprise R at a position equivalent to position 358 of SEQ ID NO: 1.

[0024] 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, AB082378.1, AT2G39830, CAN69394.1, OS03G16090, 9234.M000024, 29235.M000021, AT5G66620, AT5G66630, AT5G66610, AT5G66640, AT5G17890, SGN-U320806, AB096533.1, CAL53532.1, OS06G08400, SGN-U328968, OS03G42820 and OS12G40490 or may be variant or a fragment of one of these sequences which retains DA activity.

[0025] A DA polypeptide may comprise an amino acid sequence of AtDA1 (SEQ ID NO: 301), AtDAR1 (SEQ ID NO: 302), AtDAR2 (SEQ ID NO: 303), AtDAR3 (SEQ ID NO: 304), AtDAR4 (SEQ ID NO: 305), AtDAR5 (SEQ ID NO: 306), AtDAR6 (SEQ ID NO: 307), AtDAR7 (SEQ ID NO: 308), BrDA1a (SEQ ID NO: 309), BrDA1b (SEQ ID NO: 310), BrDAR1 (SEQ ID NO: 311), BrDAR2 (SEQ ID NO: 312), BrDAR3-7 (SEQ ID NO: 313), BrDAL1 (SEQ ID NO: 314), BrDAL2 (SEQ ID NO: 315), BrDAL3 (SEQ ID NO: 316), OsDA1 (SEQ ID NO: 317), OsDAR2 (SEQ ID NO: 318), OsDAL3 (SEQ ID NO: 319), OsDAL5 (SEQ ID NO: 320), PpDAL1 (SEQ ID NO: 321), PpDAL2 (SEQ ID NO: 322), PpDAL3 (SEQ ID NO: 323), PpDAL4 (SEQ ID NO: 324), PpDAL5 (SEQ ID NO: 325), PpDAL6 (SEQ ID NO: 326), PpDAL7 (SEQ ID NO: 327), PpDAL8 (SEQ ID NO: 328), SmDAL1 (SEQ ID NO: 329) and SmDAL2 (SEQ ID NO: 330) (as shown in Alignment E).

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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 DEDEQIARALQESMWGNSP (SEQ ID NO: 7).

[0030] A DA polypeptide which is a variant of SEQ ID NO: 1 may comprise a LIM domain having the sequence:

ICAGCNMEIGHGRFLNCLNSLWHPECFRCYGCSQPISEYEFSTSGNYPFHKAC (SEQ ID NO: 8)

[0031] 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.

[0032] 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.

[0033] 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.

[0034] Sequence comparison may be made over the full-length of the relevant sequence described herein.

[0035] The disclosure provides DA genes and nucleic acid sequences which encode DA polypeptides, as described herein.

[0036] 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, AT5G66610, AT5G66640, AT5G17890, SGN-U320806, AB096533.1, CAL53532.1, OS06G08400, SGN-U328968, OS03G42820 and OS12G40490 or may be variant or a fragment of one of these sequences.

[0037] Other database entries of nucleic acid sequences which encode DA polypeptides are shown in Table 7.

[0038] 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 this sequence which encodes a polypeptide which retains DA activity.

[0039] 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 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 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%, at least 90%, at least 95% or at least 98%. Sequence identity is described above.

[0040] A fragment or variant may comprise a sequence which encodes a functional DA polypeptide i.e. a polypeptide which retains one or more functional characteristics of the polypeptide encoded by the wild-type DA gene, for example, the ability to modulate the duration of proliferative growth.

[0041] A nucleic acid comprising a nucleotide sequence which is a variant 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.

[0042] 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 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.

[0043] An alternative, which may be particularly appropriate with plant nucleic acid preparations, is a solution of 5x SSPE (final 0.9 M 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.

[0044] 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. Alternatively, they may have been synthesised directly e.g. using an automated synthesiser.

[0045] 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 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.

[0046] The disclosure 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.

[0047] 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.

[0048] For example, a dominant negative allele of a DA polypeptide may 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 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.

[0049] 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.

[0050] 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).

[0051] "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.

[0052] 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.

[0053] Many suitable regulatory sequences are known in the art and may be used as described herein. 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 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 (1990); An, Plant Physiol. 81:86 (1986)).

[0054] 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 variety of other promoters may be employed to advantage in particular contexts.

[0055] A tissue-specific promoter may be employed to express the dominant negative form of the *DA* polypeptide in a specific tissue or organ to 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 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 (1996)).

[0056] 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.

[0057] 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).

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] Finally, the target cell type is preferably such that cells can be regenerated into whole plants.

[0065] Those skilled in the art will also appreciate that in producing 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 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, herein incorporated by reference for this purpose.

[0066] 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.

[0067] 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 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 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) *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).

[0068] 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.

[0069] Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

[0070] 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 cocultivation with Agrobacterium (EP-A-486233).

[0071] 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.

[0072] 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 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.

[0073] A method of altering the phenotype of a plant may comprise;

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

[0074] Suitable dominant-negative DA polypeptides and methods for expression in plant cells are described above.

[0075] A plant with altered phenotype produced as described above may have an extended period of proliferative growth and may display one or 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 example, in the production of biofuels.

[0076] 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.

[0077] *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 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*.

[0078] 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 in more detail above.

[0079] 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 than 50 amino acids.

[0080] In some embodiments, a *BB* polypeptide may comprise an A at a position corresponding to position 44 of SEQ ID NO: 9.

[0081] 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.

[0082] In some preferred embodiments, a nucleic acid encoding a *BB* 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.

[0083] A variant sequence may be a mutant, homologue, or allele of a 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 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] In some embodiments, the suppressor nucleic acids may be sense suppressors of expression of the BB polypeptide.

[0092] 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))

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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).

[0099] 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.

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] 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.

[0107] The construct or vector may be transformed into plant cells and expressed as described above.

[0108] 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.

[0109] A method of producing a plant with an altered phenotype may comprise:

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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] Dominant-negative DA polypeptides and BB suppressor nucleic acids are described in more detail above.

[0117] 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.

[0118] 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.

[0119] The disclosure also 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.

[0120] The dominant negative DA polypeptide may be heterologous polypeptides.

[0121] A suitable plant may be produced by a method described herein

[0122] 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 plant may have normal fertility relative to control plants.

[0123] A plant described herein may be one which does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to Plant Breeders Rights.

[0124] In addition to a plant expressing a dominant negative DA polypeptide, for example, a plant produced by a method described herein, the disclosure encompasses any clone of such a plant, seed, 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 disclosure is a plant which is a sexually or asexually propagated off-spring, clone or descendant of such a plant, or any part or propagule of said plant, off-spring, clone or descendant.

[0125] 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 more of increased life-span, increased organ size, increased duration of proliferative growth and increased seed size.

[0126] A method of altering the phenotype of a plant may comprise;

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

[0127] A method of producing a plant with an altered phenotype may comprise:

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.

[0128] The phenotype of the plant following reduction or abolition of expression is described in more detail above.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] Other aspects of the disclosure 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.

[0133] 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.

[0134] Nucleic acid encoding a DA polypeptide may be expressed in a plant cell as described above *mutatis mutandis* for dominant negative DA polypeptides.

[0135] A method of identifying a dominant negative DA polypeptide may comprise;

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.

[0136] 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.

[0137] A method of producing a dominant-negative DA polypeptide may comprise;

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.

[0138] 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.

[0139] "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.

[0140] 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.

[0141] 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.

Examples

[0142] 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 *da1-1*, is allelic to *bb*, suggesting that the 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.

[0143] 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 ubiquitin and promoting ubiquitination (Hurley, J.H. Biochem. J. 399,361-372 (2006)).

[0144] Expression of the DA1 gene is induced by the phytohormone abscisic acid (ABA), and the *da1-1* mutant is insensitive to ABA, providing 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 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 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.

[0145] By conducting genetic analysis of *abi4-1da1-1* and *abi5-1da1-1* double mutants, we found that the large organ size phenotype of *da1-1* is independent of ABI4 and ABI5 pathways.

[0146] We also show herein that suppressors of *da1-1* (*sod1*) are molecules which have a second site mutation in the *da1-1* mutant gene that are predicted to reduce gene function, indicating that the R35BK mutation in DA1 is responsible for increased seed size and that the *da1-1* allele interferes with activities of DARs.

[0147] We also show herein that the *da1-1* R358K allele also interferes with DA1 functions in a dosage dependent manner, as evidenced by the fact that plants overexpressing *da1-1* allele (35S::DA1R358K) in wild type have large seed and organ size. This result also demonstrates that the *da1-1* mutant gene (DA1R358K) may be used to genetically engineer significant increases in seed weight and biomass.

[0148] 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. Natl Acad. Sci USA 102, 3123-3128 (2005); Jofuku, K.D. Proc. Natl Acad. Sci. USA 102, 3117-3122 (2005)). However, the experiments set out below show that *da1-1* has increased seed mass, large organ size, but normal fertility, compared with wild type.

Methods

Plant materials

[0149] The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild type. All mutants are in the Col-0 background, except for *da1-1Ler* and *bb-1*, which are in Landsberg erecta background. Before analysis, *da1-1* and *da1-1Ler* 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

[0150] *da1-1* 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 *da1-1* from an ethyl-methanesulphonate (EMS)- treated M2 populations of *da1-1*, respectively. F2 mapping populations were generated from a single cross of *Ler/dal-1*, *Ler/sod1-3da1-1*, and *da1-1Ler/eod1-1da1-1*. A list of primer sequences is provided in Table 2.

Plasmids and transgenic plants

[0151] 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

[0152] Sample preparation, measurement, microscopy, and histochemical 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

[0153] To identify repressors of seed and/or organ growth, we screened for *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*. *da1-1* 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 mechanisms. Genetic analysis with reciprocal crosses between *da1-1* and wild type plants revealed that *da1-1* possesses a mutation in a single nuclear locus.

[0154] To reveal differences in seed size between wild type and *da1-1* mutant, we examined *da1-1* mutant seed size by fractionating seeds produced by individual wild type and *da1-1* plants by using a series wire mesh screens. Seeds from wild type were retained only in 180-300 μ m aperture meshes while the mutant seeds displayed a shift in range to larger exclusion sizes, 180- 355 μ m (Fig. 5a and 5b). More than 80% of the wild type seeds were retained in 250 μ m aperture meshes, whereas about 70% *da1-1* seeds were retained in 300 μ m aperture meshes. To determine whether the increase in seed size in *da1-1* reflected an alternation in embryo size, we isolated mature embryos from wild type and *da1-1* mutant seeds. *da1-1* mature embryos were significantly fatter and longer than those of wild type (Fig. 1c and 1d). We observed that the seed cavity in *da1-1* seeds is larger throughout development than that in wild type (Fig. 6). In addition, the average seed mass of *da1-1* mutant is increased to 132% of that of wild type (Table 5).

[0155] The fertility of *da1-1* plant was found to be normal and the average seed weight per *da1-1* 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.

[0156] We investigated whether DA1 acts maternally or zygotically. As shown in Table 1, the effect of the *da1-1* mutation on seed mass was observed only when maternal plants were homozygous for the mutation. Seeds produced by a *da1-1* mother, regardless of the genotype of the pollen donor, were consistently heavier than those produced by maternal wild type plants. In contrast, *da1-1* mutant and wild type pollen produced seeds whose weight was comparable to that of wild type maternal plants. These results show that *da1-1* is a maternal effect mutation that affects seed mass.

[0157] In addition to the increased seed mass, the *da1-1* mutant exhibited larger organ size than wild type (Fig. 1e-m and o). Compared with wild type, *da1-1* plants have large flowers (Fig. 1h and i) that frequently have extra petals and carpels (Fig. 7). The average size of *da1-1* petals was about 1.6-fold that of comparable wild-type petals (Fig. 1o). Siliques of the *da1-1* mutant were wide, deformed and flattened, compared with the narrow, smooth, cylindrical shape of wild type siliques (Fig. 1j and 1k). *da1-1* mutant also forms larger cotyledons and leaves, as well as thicker stems than wild type (Fig. 1e,-g , i, and m). Consistent with this, *da1-1* 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

[0158] 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 *da1-1* mutant, we analyzed cell size of embryos, petals and leaves. As shown in Fig. 1r, the size of cells from *da1-1* embryos, petals and leaves, were comparable with that of corresponding wild type cells. The epidermal cell number of stem in *da1-1* mutant is increased to 180% that of wild type stems (Fig. 1n). These results indicate that *da1-1* induced effects on seed mass and organ size are due to the increased cell number.

[0159] To determine how DA1 limits seed and organ size, we performed a kinematic analysis of embryos, petals, and leaves in wild type and the *da1-1* mutant. We manually pollinated *da1-1* 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 *da1-1* seeds developed into mature stage in 10 days after fertilization in our growth conditions, suggesting that the period of seed development of *da1-1* mutant was prolonged. Plotting the size of petal primordia and leaves over time revealed that the organ enlargement in *da1-1* mutant is mainly due to a longer growing period of time (Fig. 2a, c). Consistent with this, *da1-1* plants have younger and fresher organs in early developmental stages (Fig. 1g) and longer lifespan than wild type (Fig. 12 e, f).

[0160] 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 *da1-1* plants. The cells in *da1-1* 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 *da1-1* mutant exits cell cycle later than wild type. This result provided indication that *da1-1* exhibits prolonged cell proliferation.

DA1 encodes a novel protein containing UIM and LIM domains

[0161] 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 *da1-1* allele carries a single nucleotide mutation from G to A in the At1g19270 gene which cosegregated with the *da1-1* 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 (*3SS::DA1*) carrying 35S promoter plus At1g19270 cDNA were able to rescue the phenotypes of the *da1-1* mutant (Fig. 1i-q and Fig. 2a), confirming that that At1g19270 is indeed the *DA1* gene.

[0162] 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, Is1-1, Mec-3 (Freyd, G. Nature 344, 876-879 (1990) at the N terminus (see Sequences). The UIM is a short peptide motif with the 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.

[0163] 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 *da1-1* 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, *da1-1* 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.

[0164] A green fluorescent protein (GFP)-DA1 translational fusion under the control of 35S promoter rescued the *da1-1* 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 (35::DA1-HA) and GFP (35S::DA1-GFP) tags using western blot providing indication that the DA1 protein is readily degraded or cleaved in plants.

***da1-1* acts as a type of dominant negative mutation for DA1-related proteins**

[0165] To identify the novel components of the DA1 pathway that determines the final size of seed and organ size, we screened for suppressors of the large seed and organ size phenotypes of *da1-1* (*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 abolish gene function (Fig. 3A). That second site mutations in the *da1-1* mutant gene suppress the *da1-1* 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-kol*, *dal-ko2* and *dal-ko3*) display no obvious phenotype (Fig. 10). To determine if one amino acid change found in the original *da1-1* allele was necessary for the *da1-1* phenotype, we crossed *da1-1* with wild type or *dal-ko* lines. All heterozygotic lines (F1) from crosses between Col-0 and *da1-1* exhibited the wild type phenotype, whereas all the F1 plants from crosses between *da1-1* and T-DNA lines (*dal-kol*, *dal-ko2* and *dal-ko3*) exhibited similar phenotypes to *da1-1* (Fig. S9). In addition, the *da1-1* phenotype was also observed in wild type plants carrying the 35S::DA1R358K transgene (Fig. li-r). Therefore, the R358K mutation in DA1 is necessary and sufficient to cause the *da1-1* phenotype.

[0166] The loss-of-function alleles display no obvious phenotype. We therefore postulated that DA1 may act redundantly with DARs and expression of *da1-1* allele interferes with the ability of DARs to replace DA1. To test this hypothesis, we generated *da1-ko1dar1-1* double mutants. The *da1-ko1dar1-1* double mutant displayed the original *da1-1* phenotype (Fig.3i-1, Table 1 and Fig. 4e), indicating that *da1-1* acts as a type of recessive interfering allele for DARs. Large seed and organ size phenotypes of plants overexpressing the *da1-1* allele in Col-0 suggested that the *da1-1* 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

[0167] In enhancer screens, we isolated one allele of a recessive enhancer of *da1-1* (*eodl-1*). *eodl-1da1-1* plants exhibits larger seed and organ size, more extra petals and longer lifespan than *da1-1* (Fig. 12a,b). We mapped the *eodl-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 *eodl-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 (*eodl-2*) and *bb-1* also enhance *da1-1* 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 *da1-1* mutant. Expression of the DA1 gene in a *bb-1* mutant and of the BB gene in a *da1-1* plant is not significantly affected, compared with wild type (Fig. 12a,b).

[0168] To understand the genetic relationships between EOD1/BB and DA1, we measured seed and petal size in *eod1-2da1-1* and *bb-1da1-1Ler* double mutants and found that mutations in *EOD1/BB* synergistically enhance the phenotype of *da1-1* (Fig. 4, Fig. 11 d, e and 12a), providing indication that the two genes act in parallel pathways to limit seed and organ size in plants (Fig. 4e).

[0169] *aintegumenta* (*ant*) alleles exhibit small petals and plants over-expressing ANT exhibit organ enlargement because of a prolonged 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 CyclinD3;1 in *da1-1* mutant. DA1 mRNA levels do not show robust changes in *ant* mutants (Fig. 12b). Similarly, the levels of both ANT and Cyclin3;1 RNA are not significantly affected by the *da1-1* 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 petal size phenotype of *ant-5da1-1* mutant was essentially additive, providing indication that DA1 and ANT act in independent pathways. We also generated *axr1-12da1-1*, *arf2-7da1-1* and *ap2-7da1-1* 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. Development 137, 251-261 (2006); Ohto, M.A. Proc. Natl Acad. Sci USA 102, 3123-3128 (2005); Jofuku, K.D. Proc. Natl Acad. Sci. USA 102, 3117-3122 (2005)). Genetic analysis revealed that the petal size phenotype of *axr1-12da1-1* mutant or the seed size phenotype of *arf2-7da1-1* and *ap2-7da1-1* were essentially additive (Fig. 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*

[0170] 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, Is1-1, and 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 characterised (see Table 11).

[0171] UIM is the ubiquitin-interacting motif with two conserved serine residues required for binding and forms a short α -helix structure 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 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*.

[0172] 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 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*

[0173] 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.

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

[0174] 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=PhyPal_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=Selmol&advanced=1>), two orthologs were found, 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 DA1 protein structure is not found in lower plants.

DA1-like proteins in *Brassica rapa*

[0175] Due to the close evolutionary relationships of Arabidopsis and Brassica, nucleotide BLAST methods for identifying Brassica DA1 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*

[0176] 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*.

[0177] 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.

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

[0178] 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).

The phenotypes of *da1-1* are rescued by *BrDA1a*, *BrDA1b* and *OsDA1* genes

[0179] 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. *da1-1* plants were transformed to test whether the wildtype DAL genes from Brassica and rice could complement the large growth phenotypes of *da1-1* 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 *da1-1* 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 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.

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

[0180] 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 *da1-1* phenotypes were observed in wildtype Col-0 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 *da1-1* plants.

DA1 protein stability.

[0181] 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 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 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 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 for degradation as part of the mechanism of growth control.

Table 1 DA1 acts maternally to control seed weight

Parent Female	male	F ₁ seed weight
Col-0	Col-0	2.368 ± 0.023
Col-0	<i>da1-1</i>	2.427 ± 0.031
<i>da1-1</i>	Col-0	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 2 List of PCR-based molecular markers.

Marker	Forward Primer	Reverse Primer	Restrict ion Enzyme
T16N11	TAATTAATATTTCTTCTTCCCC	TACTTTGACTCACTTTCACCAC	
F6A14	AATTAGAATAATAATGCAGCGTTG	CGTTTCGGTATCGCTTTGCG	
F14D16-12	TTG GTT TTC GTT GGG TCA AGG	TGTTTCTGCAGAAGCGAGGG	
T29M8-26	AATCACGTGGTGTCTTAGCC	ACTCATTTTGGCAGCTTGGTG	
DA1CAPS	GACACCATGCAATGCCAACC	CTTTGAGCCTCATCCACGCA	<i>MnI</i>
F18O14-78	CTCAGGCTCAGGTAAATGCG	TTCACGTCCGAAACGCATCC	<i>BsaHI</i>
F18O14-52	CTAACACGACCCACATGATGC	CTCGAGTTTCGTGGTTACGG	<i>NspI</i>

Marker	Forward Primer	Reverse Primer	Restriction Enzyme
T20H2	AGCATCCTCAAGGTATAAGCC	GGTGCTGCATTCTGTCACC	
CER451450	GGTTGCTCTAAATCACCTAACG	CTCACC11AGAATATGCATATGTC	

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

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

Gene Name	Forward Primer	Reverse Primer
RT-DA1	ATGGGTTGGTTTAACAAGATCTTT	AACCGGGAATCTACCGGTCA
RT-DAR1	ATGGAGTTTCTTCTCTTTCTTGG	TAAATCCATTTAGGAAATGTACCG
RT-ACTIN	GAGAAGATGACTCAGATC	ATCCTTCCTGATATCGAC
QRT-DA1	GACACCATGCAATGCCAACC	CTTTGAGCCTCATCCACGCA
QRT-TUB6	GGTGAAGGAATGGACGAGAT	GTCATCTGCAGTTGCGTCTT

Table 4 List of Primers for verifying T-DNA.

T-DNA Lines	LP	RP
SALK_126092	AAGCCAGCTAAATATCATTGG	AATCCGTTTGGAATCGTTTG
SALK_110232	GAATTTGGTTCGGTTGGTTTG	TCACATGCCAGAAACAAGAGG
SALK_054295	TCCTCTTCGTTGAGAGACAAGC	TCCATTTGGGTTCTTAAACCG
SALK_067100	ATTTAGTCGAAGCCATGCATG	TTACAAGGAGCAGCATCATCC
SALK_016122	TGAGGTGGCCTATTTTBATACC	CACAACCTTAGTCACCTCAGAAGG
SALK_045169	GAGCGATGCATCTCTAACCAC	AGTAGGAACAGAAAGCAGGGG
Lba	TGGTTCACGTAGTGGGCCATCG	

Table 5 DA1 controls seed weight

Genotype	Average seed weight, mg	% increase over wild type
Col-0	2.206 ± 0.015	
da1-1	2.915 ± 0.039	+32
DA1COM # 2	2.182 ± 0.022	
DA1COM # 3	2.301 ± 0.018	
35S::DA1 ^{R358K} # 2	2.513 ± 0.026	+14
35S::DA1 ^{R358K} # 5	2.672 ± 0.019	+21
da1-ko1	2.231 ± 0.029	
dar1-1	2.199 ± 0.032	
da1-ko1 dar1-1	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 6

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]
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 1255B7181 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]
Table 7
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:J013073011, 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 AY224SS9.1 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 5853H93 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: J100024O13, full insert sequence gi 116012466 dbj AK243101.1 116012466]
Zea mays clone EL01N0524A08.d mRNA sequence gi 54653541 gb BT018760.1 54653541]

Zea mays PCO156068 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 S4651736 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 db AK250393.1 [151419042]
Vitis vinifera, whole genome shotgun sequence, contig VV78X106678.4, clone ENTAV 115 gi 123680846 emb AM488121.1 [123680846]
Vitis vinifera contig W78X222701.5, 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 W78X263569.4, 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 db 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 8

Soybean cDNA clones
gb CX711863.1 CX711863
gb BM525343.1 BM525343
gb BG156297.1 BG156297
gb BM308148.1 BM308148
gb A856660.1 A856660

Soybean cDNA clones
gb BF596520.1 BF596520
gb B472193.1 B472193
gb CO982042.1 CO982042
gb BM143278.1 BM143278
gb AW831270.1 AW831270
gb BE329874.1 BE329874
gb BG652163.1 BG652163
gb B967821.1 B967821
gb B321493.1 B321493
gb BU546579.1 BUS46579
gb CO984945.1 CO984945
gb DW247614.1 DW247614
gb BG726202.1 BG726202
gb B968915.1 B968915
gb BG043212.1 BG043212
gb BG510065.1 BG510065
gb BG043153.1 BG043153
gb AW832591.1 AW832591
gb A856369.1 A856369
gb B699452.1 B699452
gb BG650019.1 BC650019
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 B317518.1 B317518
gb 988431.1 A988431
gb CA801874.1 CA801874
gb BE057592.1 BE057592
gb AW102002.1 AW102002
gb CA938750.1 CA938750
gb AW397679.1 AW397679

Table 9 BB polypeptides identified by Blastp

emb CAO40855.1 unnamed protein product [Vitis vinifera]
gb EAY88740.1 hypothetical protein OsI_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 OSJNBa0088I22.8 [Oryza sativa (japonica cultivar-group)]
gb EAY95219.1 hypothetical protein OsI_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 CA043928.1 unnamed protein product [Vitis vinifera]
emb CAN79375.1 hypothetical protein [Vitis vinifera]

Table 10 nucleic acid encoding BB polypeptides identified by tBlastn

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 C16240_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 11

	Length	Function Domains
DA1	532aa	Two UIM, One LIM
DAR1	548aa	three UIM, one LIM
DAR2	529aa	One LIM

	Length	Function Domains
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 12

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

Sequences

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 SEDNTSNDQENEDIDRAIALSLLEENQEQTSGKYSMPVDEDEQLARALQESMVVGNSP
 RHKSGSTYDNGNAYGAGDLYGNHMYGGGNVYANGDIYYPRPITFQMDPRICAGCNMEIG
 HGRFLNCLNSLWHPFCFRYGCSPISEYEFSTSGNYPFHKACYRERYHFKCDVCSHFIP
 TNHAGLLEYRAHPPFWQKYCPSHEHDATPRCCSCERMEPRNTRYVELNDGRKLCLECLDS
 AVMDTMCQPLYLQIQNFYEGLNMKVEQEVPLLLVERQALNEAREGEKNGHYHMPETRGL
 CLSEEQTVSTVRKRSKHGTGKWAGNITEPYKLTRQCEVTAILILFGLPRLLTGSILAHEM
 MHAWMLKGFRTLSQDVEEGICQVMAHKWLDALAGSTNSNAASSSSSQGLKKGPRSQ
 YERKLGEFFKHQIESDASPVYGDGFRAGRLAVHKYGLRKTLEHIQMTGRFPV

SEQ ID NO: 1

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SEQ ID NO: 2

p---pLpBI pb.Sbp.-pp p

SEQ ID NO: 3

p---pLpBI pb.Sbp.-spp p

SEQ ID NO: 4

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, 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;

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;

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

. is absent or is any amino acid; and

- is any amino acid.

SEQ ID NO: 5

QENEDIDRAIALSLEENQE

SEQ ID NO: 6

DEDEQIARALQESMVVGNSP

SEQ ID NO: 7

ICAGCNMEIGHGRFLNCLNSLWHPECFRCYGCSQPISEYEFSTSGNYPFHKAC

SEQ ID NO: 8

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121 hseaeecian ehdpdpqvs wqddidpdtm tyeelevelge avgtesrgls gelietlptk
181 kykfgsifsr kragerxcvic qlkykigerq mnlpckhvyh seciskwisi nkvcpcvnse
241 vfgepsih
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SEQ ID NO: 9

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661 tgacattgat cctgatacaa tgacctatga ggaatttagta gagctggggg aagcagtagg
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1141 ctagtctcat ttctgcttgt gtgtgcttta agtttaacag tcactcgtat tgtatactaa
1201 atgctaagtc aaaaacgctg aatccatat
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SEQ ID NO: 10

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SEQ ID NO: 11 (OsDAR2)

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SEQ ID NO:12 (BrDAR1)

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SEQ ID NO:14 BrDAR3-7

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421 tattatccaa gacctattgc ttctcaatg gacttcagga tctgtgctgg ctgcaatatg
481 gagattggcc atggaagala tctgaattgc ctcaacgcac tatggcatcc acaatgtttt
541 cgatgctatg gctgcagtea cccaatctct gagtacgagt tctcaacgct tgggaattac
601 ctttttcaca aagcttggtt cagggaaggg ttccatccaa aatgtgatgt ctgcagcctc
661 tttatttcaa caaacctatg tggctcttatt gaatatagag cacatccctt ctgggtccag
721 aagtattgcc cttctcacga acacgatgct acgccaagat gttgcagctg tgaaagaatg
781 gagccgcgga atacaggata ttttgaactc aacgatggag ggaagctttg ccttgagtgt
841 ctagactcat cggtgatgga cacttttcaa tgccagcctc tgtacttgca gatacaagag
901 ttctatgaag gctttaacat gacggtagag caggaggttc cacttctctt agttgagcgg
961 caggcactta acgaagccag agaaggtgaa aggaatggct actatcacat gccagagaca
1021 agaggactct gtctgttcgga agaacaaact gttagaactg tgagaaagag atcgaaaggga
1081 aactggagtg ggaatatgat tacagagcaa ttcaagctaa ctctgctgat cgagggtact
1141 gccattctca tcttgtttgg tctccctagg ctactcactg gttcaattct agctcatgag
1201 atgatgcacg cgtggatgag gctcaaaagg ttccggccac tttagccaaga tgttgaagag
1261 ggggatgtgc aagtgatggc tcataagtgg ttagaagctg agttagctgc tggttcaaga
1321 aatagcaatg ctgcatcacc ttcatcattc tcttatggag gagtgaagaa gggaccaagg
1381 tctcagtagc agaggaagct tggtagtttt ttcaagcacc agatagagtc tgatgcttct
1441 ccggtttatg gagatgggtt cagggcgggg aggttagcgg ttaacaagta tggttttgtg
1501 agaacacttg agcatataca gatgactggg agattcccgg ttttaa

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SEQ ID NO:15 BrDAIb

1 atgggttggt taaacaagat cttcaaagc tctaaccaaa ggttccggct tgggaatgac

```

61 catgaccaca atggctatta ccagagttat ccacatgatg agcctagtgc tgatactgat
121 cctgatcctg atcctgatcc tgatgaaact cactactcagg aacctcttac ccttgaggag
181 gatacatccg gccaggaaaa cgaagacata gatcgtgcaa tcgcattgtc tcttatagaa
241 aacagtcagg gacagactaa taatacatgc gctgccaaag cagggaagta cgaatgggtg
301 gatgaagatg agcaacttgc tagagccata caagagagca tggtagtttg gaatacaccg
361 cgtcagaagc atggaagtga ttatgatatt gggaaatgcat atggggctgg agacgtttac
421 gggaaatggc atatgcatgg aggtggaaat gtatatgcca atggagatat ttattatcca
481 agacctactg ctttcccaat ggatttccag atttgtgctg gctgcaatat ggagattgga
541 catggaagat atctgaattg cttgaatgca ctatggcatc cagaatgttt tcgatgttat
601 ggctgtaggc accccatttc tgagtacgag ttctcaacgt ctgggaaacta cctttttcac
661 aagctttgtt ataggagag ataccatcca aaatgtgatg tctgcaacct ctttattcca
721 acaaacctatg ctggtcttat tggatatagg gcacatcctt tttgggtcca gaagtattgc
781 ccttctcagc aacacgatgc tacccaaga tgttgcaagt gcgaaagaat ggagccacgc
841 aatacaggat atgttgaact taacgatgga cggaaacttt gccttgaatg tctggactca
901 gcggtgatgg acacttttca atgccaaact ctgatctgc agatacaaga attctacgaa
961 ggtcttttca atagggtaga gcaggacgtt ccacttcttt tagttgagag gcaagcactc
1021 aacgaagcca gagaagtgta aaagaatggg cactatcaca tgcagagac aagaggactc
1081 tgcctttcag aagagcaaac tgttagcact gtaagaaaga gatcgaaagc tggcacagga
1141 aactgggctg ggaatatgat tacagagcct tacaagtga cactcaaatg cgagggtact
1201 gccattctca tctgttttgg gctccctagg ctactcaccg gttcgattct agctcatgag
1261 atgatgcacg cgtggatgag gctcaaggga ttccggagcg tgagccaaga cgttgaagaa
1321 ggaatatgtc aagtgatggc tcataagtgg ttggaagcag agttagctgc tggttcaaga
1381 aacagcaatg ttggtcacc ttcatcttct agaggagtg aagaaggacc aagatcgacg
1441 tacgagagga agcttggtag gtttttcaag caccaaatcg agtctgatgc tctccgggtt
1501 tatggagacg ggttcagggc tgggaggtta cgggttaaca agtatgggtt gccaaaaaca
1561 cttgagcata tacagatgac cggtagattc ccggttttaa

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SEQ ID NO:16 BrDAIa

```

1 atgggttggt tgaccaaat ttttagaggt tcaaccaca aaatctcggg agggcaatac
61 cacagcaaac ccgcgaggga gacgatatgg aatggaccct ctaattccgc agttgtgacg
121 gatgtcccg cagaatttga caatgaagat atcgctcgtg ctatatcact ctctctatta
181 gagggaggaac aaagaaaggg aaaggcaata gaaaaggaca tgcatattgga ggaggatgaa
241 caacttgcaa gacttatcca ggaaagtttg aatgttgaat cgctcctcgg tgctcgtgaa
301 aatggcaacg ccaatggttg caatatgtat caaccactgc cacttatgtt ttcttctgga
361 ttcaggagct gtgcgggatg tcacagttag attggtcatg ggcgtttcct tagttgcatg
421 ggagctgttt ggcattccaga atgttttctg tgcattgctt gtaatcaacc aatatatgac
481 tatgagttct ccatgtcggg aaaccatcca taccataaaa catgctacaa ggagcgtctt
541 cacccaaaat gtgatgctcg caagcaattt atctctacaa atatgaatgg cctgattgaa
601 tatagagcac atcctttctg gttacaaaaa tactgtccat cacatgaggt ggacggtact
661 ccaagatgct gtagttgtga aagaatggag ccaagggaat caagatatgt atgctggac
721 gatggtcgca aactctgctt ggagtgcctt gattctcgag ttatggatag gagcagtgct
781 caacctcttt atcttgaaat acaggaattt tatgaaggcc taaatatgaa agtggaaaca
841 caagttccct tgcttcttgt agaaagacag gctttaaatg aagccatgga aggagagaa
901 actggtcacc accacttccc agaaacaaga ggtttatgct tatcagaaga gcaaaactgtc
961 agcacgatat tgaggagacc aagaatggct ggaataaagg ttatggaaat gataacggag
1021 ccatataggt tgactcgtcg atgtgaagtg actgcaattc tcaattctta tggctctcca
1081 agattgttga caggttcaat tttagctcat gagatgatgc atgctggtt cgcacttaaa
1141 ggatattcgca cacttagtcc agacttagaa gagggcatat gccaaagttc tgcctcacatg
1201 tggattgagt cagagatcat tgcaggatca ggcagtaatg gtgcttcaac gttctcatcc
1261 tcatcagcat ccacatcacc gaaaaagggg ggaagatctc agtttgagcg aaagcttggg
1321 gatttttcca agcaccatc ttgagtcagat acctcaatgg cctatggcga tggtttttag
1381 gctggcaacc gagctgttct tcagtattgt taaagcgca cccttgagca tatccgggtt
1441 acagggaact tcccaatttg a

```

SEQ ID NO:17 OsDA1

Sequence Alignments

Alignment B

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UIM1
DR1 QENEIDR AH. SULENQ E
Q9LM05/295-314 DDTAL QO AM. SMAQAAQ A
Q74423/258-277 DSEAP QO QH. SKEDEA R
VF27_YEAST/258 DEEELIRK EL. SKESRN S
Q05785/175-194 SYQDP EK EE. SRITAE D
Q9P2G1/976-995 EDDPNILL QL. SQESGL A
AAK61871/105-1 EEEELIRK AE. SGNSCR S
16741231/229-2 DEDLO QD SL. SHOEHEK G
Q9XTL2/173-192 QEEDDIAR EL. SKENKG S
2623826/295-31 SEEDDIAY RM. SLQQMG E
Q9FJX8/64-84 HEAEQ DL QEF. SHOEHE E
Q9C9U5/139-158 EEENQIQ EL. SAREDE A
YMI8_YEAST/651 NVDEIQ EL. SLSEIN -
Q9FJX8/119-138 EEDELART EE. SKENNR R
AAK61871/80-99 TEEEQATL KM. SEQEAR V
Q20187/2-21 SDDEEQL EL. SKKTFK E
Q9HCH8/758-777 REEQEQ QO AQ. SLQEQEA W
Q9V8R1/660-679 YVDPDAM RM. SQEQRK F
Q81340/323-342 GDDQDQL QL. SVQDSAK E
Q9TOE1/138-157 EEEEMIRAE EA. SKKEAEG S
Q74749/164-183 REEEEQY AL. SLSESTA Q
Q35815/224-243 DDEDDQLR AM. SRQETDM E
Q82143/323-342 VDDQDIAL QM. SVQDAGG S
Q9HFR1/208-227 NMDPEAM RM. SLQEAQA Q
Q9FJX8/146-165 NKDEQAL QO. SLNMEY P
ENSG0000013275 EDDDLQF QQ. SLLEAGT E
YMI8_YEAST/517 ENDIQRI LE. SQEAQAR N
Q9MA77/5-24 QEDEDKLA KM. SMQYNFP E
Q9V8R1/510-529 DEEDMQY EQ. SLVETSG A
AAH11090/250-2 SEDEDQLM AY. SLSEMEA A
Q13821/260-279 NEDEDIKR EL. SKEMFQ S
Q13821/304-323 DEDEEKRA AT. SLLEAQS S
YMI8_YEAST/583 EDDEEFLR KQ. SRVEDER R
EP15_MOUSE/878 QEQDEELA AL. SKSEISE A
Q35815/325-348 SEEDVIRAT TV. SLETAKD S
PSD4_ARATH/282 EDSALDQ AM. SVGDVNM S
Q9FJX8/38-57 QHEADIQK QR. SLATHEA E
VF27_YEAST/301 EEDPDKAK QE. SLREAE E
Q74423/230-249 ENDPDQRV EE. SKRAEE D
Q82143/291-310 DDAQL QO AM. SMEEGSS G
Q9LG27/1615-16 QEDDELAO AL. SLGNSSE T
Q9HA18/233-252 GDDLRLQM EE. SKRETGG K
Q23197/65-84 FDKEEIEC AL. SLSEQEH V
Q45266/382-401 SDSIMKY EL. SLLEDSKE D
Q9H3M9/215-234 QDEDFOR EL. SRQETNR E
YHA2_YEAST/162 SDDEEQR KM. SLFEVEK Q
YMI8_YEAST/547 DEDEQRR EE. SGLIVET Q
2623826/262-28 SEEDMQY AM. SMQNNNT E
Q9HCH8/780-799 KEDDDKRT EL. SLQEFNN S
Q12518/165-184 ENDDQLR SA. SLRTAE D
Q75886/165-184 KEDEDIAR EL. SLQEQOQ Q
EP15_MOUSE/852 SEEDMIET RE. SREEEQ R
Q23197/110-129 DEDEEYMR LE. SLSEEE R
Q9HCH8/656-675 SEEDLAAY EL. SKRDASP S
Q9TOE1/170-189 EDDDDIAL TM. SKSAEE E
Q03291/171-190 QDDENRMA LE. SLQELNT N
PSD4_DROME/212 NEDPEAL RV. SMEEORQ R
Q9H3M9/235-252 REDEHRT EL. SMQSSG N
Q9VS85/226-245 SDDVRLQL SQ. SEQDFKD P

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Q05785/206-225 DEDPDFOAQL QL.SKEEEL K
 O13027/227-246 GDDLRLQOML EE.SRKGAPS K
 O88321/285-304 TEEEQIAYAM QM.SLQGAEF G
 Q9LM05/334-353 GDDQDIAYAM QM.SMQQAAA A
 Q9V8R1/685-701 QEQEMIEQEL KL.SLQEH--
 CE17317|B0205- TEEQOEWEAL RL.SMQENAP A
 Q9MA26/374-393 EEEEEIQRAL AA.SLEDNNM K
 Q17796/291-310 KEEEDIALAL AL.SQSEAEA K
 Q9D0W4/197-216 SEDEAIQRAL EL.SLAELAP Q
 Q9D0W4/221-240 QEEDDIALAL AL.SASEAEY Q
 O15286/347-366 SEEDMEQAL TM.SLQTVRN D
 O45266/775-794 IENKMIKALM KE.SLGSSED G
 Q9FJX8/244-263 DEDEQIAKAL EE.SLKGKGQ I
 Q9HFR1/347-366 DEEAATARAL AM.SLEGQEE G
 UBPP_HUMAN/97- DDKDDIQRAL AL.SLAESNR A
 CE06353|T04C10 SDDARLQOML EE.SQKDADR L
 AAH11090/207-2 GVEDDIALGE EL.SRREQQP S
 O45266/332-351 FDNIMEALAL KQ.SLLDLKG D
 Q9LM99/105-124 DEDEQIARAL QE.SMVVGN S P
 Q9FD28/140-159 EDDDDIDRAT AL.SLQGSVA G
 Q9LM99/69-88 QENEDIDRAL AL.SLLEENQ E
 PSD4_ARATH/305 DEDQDIARAL QM.SMSGES S
 O35815/244-263 DEEADIRRAL QL.SMQGSSR G
 ENSG00000132_1 RFEEQIRIAL EL.SRQEE R
 O81340/291-310 DENALIQOAL AM.SMDEPAS T
 Q9HFR1/321-340 EEDEEIKRAL AM.SRGEQGD I
 Q9ZPH8/231-250 REDEDIARAL SM.SLEAMS L
 PSD4_DROME/276 TEEAMQORAL AL.STETPED N
 Q9VTK7/621-640 NESEMQOAL QM.STRDYME D
 Q9NWK2/209-228 EEELQIQIAL AM.SREEARK P
 Consensus/60% p---pLpba1 pb.Sbp-.pp p

UIM2

DA1 DEDEQIARAL QE.SMVVGN S P
 Q9LM05/295-314 DDTALIQOAL AM.SMAQAAQ A
 Q9HA18/233-252 GDDLRLQOML EE.SKRETTG K
 Q9V8R1/685-701 QEQEMIEQEL KL.SLQEH--
 ENSG0000013275 EDDDLIQOAL QM.SLLEAGT E
 CE17317|B0205- TEEQOEWEAL RL.SMQENAP A
 YMI8_YEAST/517 ENDIQIRIAL LE.SQEAQAR N
 Q9MA77/5-24 QEDEDIKIAL KM.SMQYNPP E
 O74423/258-277 DSEAEIQRAL QL.SKEDEA R
 VP27_YEAST/258 DEEELIRKAL EL.SLKESRN S
 Q9MA26/374-393 EEEEEIQRAL AA.SLEDNNM K
 Q9V8R1/510-529 DEDDMIQOAL EQ.SLVETSG A
 Q05785/175-194 SYODDEKIAL EE.SRITAQE D
 Q9P2G1/976-995 EDDPNILLAL QL.SLQESGL A
 Q17796/291-310 KEEEDIALAL AL.SQSEAEA K
 Q23197/65-84 FKKEETECAL AL.SLSEQEH V
 AAK61871/105-1 EEEELIRKAL AE.SLNSCRP S
 Q9D0W4/197-216 SEDEAIQRAL EL.SLAELAP Q
 AAH11090/250-2 SEDEDIALAL AM.SLSEMEA A
 Q9D0W4/221-240 QEEDDIALAL AL.SASEAEY Q
 O15286/347-366 SEEDMEQAL TM.SLQTVRN D
 16741231/229-2 DEDLQIQIAL SL.SRQEHK G
 O45266/775-794 IENKMIKALM KE.SLGSSED G
 O45266/382-401 SDSIMIKYAL EL.SLLDSKE D
 O13821/260-279 NEDEDIKRAL EL.SLKEMPO S
 Q9H3M9/215-234 QEEDFQIRAL EL.SRQETNR E
 Q9FJX8/244-263 DEDEQIAKAL EE.SLKGKGQ I
 Q9HFR1/347-366 DEEAATARAL AM.SLEGQEE G
 UBPP_HUMAN/97- DDKDDIQRAL AL.SLAESNR A
 CE06353|T04C10 SDDARLQOML EE.SQKDADR L

YHA2_YEAST/162 SDDEEIQKEL KM. SLFEYEK Q
 AAH11090/207-2 GVPDDIALGL EL. SRREQQP S
 YMI8_YEAST/547 DEDEQIARRL EE. SOLIYET Q
 O45266/332-351 FDNIMLEAEL KQ. SLIDLKG D
 Q9XTL2/173-192 QEEDDIARGL EL. SLKENKG S
 Q9LM99/105-124 DEDEQIARRL QE. SMVVGNS P
 Q9FDZ8/140-159 EDDDDIDKEE AL. SLQGSVA G
 2623826/262-28 SEEAMIQCEI AM. SMQMNT E
 2623826/295-31 SEEDQIAYEL RM. SLQOMGE E
 Q13821/304-323 DEDEEIKRGL AT. SLLEAOK S
 Q9HCH8/780-799 KEDDDIKRGL EL. SLQEFNN S
 Q9FJX8/64-84 HEAEQIDLEL QEF. SRQEEEE E
 Q9C9U5/139-158 EEENQIQLEL EL. SARDEPE A
 Q12518/165-184 ENDDDIQREEL SA. SRLTAE D
 FSD4_ARATH/305 DEDQDIARGL QM. SMSGEES S
 Q9LM99/69-88 QENEDIDRGL AL. SLLEENQ E
 C75886/165-184 KEDEDIARGL EL. SLQEQKQ Q
 YMI8_YEAST/651 NVDEDIQLEL AL. SLSEIN -
 EP15_MOUSE/852 SEEDMIENK RE. SEREEEQ R
 Q9FJX8/119-138 EEDELEARTL EE. SLKENNR R
 Q23197/110-129 DEDEEYNREL LE. AAEEEEER R
 Q9HCH8/656-675 SEEELIARVL EL. SKRDASP S
 C35815/244-263 DEEADIARGL QL. SMQSSR G
 YMI8_YEAST/583 EDDEEFLRGL RQ. SRVEDER R
 EP15_MOUSE/878 QEEDIELEL AL. SKSEISE A
 Q20187/2-21 SDDEEIQLEL EL. SKKTFKD E
 AAK61871/80-99 TEEEFALGL KM. SEQEAARE V
 Q9HCH8/758-777 REEQFIQCEI AQ. SLQOEAE W
 ENSG00000132_1 REFEQIRLEL EL. SRQOE R
 Q9V8R1/660-679 YVDDPIARGL EL. SQEQRK F
 Q9TOE1/170-189 EDDDDIARGL TM. SLKSAAE E
 C35815/329-348 SEEDVIRATL TV. SLTAKD S
 Q03291/171-190 QDDENIRGL LE. SLQELNT N
 C81340/291-310 DENALIQCEI AM. SMDEPAS T
 FSD4_DROME/212 NEDPEIARGL RV. SMEEORQ R
 Q9HFR1/321-340 EEDEEIKRGL AM. SRGEGGD I
 C81340/323-342 GDDQDIARGL QL. SVQDSAK E
 Q9ZPH8/231-250 REDEDIARGL SM. SLEAMSY L
 FSD4_ARATH/282 EDSALIQCEI AM. SVGDVNM S
 Q9TOE1/138-157 IEEEMIRATL EA. SKKEAEG S
 C35815/224-243 DDEDDIARGL AM. SRQEIDM E
 C74749/164-183 REEEFIQYEL AL. SLSESTA Q
 Q9FJX8/38-57 QHEADIQKEL QR. SLATHEA E
 FSD4_DROME/276 TEEAMIQREL AL. STETPED N
 C82143/323-342 VDDQDIARGL QM. SVQDAGG S
 Q9VTK7/621-640 NESEMIQCEI QM. STRDYME D
 Q9HFR1/208-227 NMDPEIARGL RM. SLQEAQA Q
 VP27_YEAST/301 EEDPDIAKEL QE. SLREAAE A
 C74423/230-249 ENDPEIARGL EE. SRQAEE D
 Q9H3M9/235-252 REDEHIRSTL EL. SMQSSG N
 Q9VS85/226-245 SDDVRIQLEL SQ. SEQDFKD P
 Q9NWK2/209-228 EEELQIARGL AM. SREEAEK P
 Q05785/206-225 DEDPDIAKEL QL. SKEEEL K
 C82143/291-310 ODAQLIQCEI AM. SMEEGSS G
 C88321/285-304 TEEEQIAYEL QM. SLQGAEF G
 Q9LG27/1615-16 QEEDIEARGL AL. SLGNSSE T
 C13027/227-246 GDDLRIQARGL EE. SRKGAPS K
 Q9FJX8/146-165 NKDEQIALIL QE. SLNMEEY P
 Q9LM05/334-353 GDDQDIARGL QM. SMQAAA A
 Consensus/60% p---pLpba1 pb. sbp-spp p

Alignment C

[illegible]

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	□
Positive	H,K,R	+
Tiny	A,G,S	t
Aromatic	F,H,W,Y	an
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

```

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 -----
AT5G17890 MEPPAARVTPSIKADCSH5VNIICEETVLHSLVSHLSAALRREGISVVFVACGLQETKFF 60

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 SIKQHQFLTDGARLVVVVISDEVEFYDFWFKFLAVIQGWQNHGRVVVFTYGVDSLTAV 120
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 YGILSNHVLTDSELVEEIVRDVVGGLYPAERVGIIYARLLEIEKLLYKQHRDIRSTGINGM 160
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 FGIGKTTLAKAVFMHMSYDYDASGFIENFDEAFNKEGLHRLLEKRIQKYLEDEFDISSV 240
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 IMRPTLRDKLYDKRIIVVLDVDRDSLAEFTLRLOWFGSGSLIIITSVDKQVFATCGI 300
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 NQIYIVQGLNVHEALQLFSQSVEGINEPEQNDARKLSMKVIDYVNGNPLALSIYGRMLMGK 360
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----
AT5G46620 -----
AT5G46630 -----
AT5G46610 -----
AT5G46640 KSIKMETAFFELAKCPPLKIODVLKWAYSALSDEKKNIVLDIAFFTKGETVNYVMQLLEES 420
AT5G17890

AT1G19270 -----
AT4G36840 -----
AT2G39830 -----

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```

AT5G666420 -----
AT5G666430 -----
AT5G666410 -----
AT5G666440 -----
AT5G17890 HYFFRLAIDVLVDKCVLIISENTVQNNLIQDTQCEIFNSEIETCTRMWEPSPRIKYLEY 480

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```

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

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```

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

```

```

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

```

```

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

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

```

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

```

```

AT1G19270 -----
AT4G36860 -----
AT2G39830 -----
AT5G66620 -----
AT5G66630 -----
AT5G66610 -----
AT5G66640 -----
AT5G17890 -----
-----HASDYSSDDEGPE 15
-----MPISDVAISLVGGAAL 15
-----HKLIS---CFAP 9

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ATLIG19270	YHPKCVDSCHFIPTNHAGLIEYRAPHFWQVQKPSVHEHDAITPRCCSEMEFPEPNTK/VEL	285
ATG436860	HHPKPCDVCHNFIPTNHAGLIEYRAPHFWQVQKPSVHEHDAITPRCCSEMEFPEKDKYLIL	225
ATG39930	YHPKCVCHFIPTNHAGLIEYRAPHFWQVQKPSVHEHDAITPRCCSEMEFPEPNTK/VEL	197
AT566620	-ERYCVYCKSEKK--MKTNNRPFWSEWVQVPSVHEADGTPKCCSEMRFLPSFSSNYML	34
AT566630	-ERYCVYCKSEKK--MKTNNRPFWSEWVQVPSVHEADGTPKCCSEMRFLPSGRTYKGL	15
AT566610	-EYKDFHFWKQKCVPSVHEADGTPKCCSEMRFLPSGRTYKGL	278
AT566640	RNPNCVQVQKIPVMAIGERKFSDEHPFWQVQKPSVHEHDAITPRCCSEMRFLPSGRTYKGL	167
AT5617890	HPNVCYCKEPIPTAEGL-RYHEHFWMTYVQVPSVHEADGTPKCCSEMRFLPGTGYTML	131

AT1G15270	NDGKSLGLECLDSAVMTQCPQLPYLQIQNFYEGLNKNEVQEVQPLLVLRVQALNDAEVS	347
AT493660	DGSKSLGLECLDSAMTQDQCPQLYLEIEFEGGLHMKVQEQPLMLLVRSALNSAMEGS	285
AT239380	DGKSLGLECLDSMTAITDGGQCPQLYHAIIRDFEGYLNKIEEQPLMLLVQRLNDAIVGS	257
AT566620	ADGRLGLECMNSAVMSDEQPLKINRDFEGFLGNKIEEQPLLVLRVQALNDAEVS	454
AT566630	SDGNWLGLECMNS-AMSDGQCPQLYPMRDFEGFLGNKIEEQPLLVLRVQALNDAEVS	512
AT566640	DGSLVLCVRCGSAVMTQDQCPQLHFEIEFEGSLNKKIEEQPLMLLVLRVQALNDAEVS	332
AT566650	DGKSLGLECLDSAVMTQDQCPQLHFEIEFEGSLNKKIEEQPLMLLVLRVQALNDAEVS	332
AT561780	ADFNLWLCRCMSAIVMSDQCPQLHFEIEFEGFLHMKIEEQPFWYLVRLNALNAKEE	178

<i>dal-1</i> (R/K)		
ATG19270	KNGHYHMPETPGLCLSEEQTVSTVRRKSKH-GTG-KWAGNITPEYKLTQCEVTAILLIF	405
AT4C3686D	KGHMHMLPETPGLCLSEEQTVTVLRLRFL- ~~GA~~ GKYLIMDITPECLRIIRCEVTAILLY	344
AT2C39630	KNGYHYHMPETPGLCLSEEQTVSTVLLRLRL- ~~GA~~ RLVCRITPEQTRTRCEVTAILLVLY	315
AT5G66620	KIDYQGVYVTTGICLSEEQTVSDSVGRPVR- ~~GF~~ NKKLVGMATSSGKTVFCEVTAILLY	513
AT5G66610	KIDYQGVYVTTGICLSEEQTVSDSVGRPVR- ~~GF~~ NKKLVGMATSSGKTVFCEVTAILLY	513
AT5G66610	KIDNQHGVTGICLSEEQTVSDSVGRPVR- ~~GF~~ NKKLVGMATSSGKTVFCEVTAILLY	357
AT5G66640	KIDYVRAAVTGLCMCLSEEQTVSFI IKGRMGMPDQILDTIVTESQVS- ~~GF~~ ETVGILLI LY	306
AT5G17990	KIGDQGLVTVGGICLSEEQTVSDSVGRPVR- ~~ML~~ NKQLDITPEQTRVQVKEVTAILLY	1437

[illegible][illegible]

RT1G19270	KY--GLRRTLEHIQMTGRFPV----	532
RT4G38860	KH--GLRRTLDHLRLTGPFPKW----	476
RT2G39030	KY--GLRRTLDHLRLTGPFP----	448
RT5G66620	NS--SLQSTLEKILPQ-----	644
RT5G66630	NS--SLQSTLEKILRK-----	702
RT5G66610	D5--SLHXILKLSIQHWTKPQSNL--	529
RT5G66640	SNYMKIKTDLRIDIVASNATPDSV	451
RT5G617890	SNYHSLKLETLKELGISIKTPTQYSKL	1575

Alignment E (SEQ ID NOS: 301-330)

	(1101)	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
A1D1.p1	(1)	GNNGNNNN-VYDNYT	PTASRDE	PSAAD	DAAND	EPHRT	DEPST	DSNN	SXND	D	DAZAL
A1D1.p1	(2)	GAGCGRNY	-----	-----	-----	REDR	LRG	-----	YSAGSG	DFDK	IK
A1D1.p1	(3)	GAAGG	-----	-----	-----	EPFOQ	QEN	-----	Y	PAKSLG	IK
A1D1.p1	(4)	(1)	-----	-----	-----	IVRRNG	DEDEK	-----	LYRVEG	SLG	AK
A1D1.p1	(5)	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1D1.p1	(6)	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1D1.p1	(7)	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1D1.p1	(8)	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1D1.p1	(9)	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1D1.p1	(10)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(11)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(12)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(13)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(14)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(15)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(16)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(17)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(18)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(19)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(20)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(21)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(22)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(23)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(24)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(25)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(26)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(27)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(28)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(29)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(30)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(31)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(32)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(33)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(34)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(35)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(36)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(37)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(38)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(39)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(40)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(41)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(42)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	ETRAL
A1D1.p1	(43)	CGFLP	QIN	RRSR	RTTAL	LEAL	LEAL	PERKL	-----	ETP	

[illegible]

[illegible]

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Patentkrav

1. Fremgangsmåde til øgning af levetiden, organstørrelsen og/eller frøstørrelsen af en plante, omfattende:
- 5 at eksprimere en nukleinsyre, der koder for et dominant-negativt DA-polypeptid i plantens celler,
hvor det dominant-negative DA-polypeptid omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og en
- 10 mutation af R til K ved en position, der svarer til position 358 i SEQ ID NO: 1.
2. Fremgangsmåde ifølge krav 1, yderligere omfattende at reducere eller opheve eksprimering af et Big Brother (BB)-polypeptid i plantens celler,
hvor Big Brother (BB)-polypeptidet omfatter en aminosyresekvens med
- 15 mindst 50 % sekvensidentitet med SEQ ID NO: 9 og har E3 ubiquitinligaseaktivitet.
3. Fremgangsmåde til fremstilling af en plante med øget levetid, organstørrelse og/eller frøstørrelse, omfattende:
- 20 at inkorporere en heterolog nukleinsyre, der koder for et dominant-negativt DA-polypeptid i en plantecelle ved hjælp af transformation, og eventuelt at inkorporere en heterolog nukleinsyre, der eksprimerer en suppressor-nukleinsyre, der reducerer ekspression af et Big Brother (BB)-polypeptid i plantecellen ved hjælp af transformation, og
- 25 at regenerere planten fra en eller flere transformererede celler,
hvor det dominant-negative DA-polypeptid omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og en
- 30 mutation af R til K ved en position, der svarer til position 358 i SEQ ID NO: 1,
og
hvor Big Brother (BB)-polypeptidet omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 9 og har E3 ubiquitinligaseaktivitet.

4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, hvor planten er en højere plante.
5. Plante, der eksprimerer en heterolog nukleinsyre, der koder for et dominant-negativt DA-polypeptid, i sine celler og eventuelt har reduceret eller ophævet ekspression af et BB-polypeptid, hvor det dominant-negative DA-polypeptid omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og en mutation af R til K ved en position, der svarer til position 358 i SEQ ID NO: 1, og hvor BB-polypeptidet omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 9.
6. Plante ifølge krav 5, som er fremstillet ved en fremgangsmåde ifølge krav 3.
7. Fremgangsmåde til ændring af levetiden, organstørrelsen og/eller frøstørrelsen af en plante, omfattende at reducere eller ophæve ekspressionen af to eller flere nukleinsyrer, der koder for DA-polypeptider, i en eller flere af plantens celler; eller fremgangsmåde til fremstilling af en plante med en ændret levetid, organstørrelse og/eller frøstørrelse, omfattende: at reducere eller ophæve ekspressionen af to eller flere nukleinsyrer, der koder for DA-polypeptider i en plantecelle, og at regenerere planten fra plantecellen, hvor DA-polypeptiderne omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne og et LIM-domæne.
8. Fremgangsmåde til ændring af fænotypen af en plante, omfattende: at eksprimere en nukleinsyre, der koder for et DA-polypeptid, i plantens celler i forhold til kontrolplanter, hvor den ændrede fænotype er **kendetegnet ved** normal fertilitet og en eller flere blandt reduceret levetid, reduceret organstørrelse og reduceret frøstørrelse i forhold til kontrolplanter, og

hvor DA-polypeptidet omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og en R-rest ved en position, der svarer til position 358 i SEQ ID NO: 1.

5

9. Isoleret DA-polypeptid omfattende:

en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og

10 en mutation af R til K ved en position, der svarer til position 358 i SEQ ID NO:1.

10. Plante omfattende det isolerede DA-polypeptid ifølge krav 9, eller

en isoleret nukleinsyre, der koder for et DA-polypeptid ifølge krav 9, eller

15 en vektor omfattende den isolerede nukleinsyre, der er funktionelt forbundet med en promoter,

hvor vektoren eventuelt yderligere omfatter nukleinsyresekvenser, der koder for mindst en *eod*-sekvens,

hvor *eod*-sekvensen omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 9 med en mutation ved en position svarende til position 44 i SEQ ID NO:9.

20

11. Fremgangsmåde til forlængelse af vækstperioden hos en plante, som omfatter at eksprimere *DA1^{R358K}* i planten,

25 hvor *DA1^{R358K}* omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne, et LIM-domæne og

en R til K-mutation ved en position, der svarer til position 358 i SEQ ID NO: 1.

30

12. Plante omfattende:

et knockout i mindst et første gen, der koder for DA1, og i mindst et andet gen, der koder for en DAR, eller

et transgen, der eksprimerer et RNA interferens-konstrukt(er), der reducerer

35

ekspressionen af DA1 eller DAR,

- hvor varianten interfererer med begrænsningen i varighed af proliferativ vækst og øger frø- og/eller organstørrelse,
hvor planten eventuelt yderligere omfatter genetiske kombinationer med mutationer, der forstyrrer funktionerne af BB-gener,
- 5 hvor BB-generne omfatter en nukleotidsekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 10, og
hvor DA1 eller DAR omfatter en aminosyresekvens med mindst 50 % sekvensidentitet med SEQ ID NO: 1, hvilken aminosyresekvens omfatter et UIM1-domæne, et UIM2-domæne og et LIM-domæne.
- 10
- 13.** Fremgangsmåde eller plante ifølge et hvilket som helst af kravene 1 til 12, hvor det dominant-negative DA-protein, DA-polypeptid, DA1, DAR eller DA1^{R358K} omfatter en aminosyresekvens med mindst 80 % identitet med en eller flere blandt SEQ ID NOS: 1 og 301 til 330.
- 15
- 14.** Fremgangsmåde eller plante ifølge et hvilket som helst af kravene 1 til 13, hvor det dominant-negative DA-protein, DA-polypeptid, DA1, DAR eller DA1^{R358K} omfatter aminosyresekvensen ifølge en blandt SEQ ID NOS: 1 og 301 til 330.

DRAWINGS

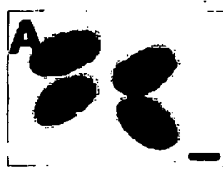


Fig. 1A

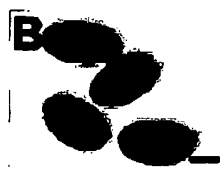


Fig. 1B



Fig. 1C



Fig. 1D



Fig. 1E



Fig. 1F



Fig. 1G

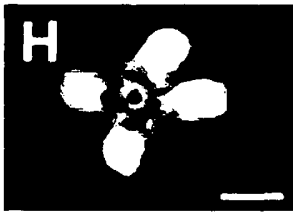


Fig. 1H

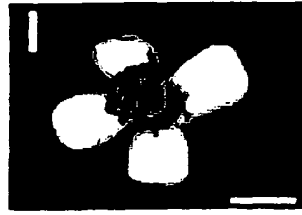


Fig. 1I



Fig. 1J



Fig. 1K

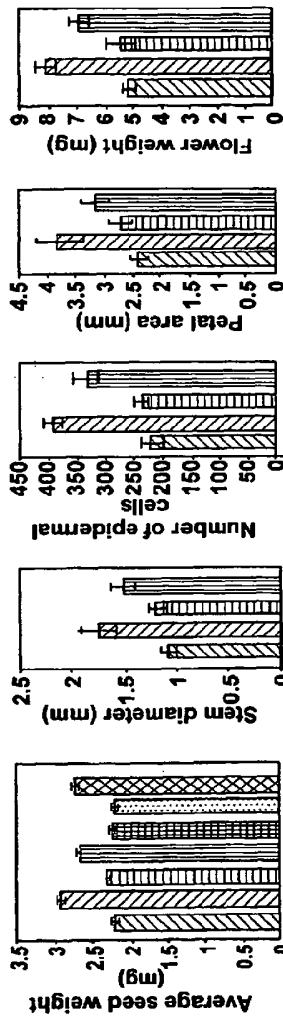


Fig. 1L

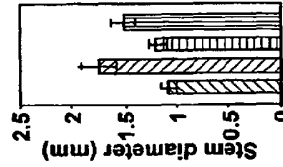


Fig. 1M

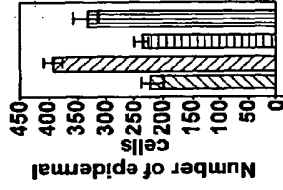


Fig. 1N

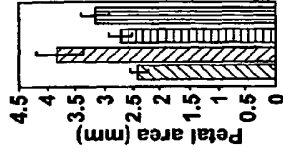


Fig. 1O

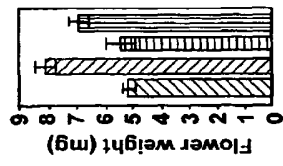


Fig. 1P

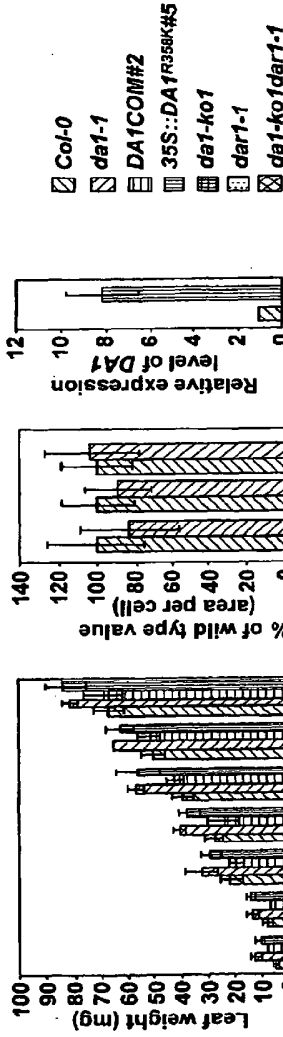


Fig. 1Q

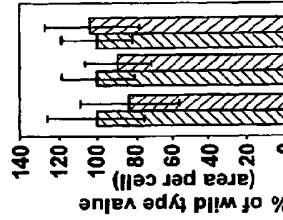


Fig. 1R

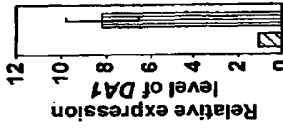


Fig. 1S

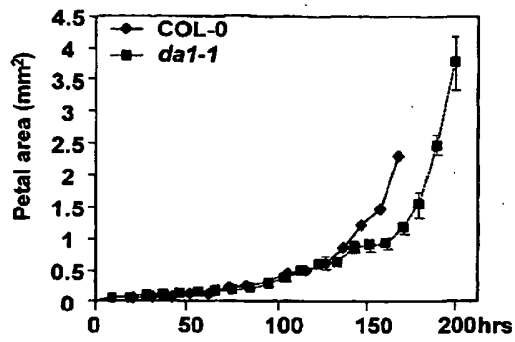


Fig. 2A

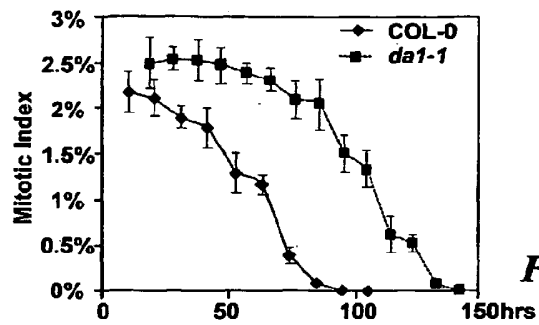


Fig. 2B

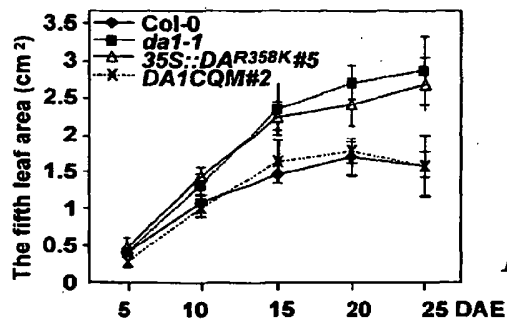


Fig. 2C

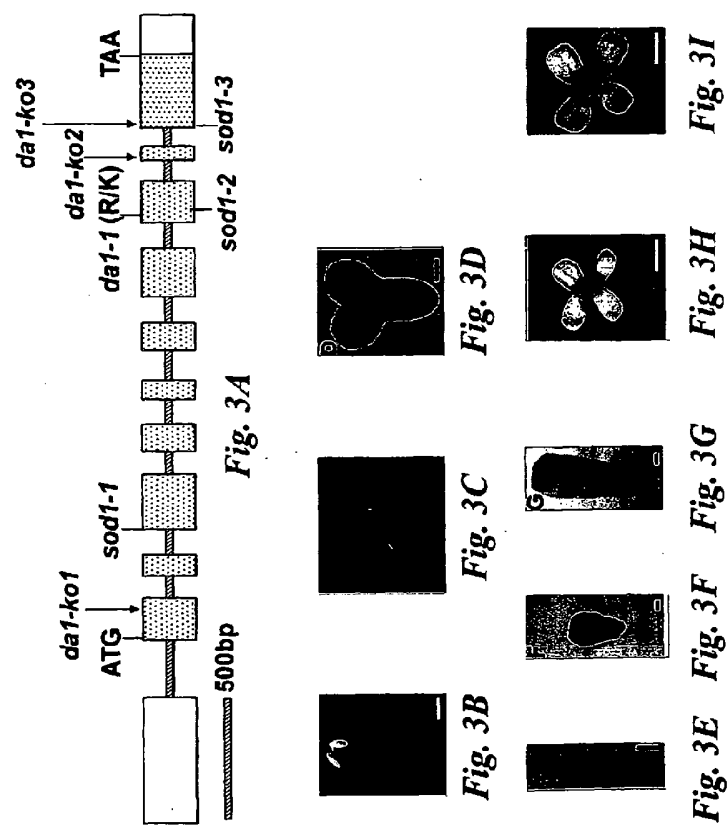




Fig. 3J

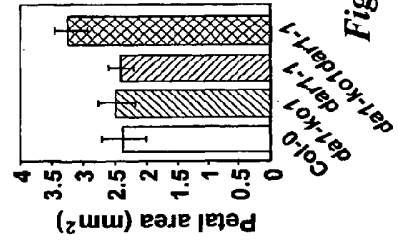


Fig. 3K

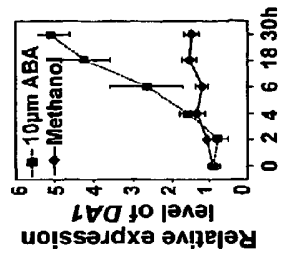


Fig. 3L



Fig. 3M



Fig. 3N



Fig. 3O

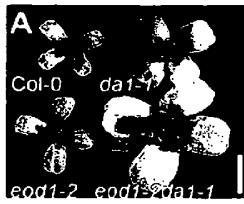


Fig. 4A



Fig. 4B

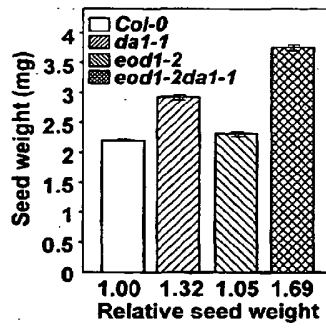


Fig. 4C

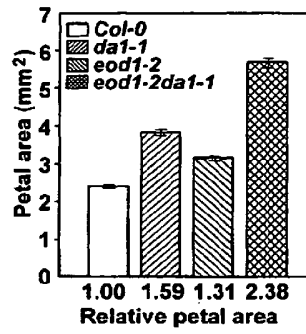


Fig. 4D

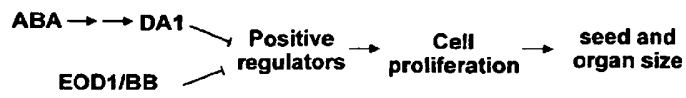


Fig. 4E

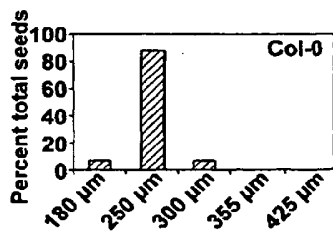


Fig. 5A

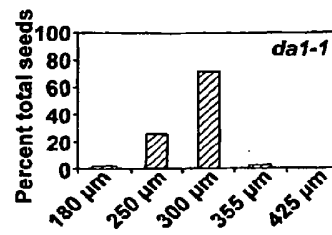


Fig. 5B

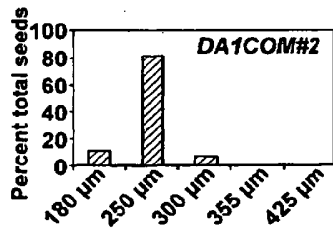


Fig. 5C

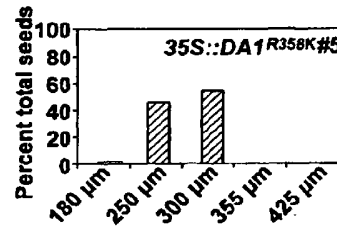


Fig. 5D

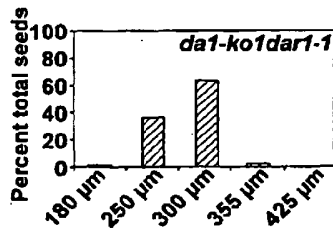


Fig. 5E

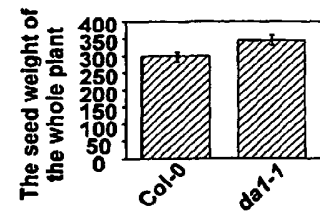


Fig. 5F



Fig. 6A



Fig. 6B



Fig. 6C



Fig. 6D

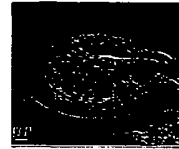


Fig. 6E



Fig. 6F



Fig. 6G

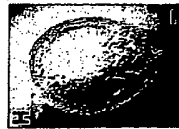


Fig. 6H

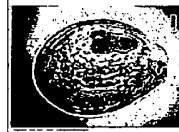


Fig. 6I



Fig. 6J



Fig. 6K



Fig. 6L

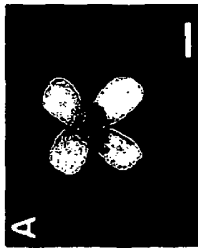


Fig. 7A

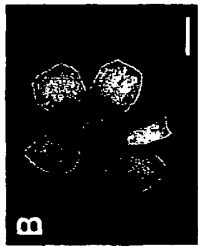


Fig. 7B



Fig. 7C



Fig. 7D



Fig. 7E



Fig. 7F



Fig. 7G

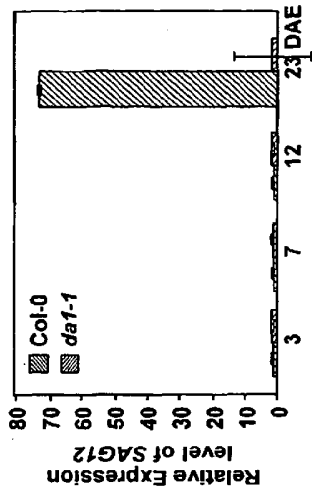


Fig. 8C



Fig. 8B

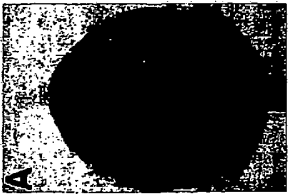


Fig. 8A

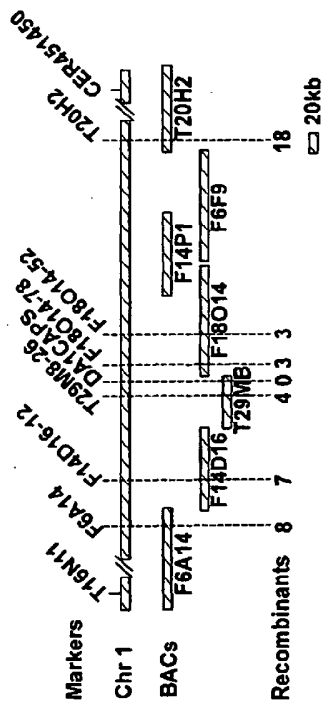


Fig. 9A



Fig. 9B

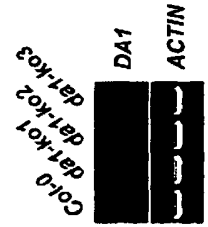


Fig. 9C



Fig. 9D

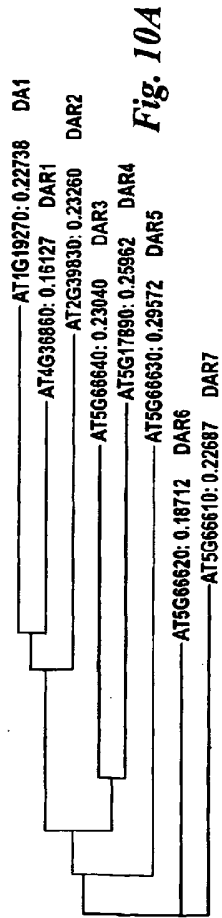


Fig. 10A

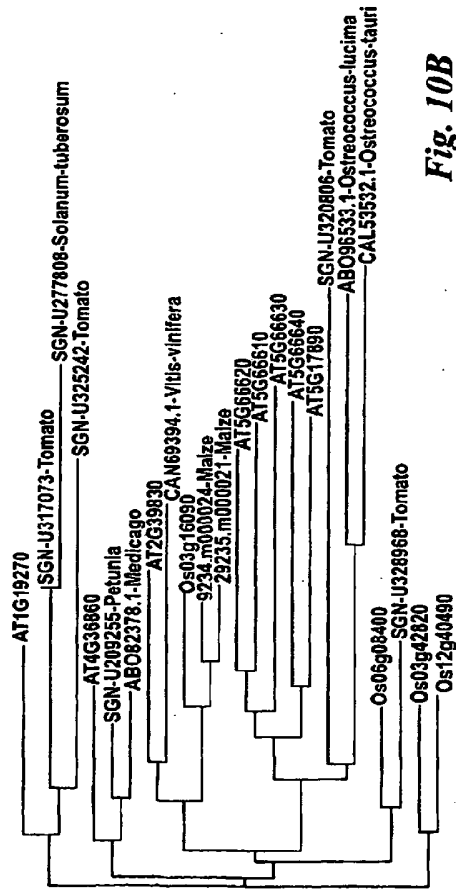


Fig. 10B

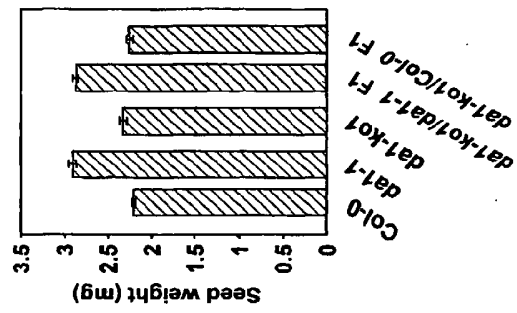


Fig. 11B

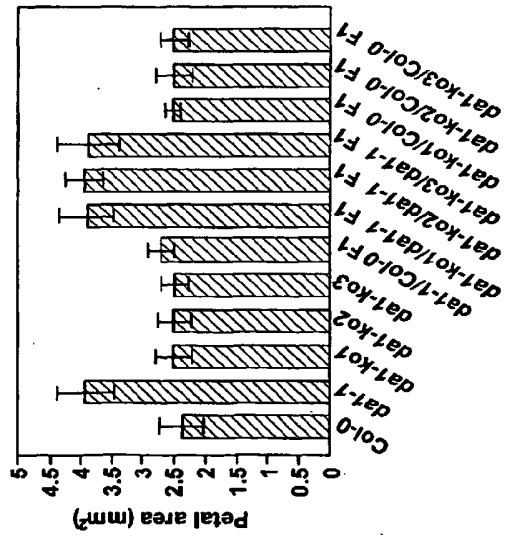


Fig. 11A

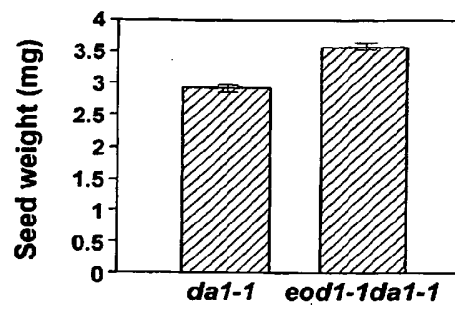


Fig. 12A



Fig. 12B

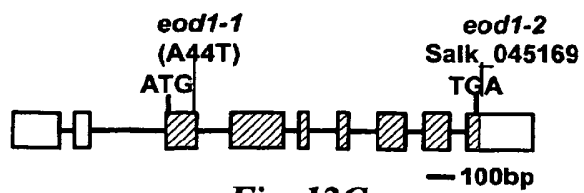


Fig. 12C



Fig. 12D

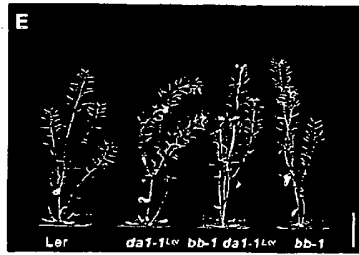


Fig. 12E

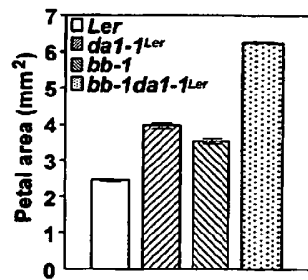


Fig. 12F

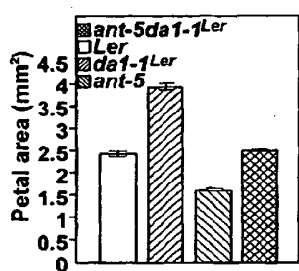


Fig. 13A

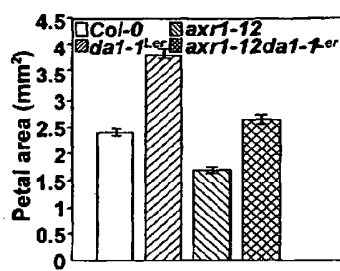


Fig. 13B

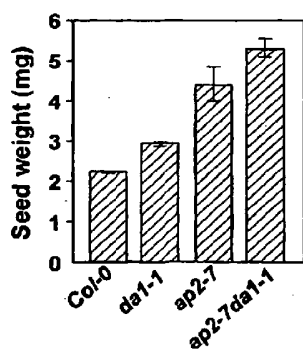


Fig. 13C

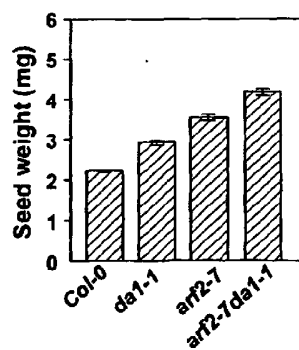


Fig. 13D

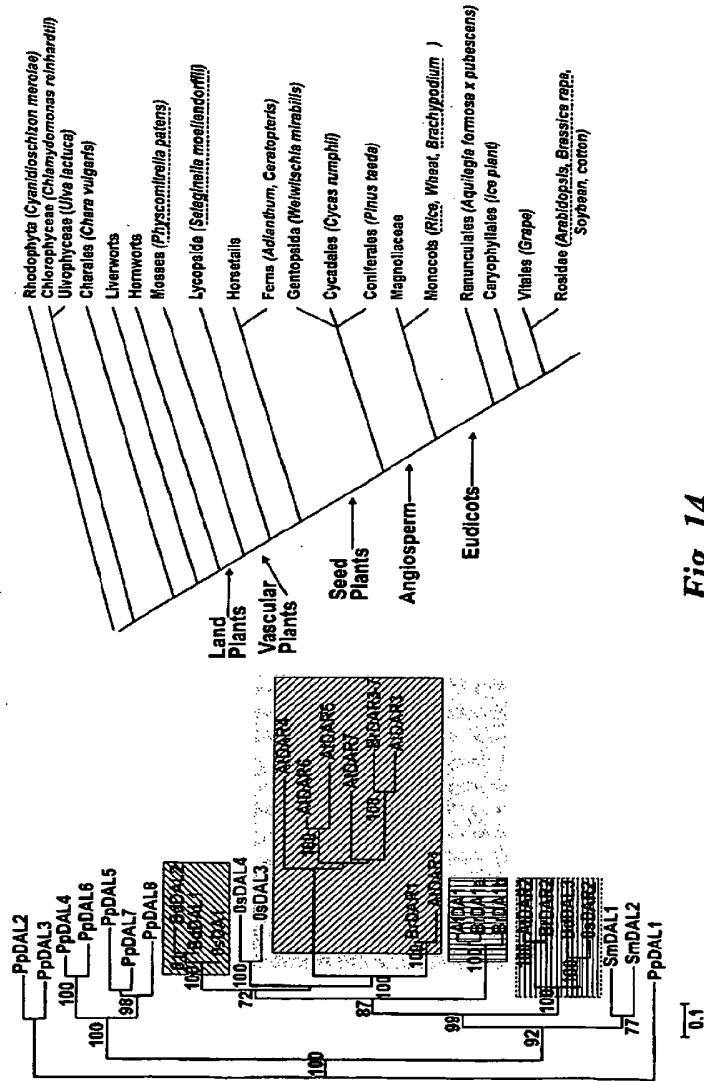


Fig. 14



Fig. 15A ***Fig. 15B*** ***Fig. 15C*** ***Fig. 15D***

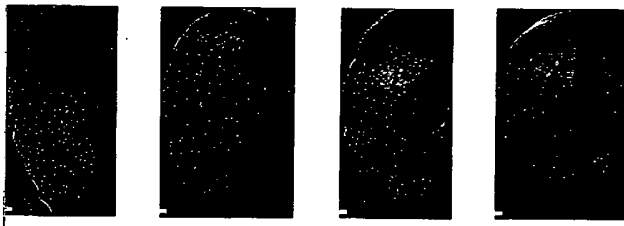


Fig. 15E ***Fig. 15F*** ***Fig. 15G*** ***Fig. 15H***

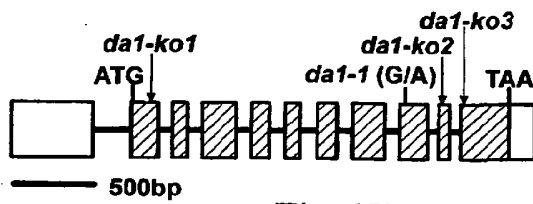


Fig. 15I