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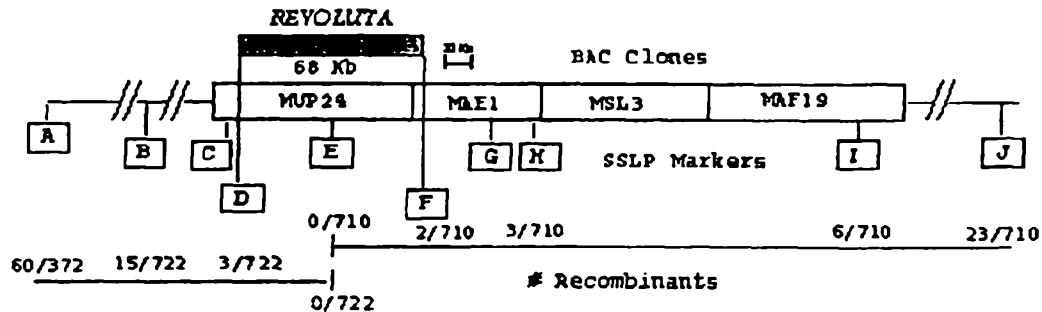
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(54) Title: COMPOSITIONS AND METHODS FOR MODULATION OF PLANT CELL DIVISION



(57) Abstract: The present invention provides compositions and methods for modulating cell division in plants. In particular, the present invention provides polynucleotides that encode REVOLUTA. In addition, REVOLUTA vectors and transformed plants are provided wherein plant cell division is modulated by expression of a REVOLUTA transgene as compared to a control population of untransformed plants. The present invention also provides methods for the isolation and identification of REVOLUTA genes from higher plants.



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## COMPOSITIONS AND METHODS FOR MODULATION OF PLANT CELL DIVISION

### Field of the Invention

5 The present invention relates generally to compositions and methods for modulating plant division and growth. More specifically, transgene vectors, cells, plants and methods for producing the same are provided that facilitate the production of plants having an increased or decreased number of cells.

### Background of the Invention

10 Elaboration of the plant body pattern depends primarily on the proper regulation of cell division versus cell differentiation at the growth sites called meristems. In seed plants, apical growth is carried out by the apical meristems. Although structurally identical, shoot apical meristems differ ontogenetically. A primary shoot apical meristem originates during embryogenesis and becomes the apex of the primary shoot. Secondary shoot apical meristems develop later on the  
15 sides of the primary shoot and form lateral shoots. In many seed plants, radial growth of the shoot is conferred by the cambium, a cylindrical meristematic layer in the shoot body. Growth of lateral "leafy" organs (i.e., leaves, petals, etc.) occurs from transient meristems formed on the flank of the apical meristem. Root growth occurs from analogous apical and cambial meristems. Presently, very little of the  
20 regulation and interaction of these different types of meristems is understood.

The commercial value of a cultivated plant is directly related to yield, i.e. to the size and number of the harvested plant part, which in turn is determined by the number of cell divisions in the corresponding plant tissues. Although a genetic approach to the study of plant development has provided important information on

pattern formation and organ morphogenesis (see, for example, Riechmann et al., 1997 *Biol. Chem.* 378:1079-1101; Barton, 1998 *Current Opin. Plant Biol.* 1:37-42; Christensen et al., 1998 *Current Biol.* 8:643-645; Hudson, 1999 *Current Opin. Plant Biol.* 2:56-60; Irish, 1999 *Dev. Biol.* 209:211-220; Scheres et al., 1999 *Current Topics Dev. Biol.* 45:207-247). Very little has been learned about how and what regulates plant cell division, and, therefore the overall size of a plant organ. Therefore, the isolation and manipulation of genes controlling organ size via regulatory effects on cell division will have a large impact on the productivity of virtually every commercial plant species.

10 Hermerly et al. (1995 *EMBO J.* 14:3925-3936) studied the effects on tobacco plant growth and development using a dominant negative mutation of an *Arabidopsis thaliana* Cdc2 kinase gene. Cdc2 kinase activity is required in all eukaryotic organisms to properly progress through the cell cycle. Hermerly et al. showed that expression of the *Arabidopsis thaliana* gene encoding the dominant negative Cdc2  
15 protein in tobacco plants resulted in plants that were morphologically normal, but were smaller in size due to a reduction in the frequency of cell division. Thus, the regulation of plant cell division can be at least partially uncoupled from plant development. However, in normal plant growth and development, Cdc2 kinase activity must be activated by other regulatory proteins in order to instigate plant cell  
20 division.

A large number of plant mutants have been isolated that display a wide variety of abnormal morphological and growth phenotypes (see, for example, Lenhard et al., 1999 *Current Opin. Plant Biol.* 2:44-50). However, it is difficult to visually identify which plant morphology phenotypes are due to mutations in the  
25 putative key controller genes that determine whether a plant cell will grow and divide versus other genes that specify the developmental fate of a cell. Furthermore, even when such a putative plant growth mutant has been identified, a great deal of effort is required to identify which DNA segment encodes the mutant gene product that functions to regulate plant cell division.

30 For example, Talbert et al. (1995 *Development* 121:2723-2735), reported *Arabidopsis thaliana* mutants defective in a gene named *revoluta* (*REV*), that appear to display an abnormal regulation of cell division in meristematic regions of mutant plants. More specifically, the *REV* gene is required to promote the growth of apical meristems, including paracade meristems, floral meristems and the primary shoot  
35 apical meristem. Simultaneously, the *REV* gene has an opposing effect on the

meristems of leaves, floral organs and stems. That is, in leaf, floral organ and stem tissues *REV* acts to limit cell division, thereby, reducing both the rate of plant growth and final size of the tissue. Loss of functional *REV* protein in leaf, floral organ and stem tissues leads to an increase in the number of cells and the size of these tissues.

5 In contrast, loss of functional *REV* protein in apical meristem cells leads to a reduction in cell division and reduced organ size. Talbert et al., (1995, incorporated herein by reference) reports the detailed morphological changes observed in homozygous *revoluta* plants. The aberrant morphologies recorded for *revoluta* mutants strongly suggest that the *REV* gene product has a role in regulating the  
10 relative growth of apical and non-apical meristems in *Arabidopsis*. The *revoluta* mutations were used to map the *REV* gene to the generally distal, but unspecified, portion of Chromosome 5 in *Arabidopsis*. However, prior to the present invention the *REV* gene sequence and methods for using polynucleotides encoding the *REV* protein to modulate cell division in transgenic plant cells were unknown.

15 In principle, mutations in a plant growth regulator gene could also be identified based upon their sequence similarity at the DNA or protein level as compared to animal or fungal genes that are known to play an important role in initiating the cell cycle (such as cyclins) or otherwise regulating growth. For example, homeobox (HB) genes are well known in animals as encoding proteins that  
20 act as master control genes that specify the body plan and otherwise regulate development of higher organisms (Gehring et al. 1994, *Annu. Rev. Biochem.* 63:487-526). The HB genes of animals encode an approximately 60 amino acid protein motif called a homeodomain (HD) that is involved in DNA binding, and the proteins that contain an HD are transcription factors which act as regulators of the  
25 expression of target genes. HD regions are highly conserved between both plants and animal. Plant homeobox genes were first identified based upon the isolation of a maize mutant called *knotted1* (*kn1*) that had a dominant mutation that altered leaf development (Vollbrecht et al. 1991, *Nature* 350:241-243). Genes encoding proteins homologous to the maize Knotted protein have been identified and cloned from a  
30 wide variety of plant species based upon their sequence homology (for a review see Chan et al., 1998 *Biochim. et. Biophys. Acta* 1442:1-19). Hybridization studies indicate that there may be about 35 to 70 different HD-containing genes in *Arabidopsis* (Schena et al., 1992 *Proc. Natl. Acad. Sci. USA* 89:3894-3898).

35 A large number of plant HD-containing genes have been isolated using degenerate oligonucleotides made from conserved HD sequences as hybridization

probes or PCR primers to identify and isolate cDNA clones (Ruberti et al., 1991 *EMBO J.* 10:1787-1791; Schena et al, 1992; Mattsson et al, 1992 *Plant Mol. Biol.* 18:1019-1022; Carabelli et al., 1993 *Plant J.* 4:469-479; Schena et al., 1994 *Proc. Natl. Acad. Sci. USA* 91:8393-8397; Soderman et al., 1994 *Plant Mol. Biol.* 26, 145-154; Kawahara et al., 1995 *Plant Molec. Biol.* 27:155-164; Meissner et al., 1995 *Planta* 195:541-547; Moon et al., 1996 *Mol. Cells* 6:366-373; Moon et al., 1996 *Mol. Cells* 6:697-703; Gonzalez et al., 1997 *Biochem. Biophys. Acta* 1351:137-149; Meijer et al., 1997 *Plant J.* 11:263-276; Sessa et al., 1998 *Plant Mol. Biol.* 38:609-622; Aso et al., 1999 *Mol. Biol. Evol.* 16:544-552). Analysis of these HD-containing genes revealed the presence of an additional large class of HD-containing genes in plants, known as HD-Zip genes because the proteins encoded by these genes contain a leucine zipper in association with the homeodomain. This class of HD genes are unique to plants (Schena et al., 1992). Based upon amino acid sequence similarity the proteins encoded by the HD-Zip genes have been divided into four HD-Zip subfamilies based upon the degree of amino acid similarity within the HD and leucine zipper protein domains (Sessa et al., 1994 In *Molecular-Genetic Analysis of Plant Development and Metabolism* [Puigdomenech, P. and Coruzzi, G., eds] Berlin: Springer Verlag, pp 411 - 426; Meijer et al., 1997). However, similar to the Knotted class of plant HD genes, the HD-Zip genes are also thought to encode proteins that function to regulate plant development (Chan et al., 1998). The presence of both HD and leucine zipper domains in the HD-Zip protein suggests very strongly that these proteins form multimeric structures via the leucine zipper domains, and then bind to specific DNA sequences via the HD regions to transcriptionally regulate target gene expression (Chan et al., 1998). This inference has been experimentally documented by *in vitro* experiments for many of the HD-Zip proteins (Sessa et al., 1993; Aoyama et al., 1995 *Plant Cell* 7:1773-1785; Ganzalez et al., 1997 *Biochim. Biophys. Acta* 1351:137-149; Meijer et al., 1997; Palena et al., 1999 *Biochem. J.* 341:81-87; Sessa et al., 1999), which publications are incorporated herein by reference.

Antisense and ectopic expression experiments have been performed with some HD-Zip subfamily I, II, III and IV genes to access the phenotypic consequences of shutting off HD-Zip gene expression and over producing HD-Zip protein throughout a plant (Schena et al., 1993; Aoyama et al., 1995; Tornero et al., 1996; Meijer et al., 1997; Altamura et al., 1998). Additional evidence regarding HD-Zip function has been inferred from *in situ* hybridization and Northern blot hybridization experiments to determine the temporal pattern of HD-Zip gene expression through

plant development as well as to locate which specific plant cells or tissues exhibit HD-Zip gene expression (See Table 1). However, as demonstrated by the information compiled in Table 1, there is no clear pattern as to what regulatory roles HD-Zip proteins play in plant growth and development either as a super family or at the subfamily level. Furthermore, there has been no recognition that a HD-Zip gene product is involved in the regulation of plant cell division.

Table 1. HD-Zip Genes And Their Proposed Functions

| <b>HD-Zip<br/>Subfamily<br/>and Gene</b> | <b>Expression Pattern</b>  | <b>Proposed Function</b>                                    | <b>Reference</b>               |
|--|--|---|--------------------------------|
| <u>Subfamily I</u>                       |  |   |                                |
| Athb-1                                   | late plant development   | activation of genes related to<br>leaf development          | Aoyama et al., 1995            |
| Athb-3                                   | root and stem cortex   | ?   | Soderman et al., 1994          |
| Athb-5                                   | leaf, root and flower  | ?   | Soderman et al., 1994          |
| Athb-6                                   | leaf, root and flower  | ?   | Soderman et al., 1994          |
| Athb-7                                   | low level throughout plant,<br>induced by abscisic acid and<br>water deficit | signal transduction pathway in<br>response to water deficit | Soderman et al., 1994,<br>1996 |
| CHB1                                     | early embryogenesis  | maintenance of indeterminate<br>cell fate                   | Kawahara et al., 1995          |
| CHB2                                     | early embryogenesis  | ?   | Kawahara et al., 1995          |
| CHB3                                     | mature tissue  | ?   | Kawahara et al., 1995          |
| CHB4                                     | hypocotyl  | ?   | Kawahara et al., 1995          |
| CHB5                                     | hypocotyl and roots  | ?   | Kawahara et al., 1995          |
| CHB6                                     | late embryogenesis, mature<br>tissue   | ?   | Kawahara et al., 1995          |
| Hahb-1                                   | stem   | ?   | Chan et al., 1994              |
| VAHOX-1                                  | phloem of adult plants   | differentiation of cambium cells<br>to phloem tissue        | Tornero et al., 1996           |

| <u>Subfamily II</u>  |   |   |  |
|----------------------|---|---|--|
| Athb-2<br>(HAT4)     | vegetative and reproductive phases of plant, induced by far-red-rich light    | involved in light perception and related responses in regulation of development | Schena et al., 1993;<br>Carabelli et al, 1993;<br>1996; Steindler et al.,<br>1999; |
| Athb-4               | vegetative and reproductive phases of plant, induced by far-red-rich light    | involved in light perception and related responses                              | Carabelli et al, 1993  |
| Hahb-10              | stems and roots   | ?   | Gonzalez et al., 1997  |
| Oshox1               | embryos, shoots of seedlings and leaves of mature plants                      | leaf developmental regulator  | Meijer et al., 1997  |
| <u>Subfamily III</u> |   |   |  |
| Athb-8               | procambial cells of the embryo, induced by auxins                             | regulation of vascular development  | Baima et al., 1995;<br>Altamura et al., 1998;<br>Sessa et al., 1998                |
| Athb-9               | mRNA slightly enriched in stem compared to leaf, root and flower              | ?   | Sessa et al., 1998   |
| Athb-14              | Strongly enriched in stem, root, slightly enriched in flower compared to leaf | ?   | Sessa et al., 1998   |
| crhb1                | expressed only in gametophyte   | ?   | Aso et al., 1999   |

| <u>Subfamily IV</u> |  |   |  |
|---------------------|--|---|--|
| Athb-10 (GI-2)      | trichome cells and non-hair root cells             | positive regulator of epidermal cell development        | Rerie et al., 1994; Di Cristina et al., 1996; Masucci et al., 1996 |
| ATML1               | Expressed in L1 layer of the shoot apical meristem | Regulation of epidermal cell fate and pattern formation | Lu et al., 1996  |
| Hahr1               | Expressed in dry seeds, hypocotyls and roots       | Early plant development?                                | Valle et al., 1997   |

The results summarized in Table 1 show that the regulatory role of any one individual HD-Zip gene product can not be predicted based upon which HD-Zip subfamily a gene is placed. The HD-Zip subfamilies were determined by alignment and comparison of the amino acid sequences found in the HD and leucine zipper domains (See Aso et al. 1999, Fig. 2 for the most recent HD-Zip region alignments). Conservation of HD-Zip regulatory function can be expected in many cases to depend on the extent of amino acid sequence similarity found in conserved protein domains found outside of the HD-Zip regions. That is, HD-Zip gene products from different plant species that are functional homologues to each other (i.e., perform the same biological function) are expected to not only share conserved HD-Zip regions, but show more amino acid sequence similarity over the entire length of the protein compared to other HD-Zip proteins that perform different biological roles. Thus, it is not surprising that the data summarized in Table 1 shows that there is no consistent pattern as to the inferred biological functions for individual HD-Zip I, II, III and IV gene products. Nonetheless, there is still wide - spread speculation that the proteins of the HD-Zip super family play important roles in regulating plant development (See, for example, Chan et al. 1998).

Given the agronomic importance of plant growth, there is a strong need for transgene compositions containing gene sequences which when expressed in a transgenic plant allow the growth of the plant to be modulated. The compositions and methods of the present invention allow useful transgenic plants to be created wherein cell division is modulated due to expression of a *REVOLUTA* transgene. Compositions and methods, such as those provided by the present invention, allow

for controlling (including increasing) plant size via the ability to control (e.g., increase) the number of cell divisions in specific plant tissues.

5 The inventive compositions and methods provide another way to meet the ever-increasing need for food and plant fiber due to the continual increase in world population and the desire to improve the standard of living throughout the world. Despite the recent agricultural success in keeping food production abreast of population growth, there are over 800 million people in the world today who are chronically undernourished and 180 million children who are severely underweight for their age. 400 million women of childbearing age suffer from iron deficiency and the anemia it causes results in infant and maternal mortality. An extra 2 billion persons will have to be fed by the year 2020, and so many more that will be chronically undernourished. For example, in forest trees the cambium is responsible for girth growth. In tomato (and many other plants), the ovary walls are responsible, not only for mature fruit size but also for soluble solids content. In cereal crops, the endosperm contributes to seed size. In some cases, increased yield may be achieved by lengthening a fruit-bearing structure, such as maize where the ear is a modified stem whose length determines the number of kernels. Moreover, possible use of transgenic plants as a source of pharmaceuticals and industrial products may require control of organ specific growth modulation.

10 20 The potential of a designer growth-increasing or growth-decreasing technology in agriculture is very large. A yield increase as small as a few percent would be highly desirable in each crop. Conversely, in many fruit crops it is highly desirable to have seedless fruits. The present invention, in addition to being applicable to all existing plant varieties, could also change the way crops are bred. Plant breeding could concentrate on stress and pest resistance as well as nutritional and taste quality. The growth-conferring quality of the present invention could then be introduced in advanced elite lines to boost their yield potential.

#### Summary of the Invention

30 The present invention provides compositions and methods for modulating plant cell division by altering the level of *REVOLUTA* protein within transgenic plants. In particular, the present invention relates to the use of *REVOLUTA* transgenes to increase or decrease the expression of biologically active *REVOLUTA* protein and thereby modulate plant cell division.

35 In one embodiment of the present invention, a DNA molecule comprising a polynucleotide sequence that encodes a *REVOLUTA* protein that is at least about

70% identical to the *Arabidopsis REVOLUTA* protein sequence [SEQ ID NO:2] is provided. According to certain embodiments, the protein is at least about 80% identical to SEQ ID NO:2. Preferably, the DNA molecule comprises a polynucleotide sequence that encodes a *REVOLUTA* protein that has the same biological activity as the *Arabidopsis REVOLUTA* protein, i.e. it modulates plant cell division. According to certain preferred embodiments, the protein encoded by the DNA molecule confers a REV phenotype.

According to certain preferred embodiments, the invention provides a polynucleotide at least 80% identical to at least one exon of the *Arabidopsis REVOLUTA* nucleic acid sequence, selected from exons 3-18 (nucleotides 3670-3743, 3822-3912, 4004-4099, 4187-4300, 4383-4466, 4542-4697, 4786-4860, 4942-5048, 5132-5306, 5394-5582, 5668-5748, 5834-5968, 6051-6388, 6477-6585, 6663-6812, and 6890-7045 of SEQ ID NO:1).

Similarly, the present invention provides an isolated DNA molecule comprising a polynucleotide sequence at least about 80% identical to at least one exon of the tomato Rev gene, or a polynucleotide, selected from the group consisting of SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, and SEQ ID NO:196.

According to other embodiments, the present invention provides an isolated DNA molecule comprising a polynucleotide sequence which encodes a protein comprised of an amino acid sequence at least about 95% identical to certain regions of a REV gene product, especially a sequence selected from the group consisting of SEQ ID NO:130; SEQ ID NO:131; SEQ ID NO:132; SEQ ID NO:133; SEQ ID NO:134; SEQ ID NO:135; SEQ ID NO:136; and SEQ ID NO:137.

According to certain embodiments of the present invention, the encoded protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:164, SEQ ID NO:171 and SEQ ID NO:173.

The present invention also provides embodiments where the polynucleotide sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:169, SEQ ID NO:170, and SEQ ID NO:172.

Another embodiment of the present invention provides a polynucleotide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11, wherein the polynucleotide sequence encodes a mutant *revoluta* protein that is defective in normal regulation of cell division as compared to the wild-type *REV* protein. The present invention also provides an isolated protein comprising an amino sequence selected from the group consisting of wild-type *Arabidopsis REVOLUTA* protein [SEQ ID NO:2], and *revoluta* mutant proteins designated *rev-1*, *rev-2,4*, *rev-3*, *rev-5* and *rev-6*, as set forth in FIGURE 3. Other inventive *REVOLUTA* proteins are provided that comprise amino acid sequences that are at least about 70% identical to the *Arabidopsis REV* amino acid sequence as set forth in SEQ ID NO:2.

In yet another embodiment of the present invention, transgenic vectors are provided that comprise a replicon and a *REVOLUTA* transgene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11. Preferably, the transgenic vectors of the present invention also include either a constitutive or a tissue specific promoter region that directs the expression of the *REVOLUTA* transgene, and a polyA addition region. The expression of the *REVOLUTA* transgene results in a modulation of cell division in plant cells transformed with the inventive transgenic vector. In another embodiment the transgene vectors of the present invention contain a polynucleotide sequence comprising a sequence that encodes a protein that is at least about 70% identical to the wild-type *Arabidopsis REVOLUTA* protein sequence [SEQ ID NO:2]. In yet another embodiment of the present invention, the polynucleotide sequence encodes a protein that has a peptide region that is at least about 70% identical to a region of wild-type *Arabidopsis REVOLUTA* protein that is defined by amino acid 114 up to and including amino acid 842 of the protein in SEQ ID NO:2.

In another aspect of the invention transgenic plants are provided that comprise at least one of the above described polynucleotides and *REVOLUTA* transgene vectors. In one aspect of the invention the transformed plants exhibit a modulation of cell division, as compared to untransformed plants, when the inventive *REVOLUTA* transgenes are expressed within the transformed cells. In particular, the present invention provides transgenic plants (genetically transformed with a nucleic acid sequence comprising a *REVOLUTA* transgene selected from the group consisting of a sense gene, an anti-sense gene, an inverted repeat gene or a ribozyme gene) that

exhibit modulated cell division as compared to a control population of untransformed plants. The transgenic plants of the present invention can be further propagated to generate genetically true-breeding populations of plants possessing the modulated cell division trait. Further, the transgenic plants of the present invention can be  
5 crossed with other plant varieties, having one or more desirable phenotypic traits, such as for example, stress and pest resistance or nutritional and taste quality, to generate novel plants possessing the aforementioned desirable traits in combination with the transgenic trait that modulates cell division.

In another aspect, the present invention provides methods for modulating  
10 plant cell division comprising the steps of introducing a *REVOLUTA* transgene into at least one plant cell. The methods of the present invention include the further step of regenerating one or more plants from the cells transformed with the *REVOLUTA* transgene. Optionally, the regenerated plants may be screened to identify plants exhibiting modulated cell division as compared to untransformed plants. Transgenic  
15 plants having a modulated cell division have at least one plant organ or tissue that is larger or smaller in size (due to an increased or decreased number of cells) as compared to untransformed plants. The presently preferred *REVOLUTA* transgenes for practicing the inventive methods are the polynucleotide sequences previously described above. In addition, the inventive methods can be practiced with a  
20 *REVOLUTA* transgene that is selected from the group consisting of a sense gene, an anti-sense gene, an inverted repeat gene and a *REVOLUTA* ribozyme gene.

In yet another embodiment of the present invention, a method is provided for isolating a *REVOLUTA* gene from a plant. More specifically the inventive method comprises the steps of:

- 25 a) amplifying a plant polynucleotide sequence using a forward and a reverse oligonucleotide primer, said primers encoding an amino acid sequence that is at least about 50% identical to a corresponding amino acid sequence found in SEQ ID NO:2;
- b) hybridizing said amplified plant polynucleotide to a library of recombinant plant DNA clones;
- 30 c) isolating a DNA molecule from a recombinant DNA clone that hybridizes to said amplified plant polynucleotide;
- d) transforming with a vector comprising said amplified plant polynucleotide or said DNA molecule into a plant; and
- e) determining that cell division in the transformed plant is modulated by  
35 comparing the transformed plant with an untransformed plant.

Preferably, the DNA isolated by the inventive method encodes an amino acid sequence that is at least about 70% identical to an amino acid sequence within the *REVOLUTA* protein having the sequence of SEQ ID NO: 2. In addition, modulation of cell division is preferably determined by comparing the size of a transgenic plant, tissue, or organ thereof with a corresponding untransformed plant, tissue or organ. An increase or decrease in the number of cells in the transgenic plant, tissue or organ as compared to the untransformed plant, tissue or organ indicates that the isolated DNA molecule encodes a *REVOLUTA* gene of the present invention.

The present invention also provides a plant comprising a chimeric plant gene having a promoter sequence that functions in plant cells; a coding sequence which causes the production of RNA encoding a fusion polypeptide or an RNA transcript that causes homologous gene suppression such that expression of the chimeric plant gene modulates plant growth, e.g. by modulating cell division; and a 3' non-translated region that encodes a polyadenylation signal which functions in plant cells to cause the addition of polyadenylated nucleotides to the 3' end of the RNA, where the promoter is heterologous with respect to the coding sequence and adapted to cause sufficient expression of the chimeric plant gene to modulate plant growth of a plant transformed with the chimeric gene.

The present invention moreover provides an isolated DNA molecule consisting of a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 70% identical to SEQ ID NO: 2, wherein said protein has a *REV* biological activity, and wherein said DNA molecule can optionally contain intervening sequences that do not encode said protein.

The present invention also provides an isolated DNA molecule consisting of a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 80% identical to SEQ ID NO: 2, wherein said protein has a *REV* biological activity, and wherein said DNA molecule can optionally contain intervening sequences that do not encode said protein.

The present invention also provides for a DNA sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:169, SEQ ID NO:170, and SEQ ID NO:172.

The present invention further provides an isolated protein comprising an amino sequence that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has a biological activity that is the same as SEQ ID NO: 2.

The present invention also provides a vector comprising a replicon and a polynucleotide comprising a sequence that encodes a protein that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has the biological activity of SEQ ID NO: 2.

The present invention further provides a transgenic plant comprising a *REVOLUTA* transgene, said transgenic plant comprising cells having modulated cell division as compared to an untransformed plant, wherein said *REVOLUTA* transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO: 2, and wherein said protein has the biological activity of SEQ ID NO: 2.

The present invention moreover provides a method for modulating plant cell division comprising introducing into a plant cell a *REVOLUTA* transgene, wherein said transgene comprises a polynucleotide from a *REVOLUTA* gene, wherein said *REVOLUTA* gene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO: 2 and has the biological activity of SEQ ID NO: 2, an inverted repeat of a polynucleotide sequence from said *REVOLUTA* gene, an anti-sense orientation of a polynucleotide from said *REVOLUTA* gene or a ribozyme comprising a polynucleotide sequence from said *REVOLUTA* gene.

The present invention further provides a method of isolating a *REVOLUTA* gene comprising amplifying a plant polynucleotide sequence using a forward and a reverse oligonucleotide primer, said primers selected from a polynucleotide sequence, or its complementary sequence, that encodes a protein that is at least about 70% identical to SEQ ID NO: 2 and which protein has the biological activity of SEQ ID NO: 2, hybridizing said amplified plant polynucleotide to a library of recombinant plant DNA clones, isolating a DNA molecule from a recombinant DNA clone that hybridizes to said amplified plant polynucleotide, transforming with a vector comprising said amplified plant polynucleotide or said DNA molecule into a plant cell and determining that cell division in the transformed plant is modulated by comparing the transformed plant cell with an untransformed plant cell.

The present invention also provides a plant comprising a chimeric plant gene having a promoter sequence which functions in plant cells, a coding sequence which causes the production of RNA encoding a fusion polypeptide which modulates plant growth; and wherein said fusion polypeptide comprises SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8,

SEQ ID NO: 10 and SEQ ID NO: 12 or an isolated protein comprising an amino sequence that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has a biological activity that is the same as SEQ ID NO: 2, and a 3' non-translated region which encodes a polyadenylation signal which functions in plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, where the promoter is heterologous with respect to the coding sequence and adapted to cause sufficient expression of the fusion polypeptide to modulate plant growth of a plant transformed with said gene.

The present invention further provides an isolated polynucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, and SEQ ID NO: 125.

The present invention also provides a transgenic seed of, or obtained from, the transgenic plant hereinbefore described, wherein the seed comprises a DNA molecule, a protein, a vector or a transformed plant cell as hereinbefore described.

The present invention additionally provides an isolated nucleotide sequence comprising a heterologous promoter and a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 70% identical to SEQ ID NO:2, wherein said protein has a REV biological activity.

#### Brief Description of the Drawings

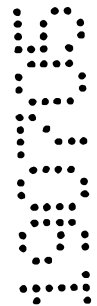
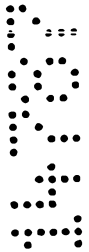
The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 presents a genetic map of a 1.95 Mb region of chromosome 5 from *Arabidopsis thaliana*.

FIGURE 2 shows an expanded region of the genetic map presented in Fig. 1 and the location of the *REVOLUTA* gene as determined by genetic crosses using simple sequence length polymorphism markers.

FIGURE 3 shows the complete protein sequence [SEQ ID NO: 2] deduced from a *REVOLUTA* gene [SEQ ID NO: 1] isolated from *Arabidopsis thaliana*. Open triangles indicate splice junctions between the exon and intron nucleotide sequences in the DNA sequence [SEQ ID NO: 1] that encodes *REVOLUTA*. Conserved homeodomain and leucine

zipper motifs are indicated in shaded boxes. The underlined amino acid region indicates a second potential leucine zipper motif.



Intron-exon junctions are indicated by an inverted triangle. The *rev-4* mutant amino acid C-terminal extension is indicated in bold under the wild-type sequence.

FIGURE 4 shows an alignment of HD-Zip III protein family of *Arabidopsis*. Protein sequences were aligned using a multiple sequence alignment program (http://dot.imgen.bcm.tmc.edu:9331/multi-align/multi-align.html) and boxshade (http://www.ch.embnet.org/software/BOX\_form.html). Residues highlighted in black are identical; conserved residues are highlighted in gray.

FIGURE 5 shows the partial complementation of *rev-1* mutants. Panel (A) shows a comparison of *rev-1* transgenic plants transformed with either the empty vector (left) or with the 5' REV construct containing the wild-type REV gene under the control of the endogenous promoter (right). Panel (B) shows a close-up of the *rev-1* vector-transformed plant showing empty axils and enlarged flowers characteristic of *rev* mutant plants. Panel (C) shows a close-up of the *rev-1* plant transformed with the 5' REV construct. Many of the leaf axils have axillary shoots and the flowers are smaller, similar to wild type. Panel (D) shows a control plant (empty vector) for the inverted-repeat constructs, Columbia ecotype. Panels (E-G) show Columbia plants transformed with the 35S-REVIR construct, showing some characteristics of the *Rev-* phenotype including empty axils (arrowheads). The flowers were similar in size to wild-type flowers, not enlarged like *rev* flowers. Panel (H) shows a comparison of 35S-REVIR transgenic plant (left) to a parent Columbia plant (right).

FIGURE 6 shows a semi-quantitative RT-PCR analysis of *REV* mRNA levels.

FIGURE 7 shows the expression pattern of *REV* mRNA. Panels (A-C) show longitudinal sections through inflorescence apices. Arrow indicates an axillary meristem in (A) showing *REV* expression. (B) is an axillary inflorescence meristem. The numbers indicate the stage of the developing flower primordia. im, inflorescence meristem; g, gynoecium; s, stamen; se, sepal. Panel (D) shows a longitudinal section of stage 10 gynoecium. op, ovule primordia; st, stigma. Panel (E) shows a longitudinal section of a young cauline leaf. Panel (F) shows a longitudinal section of a stage 4 flower showing highest expression in anthers and gynoecium. Panel (G) shows transverse section through a stage 9 flower showing highest expression in anthers and gynoecium. t, tapetum; PMC, pollen mother cells. Panel (H) shows a longitudinal section through a stage 8 flower showing *REV* expression in the stamens and petal. pe, petal primordia. Panel (I) shows a longitudinal section through a stage

9 flower showing REV expression in the stamens and petal. Panel (J) shows a longitudinal section through a developing seed, showing expression in the endosperm. Panel (K) shows a longitudinal section through a developing seed, showing REV expression in the endosperm. Arrow indicates the suspensor. Panel  
5 (L) shows a longitudinal section through a developing seed, showing expression in an early heart stage embryo. Panel (M) shows Histone H4 expression in a developing torpedo stage embryo. Panel (N) shows REV sense probe in a developing late heart stage embryo. Panel (O) shows a longitudinal section of an inflorescence apex with REV sense probe. Panel (P) shows a cross-section of a stem probed with REV  
10 antisense. co, cortex. Panel (Q) shows a cross-section of a stem with REV sense probe. Panel (R) shows a bright-field image of a cross-section of a rev-1 stem stained in safranin O and fast green FCF as described in Talbert et al., (1995). Panel (S) shows a bright-field image of a cross-section of a wild-type stem.

FIGURE 8 shows Histone H4 and FIL expression in rev-1 and wild-type  
15 tissue. Panel (A) shows Histone H4 expression in a longitudinal section of a wild-type inflorescence apex. Panel (B) shows Histone H4 expression in a longitudinal section of a rev-1 inflorescence apex. Panel (C) shows an enlarged view from A. Panel (D) shows an enlarged view from B, showing the increased number of H4  
20 expressing cells in the adaxial side of the leaf. Panel (E) shows FIL expression in a transverse section of a wild-type inflorescence meristem (im), including stamen (st) and sepal (se) primordia of stage 3 and 5 flowers. Panel (F) shows transverse section through wild-type flower showing FIL expression in the abaxial sides of carpel  
25 valves (va) and petals (pe). Panel (G-H) shows FIL expression in a longitudinal section through rev-1 inflorescence apex, and developing stage 7 flower in the abaxial side of developing stamens (st). Panel (I) shows FIL expression in a  
30 transverse section through a rev-1 flower showing FIL expression in the abaxial sides of valves (va) and petal (pe).

FIGURE 9 shows rev double mutants. Panel (A) shows a rev lfy double  
35 mutant with severely shortened inflorescence terminating in a brush of filaments. The plant also has revolute leaves. Inset: an enlarged view of small filamentous appendages found on the stem of rev lfy plants. Panel (B) shows a rev fil double mutant with severely shortened inflorescence and revolute leaves. Panel (C) shows small filamentous appendages present on the stem of rev lfy plants which resemble a structure frequently seen in axils of rev mutant plants. Panel (D) shows an axil of  
35 rev-1 mutant plant with small filamentous appendage. Panel (E) shows the

inflorescence structure of a rev lfy plant with a cluster of flowerless filaments that can have stellate trichomes or carpelloid features. Panel (F) shows the inflorescence structure of a rev fil plant with a cluster of smooth flowerless filaments. Panel (G) shows an SEM of a rev fil inflorescence. Bar is 1mm. Panel (H) shows an SEM of a rev lfy inflorescence with carpelloid features. Panel (I) shows an SEM of a rev lfy inflorescence.

FIGURE 10 shows shows a comparison of seed size produced by a typical plant transformed with the empty vector (C) and in a plant transformed with the 35S-REV gene (LS).

FIGURE 11 provides examples of rosette and leaf sizes produced by plants transformed with the empty vector (C) and plants transformed with the 35S-REV gene (LS).

FIGURE 12 provides examples of inflorescence stem and cauline leaf sizes produced by plants transformed with the empty vector (C) and plants transformed with the 35S-REV gene (LS).

Detailed Description of the Preferred Embodiments

As used herein, the terms "amino acid" and "amino acids" refer to all naturally occurring L- $\alpha$ -amino acids or their residues. The amino acids are identified by either the single-letter or three-letter designations:

|    |     |   |               |     |   |               |
|----|-----|---|---------------|-----|---|---------------|
| 20 | Asp | D | aspartic acid | Ile | I | isoleucine    |
|    | Thr | T | threonine     | Leu | L | leucine       |
|    | Ser | S | serine        | Tyr | Y | tyrosine      |
|    | Glu | E | glutamic acid | Phe | F | phenylalanine |
|    | Pro | P | proline       | His | H | histidine     |
| 25 | Gly | G | glycine       | Lys | K | lysine        |
|    | Ala | A | alanine       | Arg | R | arginine      |
|    | Cys | C | cysteine      | Trp | W | tryptophan    |
|    | Val | V | valine        | Gln | Q | glutamine     |
|    | Met | M | methionine    | Asn | N | asparagine    |

As used herein, the term "nucleotide" means a monomeric unit of DNA or RNA containing a sugar moiety (pentose), a phosphate and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of pentose) and that combination of base and sugar is called a nucleoside. The base characterizes the nucleotide with the four bases of DNA being adenine ("A"), guanine ("G"), cytosine ("C") and thymine ("T"). Inosine ("I") is a synthetic

base that can be used to substitute for any of the four, naturally-occurring bases (A, C, G or T). The four RNA bases are A, G, C and uracil ("U"). The nucleotide sequences described herein comprise a linear array of nucleotides connected by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses.

5           The terms "DNA sequence encoding," "DNA encoding" and "nucleic acid encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the translated polypeptide chain. The DNA sequence thus codes for the amino acid sequence.

10           The term "recombinant DNA molecule" refers to any DNA molecule that has been created by the joining together of two or more DNA molecules *in vitro* into a recombinant molecule. A "library of recombinant DNA molecules" refers to any clone bank comprising a number of different recombinant DNA molecules wherein the recombinant DNA molecules comprise a replicable vector and DNA sequence  
15 derived from a source organism.

"Oligonucleotide" refers to short length single or double stranded sequences of deoxyribonucleotides linked via phosphodiester bonds. The oligonucleotides are chemically synthesized by known methods and purified, for example, on polyacrylamide gels.

20           The term "plant" includes whole plants, plant organs (e.g., leaves, stems, flowers, roots, etc.), seeds and plant cells (including tissue culture cells) and progeny of same. The class of plants which can be used in the method of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants, as well as  
25 certain lower plants such as algae. It includes plants of a variety of ploidy levels, including polyploid, diploid and haploid.

A "heterologous sequence" is one that originates from a foreign species, or, if from the same species, is substantially modified from its original form. For example, a heterologous promoter operably linked to a structural gene is from a species  
30 different from that from which the structural gene was derived, or, if from the same species, is substantially modified from its original form.

The terms "*REVOLUTA* gene" or "*REVOLUTA* transgene" are used herein to mean any polynucleotide sequence that encodes or facilitates the expression and/or production of a *REVOLUTA* protein. Thus, the terms "*REVOLUTA* gene" and  
35 "*REVOLUTA* transgene" include sequences that flank the *REVOLUTA* protein

encoding sequences. More specifically, the terms "*REVOLUTA* gene" and "*REVOLUTA* transgene" include the nucleotide sequences that are protein encoding sequences (exons), intervening sequences (introns), the flanking 5' and 3' DNA regions that contain sequences required for the normal expression of the *REVOLUTA* gene (i.e. the promoter and polyA addition regions, respectively, and any enhancer sequences).

The terms "*REVOLUTA* protein," "*REVOLUTA* homolog" or "*REVOLUTA* ortholog" are used herein to mean proteins having the ability to regulate plant cell division (when utilized in the practice of the methods of the present invention) and that have an amino acid sequence that is at least about 70% identical, more preferably at least about 75% identical, most preferably at least about 80% identical to amino acid residues 1 to 842, inclusive, of SEQ ID NO:2. A *REVOLUTA* protein of the present invention is also at least about 70% identical, more preferably at least about 75% identical, most preferably at least about 80% identical to an amino acid region defined by amino acids 114 to 842, inclusive, of SEQ ID NO:2. A *REVOLUTA* protein of the present invention is also identified as a protein that is at least about 70% identical, more preferably at least about 75% identical, most preferably at least about 80% identical to an amino acid region defined by amino acids 433 to 842, inclusive, of SEQ ID NO:2. A *REVOLUTA* protein of the present invention is also identified as a protein that is at least about 70% identical, more preferably at least about 75% identical, most preferably at least about 80% identical to an amino acid region defined by amino acids 611 to 745, inclusive, of SEQ ID NO:2.

Amino acid sequence identity can be determined, for example, in the following manner. The portion of the amino acid sequence of *REVOLUTA* (shown in FIGURE 3) extending from amino acid 1 up to and including amino acid 842 is used to search a nucleic acid sequence database, such as the Genbank database, using the program BLASTP version 2.0.9 (Altschul et al., 1997 *Nucleic Acids Res.* 25:3389-3402). Alternatively, the search can be performed with a *REVOLUTA* protein sequence extending from amino acid 114 up to and including amino acid 842, or amino acid 433 up to and including amino acid 842 or amino acid 611 up to and including 745 of SEQ ID NO:2. The program is used in the default mode. Those retrieved sequences that yield identity scores of at least about 70% when compared to any of the above identified regions of SEQ ID NO:2, are considered to be *REVOLUTA* proteins.

Sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a segment of at least  
5 about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted by local identity or similarity algorithms such as those described in Smith and Waterman,  
10 1981 *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch, 1970 *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, 1988 *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, BLASTP2.0.9, TBLASTN, FASTA, and TFASTA in the Wisconsin Genetics Software Package,  
15 Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.; Atschul et al., 1997), or by inspection.

The term "percent identity" means the percentage of amino acids or nucleotides that occupy the same relative position when two amino acid sequences, or two nucleic acid sequences are aligned side by side using the BLASTP2.0.9  
20 program at <http://www.ncbi.nlm.nih.gov/gorf/wblast2.cgi>. "Percent amino acid sequence identity," as used herein, is determined using the BLASTP2.0.9 program with the default matrix: BLOSUM62 (Open Gap = 11, Gap extension penalty = 1). The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the  
25 number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Neither N- or C- terminal extensions nor insertions shall be construed as reducing sequence identity.

The term "percent similarity" is a statistical measure of the degree of  
30 relatedness of two compared protein sequences. The percent similarity is calculated by a computer program that assigns a numerical value to each compared pair of amino acids based on chemical similarity (e.g., whether the compared amino acids are acidic, basic, hydrophobic, aromatic, etc.) and/or evolutionary distance as measured by the minimum number of base pair changes that would be required to  
35 convert a codon encoding one member of a pair of compared amino acids to a codon

encoding the other member of the pair. Calculations are made after a best fit alignment of the two sequences have been made empirically by iterative comparison of all possible alignments. (Henikoff et al., 1992 *Proc. Natl. Acad. Sci. USA* 89:10915-10919).

5           The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 60% sequence identity, preferably at least 70%, more preferably at least 80% and most preferably at least 90%, compared to a reference sequence using the programs described above (preferably BLAST2) using standard parameters. One of skill will recognize that  
10 these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, preferably at least 70%, more preferably at least  
15 80%. Polypeptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and  
20 isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side  
25 chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other, or a third nucleic acid, under stringent  
30 conditions. Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5°C. lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.  
35 Typically, stringent conditions will be those in which the salt concentration is about

0.02 molar at pH 7 and the temperature is at least about 60°C. Moderately stringent conditions are more preferred when heterologous hybridizations are performed between polynucleotide sequences isolated from different species.

5 Exemplary high stringency hybridization and wash conditions useful for identifying (by Southern blotting) additional nucleic acid molecules encoding *REVOLUTA*-homologues are: hybridization at 68°C in 0.25 M Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.2) containing 1 mM Na<sub>2</sub>EDTA, 20% sodium dodecyl sulfate; washing (three washes of twenty minutes each at 65°C) is conducted in 20 mM Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.2) containing 1 mM Na<sub>2</sub>EDTA, 1% (w/v) sodium dodecyl sulfate.

10 Exemplary moderate stringency hybridization and wash conditions useful for identifying (by Southern blotting) additional nucleic acid molecules encoding *REVOLUTA*-homologues are: hybridization at 45°C in 0.25 M Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.2) containing 1 mM Na<sub>2</sub>EDTA, 20% sodium dodecyl sulfate; washing is conducted in 5X SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C. The  
15 abbreviation "SSC" refers to a buffer used in nucleic acid hybridization solutions. One liter of the 20X (twenty times concentrate) stock SSC buffer solution (pH 7.0) contains 175.3 g sodium chloride and 88.2 g sodium citrate.

In the case of both expression of transgenes and inhibition of endogenous genes (e.g., by antisense, ribozyme, inverted repeat, sense suppression or transgene  
20 directed homologous recombination) one of skill will recognize that the inserted polynucleotide sequence need not be identical and may be "substantially identical" to a sequence of the gene from which it was derived. As explained below, these variants are specifically covered by this term.

In the case where the inserted polynucleotide sequence is transcribed and  
25 translated to produce a functional polypeptide, one of skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same polypeptide. These variants are specifically covered by the terms "*REVOLUTA* gene" and "*REVOLUTA* transgene." In addition, these terms specifically includes those full length sequences substantially identical (determined as described below)  
30 with a gene sequence and that encode a proteins that retain the function of the *REVOLUTA* gene product.

In the case of polynucleotides used to inhibit expression of an endogenous gene, the introduced sequence need not be perfectly identical to a sequence of the  
35 target endogenous gene. The introduced polynucleotide sequence will typically be at least substantially identical (as determined below) to the target endogenous sequence.

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described above. The term "complementary to" is used herein to mean that the complementary sequence is identical to all or a portion of a reference polynucleotide sequence.

The term "modulation of cell division" means any change in the number of cell divisions that occur in a plant or any plant tissue or plant organ as compared to a control set of plants. For example, modulation of cell division in a transgenic plant of the present invention may result in leaf that is larger than a leaf on an untransformed plant due to an increased number of leaf cells. Modulation of cell division may occur in one plant tissue or organ cell type or through out the plant depending upon the promoter that is responsible for the expression of a *REVOLUTA* transgene. In addition, modulation of cell division by a *REVOLUTA* transgene may result in a transgenic plant or tissue that has arrested plant growth due to a cessation or diminution of cell division. Modulation of cell division can be determined by a variety of methods well known in the art of plant anatomy (see, for example, Esau, *Anatomy of Seed Plants* [2nd ed.] 1977 John Wiley & Sons, Inc. New York). For example, the overall mass of a transgenic plant may be determined or organ or tissue cell counts may be conducted whereby all of the cells in a representative tissue or organ cross-section are counted. Where the *REVOLUTA* transgene is expressed in the embryo, modulation of cell division may be also be determined by measuring the size of the seed containing the transgenic embryo as a measure of the number of cells within the embryo.

The terms "alteration", "amino acid sequence alteration", "variant" and "amino acid sequence variant" refer to *REVOLUTA* proteins with some differences in their amino acid sequences as compared to the corresponding, native, i.e., naturally-occurring, *REVOLUTA* protein. Ordinarily, the variants will possess at least about 67% identity with the corresponding native *REVOLUTA* protein, and preferably, they will be at least about 80% identical with the corresponding, native *REVOLUTA* protein. The amino acid sequence variants of the *REVOLUTA* protein falling within this invention possess substitutions, deletions, and/or insertions at certain positions. Sequence variants of *REVOLUTA* may be used to attain desired enhanced or reduced DNA binding, protein oligomerization, ability to engage in specific protein-protein interactions or modifications, transcriptional regulation activity, or modified ability to regulate plant cell division.

Substitutional *REVOLUTA* protein variants are those that have at least one amino acid residue in the native *REVOLUTA* protein sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule. Substantial changes in the activity of the *REVOLUTA* protein molecules of the present invention may be obtained by substituting an amino acid with another whose side chain is significantly different in charge and/or structure from that of the native amino acid. This type of substitution would be expected to affect the structure of the polypeptide backbone and/or the charge or hydrophobicity of the molecule in the area of the substitution.

Moderate changes in the functional activity of the *REVOLUTA* proteins of the present invention would be expected by substituting an amino acid with a side chain that is similar in charge and/or structure to that of the native molecule. This type of substitution, referred to as a conservative substitution, would not be expected to substantially alter either the structure of the polypeptide backbone or the charge or hydrophobicity of the molecule in the area of the substitution. However, it is predictable that even conservative amino acid substitutions may result in dramatic changes in protein function when such changes are made in amino acid positions that are critical for protein function.

Insertional *REVOLUTA* protein variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in the native *REVOLUTA* protein molecule. Immediately adjacent to an amino acid means connected to either the  $\alpha$ -carboxy or  $\alpha$ -amino functional group of the amino acid. The insertion may be one or more amino acids. Ordinarily, the insertion will consist of one or two conservative amino acids. Amino acids similar in charge and/or structure to the amino acids adjacent to the site of insertion are defined as conservative. Alternatively, this invention includes insertion of an amino acid with a charge and/or structure that is substantially different from the amino acids adjacent to the site of insertion.

Deletional variants are those where one or more amino acids in the native *REVOLUTA* protein molecules have been removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the *REVOLUTA* protein molecule.

The terms "biological activity", "biologically active", "activity", "active" "biological function", "REV biological activity" and "functionality" refer to the ability of the *REVOLUTA* proteins of the present invention to dimerize (or otherwise assemble into protein oligomers), or the ability to modulate or otherwise effect the dimerization of native wild type (e.g., endogenous) *REVOLUTA* proteins. However the terms are also intended to encompass the ability of the *REVOLUTA* proteins of the present invention to bind and/or interact with other molecules and which binding and/or interaction event(s) mediate plant cell division and ultimately confer a *REV* phenotype, or the ability to modulate or otherwise effect the binding and/or interaction of other molecules with native wild type *REVOLUTA* proteins (e.g., endogenous) and which binding and/or interaction event(s) mediate plant cell division and ultimately confer a *REV* phenotype. Examples of such molecules include, for example, other members of the HD-Zip III family.

Biological activity as used herein in reference to a nucleic acid of the invention is intended to refer to the ability the nucleic acid to modulate or effect the transcription and/or translation of the nucleic acid and/or ultimately confer a *REV* phenotype. Biological activity as used herein in reference to a nucleic acid of the invention is also intended to encompass the ability the nucleic acid to modulate or affect the transcription and/or translation of a native wild type *REVOLUTA* (e.g., endogenous) nucleic acid and/or ultimately confer a *REV* phenotype.

*REV* phenotype as used herein is intended to refer to a phenotype conferred by a *REV* nucleic acid or protein of the present invention and particularly encompasses the characteristic wherein an effect, relative to wild type, on organ or tissue size (e.g., increased size of seed, leaves, fruit, or root) is exhibited. Typically, a *REV* phenotype is determined by examination of the plant, where the number of cells contained in various tissues is compared to the number of cells in the corresponding tissues of a parental plant. Plants having a *REV* phenotype have a statistically significant change in the number of cells within a representative cross sectional area of the tissue.

The biological activities of *REVOLUTA* proteins of the present invention can be measured by a variety of methods well known in the art, such as: a transcription activity assay, a DNA binding assay, or a protein oligomerization assay. Such assays in the context of HD-Zip proteins, have been described in Sessa et al., 1993; 1997 *J. Mol. Biol.* 274:303-309; 1999; Gonzalez et al., 1997; and Palena et al., 1999 *Biochem. J.* 341:81-87 (contents of said publications incorporated herein by

reference). Amino acid sequence variants of the *REVOLUTA* proteins of the present invention may have desirable altered biological activity including, for example, increased or decreased binding affinity to DNA target sites, increased or decreased ability to form homo- and/or heter-protein oligomers, and altered regulation of target genes.

The terms "vector", "expression vector", refer to a piece of DNA, usually double-stranded, which may have inserted into it a piece of foreign DNA. The vector or replicon may be for example, of plasmid or viral origin. Vectors contain "replicon" polynucleotide sequences that facilitate the autonomous replication of the vector in a host cell. The term "replicon" in the context of this disclosure also includes sequence regions that target or otherwise facilitate the recombination of vector sequences into a host chromosome. In addition, while the foreign DNA may be inserted initially into a DNA virus vector, transformation of the viral vector DNA into a host cell may result in conversion of the viral DNA into a viral RNA vector molecule. Foreign DNA is defined as heterologous DNA, which is DNA not naturally found in the host cell, which, for example, replicates the vector molecule, encodes a selectable or screenable marker or transgene. The vector is used to transport the foreign or heterologous DNA into a suitable host cell. Once in the host cell, the vector can replicate independently of or coincidental with the host chromosomal DNA, and several copies of the vector and its inserted (foreign) DNA may be generated. Alternatively, the vector may target insert of the foreign DNA into a host chromosome. In addition, the vector also contains the necessary elements that permit transcription of the foreign DNA into a mRNA molecule or otherwise cause replication of the foreign DNA into multiple copies of RNA. Some expression vectors additionally contain sequence elements adjacent to the inserted foreign DNA that allow translation of the mRNA into a protein molecule. Many molecules of the mRNA and polypeptide encoded by the foreign DNA can thus be rapidly synthesized.

The term "transgene vector" refers to a vector that contains an inserted segment of foreign DNA, the "transgene," that is transcribed into mRNA or replicated as a RNA within a host cell. The term "transgene" refers not only to that portion of foreign DNA that is converted into RNA, but also those portions of the vector that are necessary for the transcription or replication of the RNA. In addition, a transgene need not necessarily comprise a polynucleotide sequence that contains an open reading frame capable of producing a protein.

The terms "transformed host cell," "transformed" and "transformation" refer to the introduction of DNA into a cell. The cell is termed a "host cell", and it may be a prokaryotic or a eukaryotic cell. Typical prokaryotic host cells include various strains of *E. coli*. Typical eukaryotic host cells are plant cells, such as maize cells, yeast cells, insect cells or animal cells. The introduced DNA is usually in the form of a vector containing an inserted piece of DNA. The introduced DNA sequence may be from the same species as the host cell or from a different species from the host cell, or it may be a hybrid DNA sequence, containing some foreign DNA and some DNA derived from the host species.

In addition to the native *REVOLUTA* amino acid sequences, sequence variants produced by deletions, substitutions, mutations and/or insertions are intended to be within the scope of the invention except insofar as limited by the prior art. The *REVOLUTA* acid sequence variants of this invention may be constructed by mutating the DNA sequences that encode the wild-type *REVOLUTA*, such as by using techniques commonly referred to as site-directed mutagenesis. Nucleic acid molecules encoding the *REVOLUTA* proteins of the present invention can be mutated by a variety of polymerase chain reaction (PCR) techniques well known to one of ordinary skill in the art. See, e.g., "PCR Strategies", M.A. Innis, D.H. Gelfand and J.J. Sninsky, eds., 1995, Academic Press, San Diego, CA (Chapter 14); "PCR Protocols: A Guide to Methods and Applications", M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds., Academic Press, NY (1990).

By way of non-limiting example, the two primer system utilized in the Transformer Site-Directed Mutagenesis kit from Clontech, may be employed for introducing site-directed mutants into the *REVOLUTA* genes of the present invention. Following denaturation of the target plasmid in this system, two primers are simultaneously annealed to the plasmid; one of these primers contains the desired site-directed mutation, the other contains a mutation at another point in the plasmid resulting in elimination of a restriction site. Second strand synthesis is then carried out, tightly linking these two mutations, and the resulting plasmids are transformed into a *mutS* strain of *E. coli*. Plasmid DNA is isolated from the transformed bacteria, restricted with the relevant restriction enzyme (thereby linearizing the unmutated plasmids), and then retransformed into *E. coli*. This system allows for generation of mutations directly in an expression plasmid, without the necessity of subcloning or generation of single-stranded phagemids. The tight linkage of the two mutations and the subsequent linearization of unmutated plasmids results in high mutation

efficiency and allows minimal screening. Following synthesis of the initial restriction site primer, this method requires the use of only one new primer type per mutation site. Rather than prepare each positional mutant separately, a set of "designed degenerate" oligonucleotide primers can be synthesized in order to  
5 introduce all of the desired mutations at a given site simultaneously. Transformants can be screened by sequencing the plasmid DNA through the mutagenized region to identify and sort mutant clones. Each mutant DNA can then be restricted and analyzed by electrophoresis on Mutation Detection Enhancement gel (J.T. Baker) to confirm that no other alterations in the sequence have occurred (by band shift  
10 comparison to the unmutagenized control). Alternatively, the entire DNA region can be sequenced to confirm that no additional mutational events have occurred outside of the targeted region.

The verified mutant duplexes in the pET (or other) overexpression vector can be employed to transform *E. coli* such as strain *E. coli* BL21(DE3)pLysS, for high  
15 level production of the mutant protein, and purification by standard protocols. The method of FAB-MS mapping can be employed to rapidly check the fidelity of mutant expression. This technique provides for sequencing segments throughout the whole protein and provides the necessary confidence in the sequence assignment. In a mapping experiment of this type, protein is digested with a protease (the choice will  
20 depend on the specific region to be modified since this segment is of prime interest and the remaining map should be identical to the map of unmutagenized protein). The set of cleavage fragments is fractionated by microbore HPLC (reversed phase or ion exchange, again depending on the specific region to be modified) to provide several peptides in each fraction, and the molecular weights of the peptides are  
25 determined by FAB-MS. The masses are then compared to the molecular weights of peptides expected from the digestion of the predicted sequence, and the correctness of the sequence quickly ascertained. Since this mutagenesis approach to protein modification is directed, sequencing of the altered peptide should not be necessary if the MS agrees with prediction. If necessary to verify a changed residue,  
30 CAD-tandem MS/MS can be employed to sequence the peptides of the mixture in question, or the target peptide purified for subtractive Edman degradation or carboxypeptidase Y digestion depending on the location of the modification.

In the design of a particular site directed mutagenesis, it is generally desirable to first make a non-conservative substitution (e.g., Ala for Cys, His or Glu) and  
35 determine if activity is greatly impaired as a consequence. The properties of the

mutagenized protein are then examined with particular attention to DNA target site binding and HD-Zip protein oligomerization which may be deduced by comparison to the properties of the native *REVOLUTA* protein using assays previously described. If the residue is by this means demonstrated to be important by activity impairment, or knockout, then conservative substitutions can be made, such as Asp for Glu to alter side chain length, Ser for Cys, or Arg for His. For hydrophobic segments, it is largely size that is usefully altered, although aromatics can also be substituted for alkyl side chains. Changes in the DNA binding and protein multimerization process will reveal which properties of *REVOLUTA* have been altered by the mutation.

Other site directed mutagenesis techniques may also be employed with the nucleotide sequences of the invention. For example, restriction endonuclease digestion of DNA followed by ligation may be used to generate deletion variants of *REVOLUTA*, as described in section 15.3 of Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Ed., 1989 Cold Spring Harbor Laboratory Press, New York, NY). A similar strategy may be used to construct insertion variants, as described in section 15.3 of Sambrook et al., *supra*. More recently Zhu et al. (1999, *Proc. Natl. Acad. Sci. USA* 96:8768-8773) have devised a method of targeting mutations to plant genes *in vivo* using chimeric RNA/DNA oligonucleotides.

Oligonucleotide-directed mutagenesis may also be employed for preparing substitution variants of this invention. It may also be used to conveniently prepare the deletion and insertion variants of this invention. This technique is well known in the art as described by Adelman et al. (1983 *DNA* 2:183); Sambrook et al., *supra*; "Current Protocols in Molecular Biology", 1991, Wiley (NY), F.T. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.D. Seidman, J.A. Smith and K. Struhl, eds.

Generally, oligonucleotides of at least 25 nucleotides in length are used to insert, delete or substitute two or more nucleotides in the nucleic acid molecules encoding *REVOLUTA* proteins of the invention. An optimal oligonucleotide will have 12 to 15 perfectly matched nucleotides on either side of the nucleotides coding for the mutation. To mutagenize nucleic acids encoding wild-type *REVOLUTA* genes of the invention, the oligonucleotide is annealed to the single-stranded DNA template molecule under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of *E. coli* DNA polymerase I, is then added. This enzyme uses the oligonucleotide as a primer to complete the synthesis of the mutation-bearing strand of DNA. Thus, a heteroduplex molecule is formed such that one strand of DNA encodes the wild-type *REVOLUTA* inserted in the vector, and the

second strand of DNA encodes the mutated form of the *REVOLUTA* inserted into the same vector. This heteroduplex molecule is then transformed into a suitable host cell.

5 Mutants with more than one amino acid substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If however, the amino acids are located some distance from each other (separated by more than ten amino acids, for example) it is more difficult to generate a single oligonucleotide that encodes all of the desired  
10 changes. Instead, one of two alternative methods may be employed. In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions. An alternative method  
15 involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type *REVOLUTA* DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first  
20 round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

#### 25 Transgenic Plants

Transgenic plants can be obtained, for example, by transferring transgenic vectors (e.g. plasmids, virus etc.) that encode *REVOLUTA* into a plant. Preferably, when the vector is a plasmid the vector also includes a selectable marker gene, e.g., the kan gene encoding resistance to kanamycin. The most common method of plant  
30 transformation is performed by cloning a target transgene into a plant transformation vector that is then transformed into *Agrobacterium tumifaciens* containing a helper Ti-plasmid as described in Hoeckema et al., (1983 *Nature* 303:179-181). The *Agrobacterium* cells containing the transgene vector are incubated with leaf slices of the plant to be transformed as described by An et al., 1986 *Plant Physiology*  
35 81:301-305 (See also Hooykaas, 1989 *Plant Mol. Biol.* 13:327-336). Transformation

of cultured plant host cells is normally accomplished through *Agrobacterium tumifaciens*, as described above. Cultures of host cells that do not have rigid cell membrane barriers are usually transformed using the calcium phosphate method as originally described by Graham et al. (1978 *Virology* 52:546) and modified as described in sections 16.32-16.37 of Sambrook et al., *supra*. However, other methods for introducing DNA into cells such as Polybrene (Kawai et al., 1984 *Mol. Cell. Biol.* 4:1172), protoplast fusion (Schaffner, 1980 *Proc. Natl. Acad. Sci. USA* 77:2163), electroporation (Neumann et al., 1982 *EMBO J.* 1:841), and direct microinjection into nuclei (Capecchi, 1980 *Cell* 22:479) may also be used.

10 Transformed plant calli may be selected through the selectable marker by growing the cells on a medium containing, e.g., kanamycin, and appropriate amounts of phytohormone such as naphthalene acetic acid and benzyladenine for callus and shoot induction. The plant cells may then be regenerated and the resulting plants transferred to soil using techniques well known to those skilled in the art.

15 In addition to the methods described above, a large number of methods are known in the art for transferring cloned DNA into a wide variety of plant species, including gymnosperms, angiosperms, monocots and dicots (see, e.g., Glick and Thompson, eds., 1993 *Methods in Plant Molecular Biology*, CRC Press, Boca Raton, Florida; Vasil, 1994 *Plant Mol. Biol.* 25:925-937; and Komari et al., 1998 *Current*

20 *Opinions Plant Biol.* 1:161-165 (general reviews); Loopstra et al., 1990 *Plant Mol. Biol.* 15:1-9 and Brasileiro et al., 1991 *Plant Mol. Biol.* 17:441-452 (transformation of trees); Eimert et al., 1992 *Plant Mol. Biol.* 19:485-490 (transformation of *Brassica*); Hiei et al., 1994 *Plant J.* 6:271-282; Hiei et al., 1997 *Plant Mol. Biol.* 35:205-218; Chan et al., 1993 *Plant Mol. Biol.* 22:491-506; U.S. Patent Nos.: 5,516,668 and 5,824,857 (rice transformation); and U.S. Patent Nos.: 5,955,362

25 (wheat transformation); 5,969,213 (monocot transformation); 5,780,798 (corn transformation); 5,959,179 and 5,914,451 (soybean transformation). Representative examples include electroporation-facilitated DNA uptake by protoplasts (Rhodes et al., 1988 *Science* 240(4849):204-207; Bates, 1999 *Methods Mol. Biol.* 111:359-366;

30 D'Halluin et al., 1999 *Methods Mol. Biol.* 111:367-373; U.S. Patent No.: 5,914,451); treatment of protoplasts with polyethylene glycol (Lyznik et al., 1989 *Plant Molecular Biology* 13:151-161; Datta et al., 1999 *Methods Mol. Biol.*, 111:335-34); and bombardment of cells with DNA laden microprojectiles (Klein et al., 1989 *Plant Physiol.* 91:440-444; Boynton et al., 1988 *Science* 240(4858):1534-1538; Register et al., 1994 *Plant Mol. Biol.* 25:951-961; Barcelo et al., 1994 *Plant J.* 5:583-592;

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Vasil et al., 1999 *Methods Mol. Biol.*, 111:349-358; Christou, 1997 *Plant Mol. Biol.* 35:197-203; Finer et al., 1999 *Curr. Top. Microbiol. Immunol.* 240:59-80).

Additionally, plant transformation strategies and techniques are reviewed in Birch, R.G., 1997 *Ann Rev Plant Phys Plant Mol Biol* 48:297; Forester et al., 1997 *Exp. Agric.* 33:15-33. Minor variations make these technologies applicable to a broad range of plant species.

In the case of monocot transformation, particle bombardment appears to be the method of choice for most commercial and university laboratories. However, monocots such as maize can also be transformed by using *Agrobacterium* transformation methods as described in United States Patent 5,591,616 to Hiei et al, issued January 7, 1997 "Method for transforming monocotyledons." Another method to effect corn transformation mixes cells from embryogenic suspension cultures with a suspension of fibers (5% w/v, Silar SC-9 whiskers) and plasmid DNA (1 µg/ul) and then placed either upright in a multiple sample head on a Vortex Genie II vortex mixer (Scientific Industries, Inc., Bohemia, NY, USA) or horizontally in the holder of a Mixomat dental amalgam mixer (Degussa Canada Ltd., Burlington, Ontario, Canada). Transformation is then carried out by mixing at full speed for 60 seconds (Vortex Genie II) or shaking at fixed speed for 1 second (Mixomat). This process results in the production of cell populations out of which stable transformants can be selected. Plants are regenerated from the stably transformed calluses and these plants and their progeny can be shown by Southern hybridization analysis to be transgenic. The principal advantages of the approach are its simplicity and low cost. Unlike particle bombardment, expensive equipment and supplies are not required. The use of whiskers for the transformation of plant cells, particularly maize, is described in United States Patent 5,464,765 to Coffee et al, issued November 7, 1995 "Transformation of plant cells."

United States Patent 5,968,830 to Dan et al published October 19, 1999 "Soybean transformation and regeneration methods" describes methods of transforming and regenerating soybean. United States Patent 5,969,215 to Hall et al, issued October 19, 1999, describes transformation techniques for producing transformed *Beta vulgaris* plants, such as the sugar beet.

Each of the above transformation techniques has advantages and disadvantages. In each of the techniques, DNA from a plasmid is genetically engineered such that it contains not only the gene of interest, but also selectable and screenable marker genes. A selectable marker gene is used to select only those cells

that have integrated copies of the plasmid (the construction is such that the gene of interest and the selectable and screenable genes are transferred as a unit). The screenable gene provides another check for the successful culturing of only those cells carrying the genes of interest.

5           Traditional *Agrobacterium* transformation with antibiotic resistance selectable markers is problematical because of public opposition that such plants pose an undue risk of spreading antibiotic tolerance to animals and humans. Such antibiotic markers can be eliminated from plants by transforming plants using the *Agrobacterium* techniques similar to those described in United States Patent 5,731,179 to Komari et al, issued March 24, 1998 "Method for introducing two T-DNAs into plants and vectors therefor." Antibiotic resistance issues can also be effectively avoided by the use of *bar* or *pat* coding sequences, such as is described in United States Patent 10           Number 5,712,135, issued January 27, 1998 "Process for transforming monocotyledonous plants." These preferred marker DNAs encode second proteins or 15           polypeptides inhibiting or neutralizing the action of glutamine synthetase inhibitor herbicides phosphinothricin (glufosinate) and glufosinate ammonium salt (Basta, Ignite).

          The plasmid containing one or more of these genes is introduced into either plant protoplasts or callus cells by any of the previously mentioned techniques. If the 20           marker gene is a selectable gene, only those cells that have incorporated the DNA package survive under selection with the appropriate phytotoxic agent. Once the appropriate cells are identified and propagated, plants are regenerated. Progeny from the transformed plants must be tested to insure that the DNA package has been successfully integrated into the plant genome.

25           There are numerous factors which influence the success of transformation. The design and construction of the exogenous gene construct and its regulatory elements influence the integration of the exogenous sequence into the chromosomal DNA of the plant nucleus and the ability of the transgene to be expressed by the cell. A suitable method for introducing the exogenous gene construct into the plant cell 30           nucleus in a non-lethal manner is essential. Importantly, the type of cell into which the construct is introduced must, if whole plants are to be recovered, be of a type which is amenable to regeneration, given an appropriate regeneration protocol.

          Prokaryotes may also be used as host cells for the initial cloning steps of this invention. They are particularly useful for rapid production of large amounts of 35           DNA, for production of single-stranded DNA templates used for site-directed

mutagenesis, for screening many mutants simultaneously, and for DNA sequencing of the mutants generated. Suitable prokaryotic host cells include *E. coli* K12 strain 94 (ATCC No. 31,446), *E. coli* strain W3110 (ATCC No. 27,325) *E. coli* X1776 (ATCC No. 31,537), and *E. coli* B; however many other strains of *E. coli*, such as  
5 HB101, JM101, NM522, NM538, NM539, and many other species and genera of prokaryotes including bacilli such as *Bacillus subtilis*, other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various *Pseudomonas* species may all be used as hosts. Prokaryotic host cells or other host cells with rigid cell walls are preferably transformed using the calcium chloride method as described in  
10 section 1.82 of Sambrook et al., *supra*. Alternatively, electroporation may be used for transformation of these cells. Prokaryote transformation techniques are set forth in Dower, W.J., in Genetic Engineering, Principles and Methods, 12:275-296, Plenum Publishing Corp., 1990; Hanahan et al., 1991 *Meth. Enzymol.*, 204:63.

As will be apparent to those skilled in the art, any plasmid vector containing  
15 replicon and control sequences that are derived from species compatible with the host cell may also be used in the practice of the invention. The vector usually has a replication site, marker genes that provide phenotypic selection in transformed cells, one or more promoters, and a polylinker region containing several restriction sites for insertion of foreign DNA. Plasmids typically used for transformation of *E. coli*  
20 include pBR322, pUC18, pUC19, pUC118, pUC119, and Bluescript M13, all of which are described in sections 1.12-1.20 of Sambrook et al., *supra*. However, many other suitable vectors are available as well. These vectors contain genes coding for ampicillin and/or tetracycline resistance which enables cells transformed with these vectors to grow in the presence of these antibiotics.

25 The promoters most commonly used in prokaryotic vectors include the  $\beta$ -lactamase (penicillinase) and lactose promoter systems (Chang et al. 1978 *Nature* 375:615; Itakura et al., 1977 *Science* 198:1056; Goeddel et al., 1979 *Nature* 281:544) and a tryptophan (*trp*) promoter system (Goeddel et al., 1980 *Nucl. Acids Res.* 8:4057; EPO Appl. Publ. No. 36,776), and the alkaline phosphatase  
30 systems. While these are the most commonly used, other microbial promoters have been utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally into plasmid vectors (see Siebenlist et al., 1980 *Cell* 20:269).

35 Many eukaryotic proteins normally secreted from the cell contain an endogenous secretion signal sequence as part of the amino acid sequence. Thus,

proteins normally found in the cytoplasm can be targeted for secretion by linking a signal sequence to the protein. This is readily accomplished by ligating DNA encoding a signal sequence to the 5' end of the DNA encoding the protein and then expressing this fusion protein in an appropriate host cell. The DNA encoding the signal sequence may be obtained as a restriction fragment from any gene encoding a protein with a signal sequence. Thus, prokaryotic, yeast, and eukaryotic signal sequences may be used herein, depending on the type of host cell utilized to practice the invention. The DNA and amino acid sequence encoding the signal sequence portion of several eukaryotic genes including, for example, human growth hormone, proinsulin, and proalbumin are known (see Stryer, 1988 *Biochemistry* W.H. Freeman and Company, New York, NY, p. 769), and can be used as signal sequences in appropriate eukaryotic host cells. Yeast signal sequences, as for example acid phosphatase (Arima et al., 1983 *Nuc. Acids Res.* 11:1657),  $\alpha$ -factor, alkaline phosphatase and invertase may be used to direct secretion from yeast host cells. Prokaryotic signal sequences from genes encoding, for example, LamB or OmpF (Wong et al., 1988 *Gene* 68:193), MalE, PhoA, or beta-lactamase, as well as other genes, may be used to target proteins expressed in prokaryotic cells into the culture medium.

The construction of suitable vectors containing DNA encoding replication sequences, regulatory sequences, phenotypic selection genes and the *REVOLUTA* DNA of interest are prepared using standard recombinant DNA procedures. Isolated plasmids, virus vectors and DNA fragments are cleaved, tailored, and ligated together in a specific order to generate the desired vectors, as is well known in the art (see, for example, Maniatis, *supra*, and Sambrook et al., *supra*).

As discussed above, *REVOLUTA* variants are produced by means of mutation(s) that are generated using the method of site-specific mutagenesis. This method requires the synthesis and use of specific oligonucleotides that encode both the sequence of the desired mutation and a sufficient number of adjacent nucleotides to allow the oligonucleotide to stably hybridize to the DNA template.

The present invention comprises compositions and methods for modulating plant cell division. A wide variety of transgenic vectors containing a *REVOLUTA* derived polynucleotide can be used to practice the present invention. When the *REVOLUTA* transgenes of the present invention are introduced into plants and expressed either a RNA or RNA and then protein, plant cell division is modulated. Provided below are examples of a number of different ways in which a *REVOLUTA*

transgene may be used to increase or decrease the amount of *REVOLUTA* protein within a transgenic plant. The altered *REVOLUTA* protein levels may occur throughout the plant or in a tissue or organ specific manner depending upon the type of promoter sequence operably linked to the *REVOLUTA* transgene.

5       The present invention also provides a transgenic plant comprising a chimeric plant gene having a promoter sequence that functions in plant cells; a coding sequence which causes the production of RNA encoding a fusion polypeptide or an RNA that causes homologous gene suppression such that expression of the chimeric plant gene modulates plant growth. The chimeric plant gene also has a 3' non-  
10 translated region immediately adjacent to the 3' end of the gene that encodes a polyadenylation signal. The polyadenylation signal functions in plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA. The 5' promoter sequence used to transcriptionally activate the chimeric plant gene is a promoter that is heterologous with respect to the coding sequence and adapted to cause sufficient  
15 expression of the chimeric gene to modulate plant growth of a plant transformed with the gene.

#### Inhibition of *REVOLUTA* gene expression

A number of methods can be used to inhibit gene expression in plants. For instance, antisense RNA technology can be conveniently used. The successful  
20 implementation of anti-sense RNA in developmental systems to inhibit the expression of unwanted genes has previously been demonstrated (Van der Krol et al., 1990 *Plant Mol. Biol.* 14:457; Visser et al., 1991, *Mol. Gen. Genet.* 225:289; Hamilton et al., 1990, *Nature* 346:284; Stockhaus et al., 1990, *EMBO J.* 9:3013; Hudson et al., 1992, *Plant Physiol.* 98:294; U.S. Patent Nos.: 4,801,340, 5,773,692,  
25 5,723,761, and 5,959,180). For example, polygalacturonase is responsible for fruit softening during the latter stages of ripening in tomato (Hiatt et al., 1989 in *Genetic Engineering*, Setlow, ed. p. 49; Sheehy et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:8805; Smith et al., 1988, *Nature* 334:724). The integration of anti-sense constructs into the genome, under the control of the CaMV 35S promoter, has  
30 inhibited this softening. Examination of the polygalacturonase mRNA levels showed a 90% suppression of gene expression.

The anti-sense gene is a DNA sequence produced when a sense gene is inverted relative to its normal presentation for transcription. The "sense" gene refers to the gene which is being targeted for control using the anti-sense technology, in its  
35 normal orientation. An anti-sense gene may be constructed in a number of different

ways provided that it is capable of interfering with the expression of a sense gene. Preferably, the anti-sense gene is constructed by inverting the coding region of the sense gene relative to its normal presentation for transcription to allow the transcription of its complement, hence the RNAs encoded by the anti-sense and sense gene are complementary. It is understood that a portion of the anti-sense gene incorporated into an anti-sense construct, of the present invention, may be sufficient to effectively interfere with the expression of a sense gene and thus the term "anti-sense gene" used herein encompasses any functional portion of the full length anti-sense gene. By the term "functional" it is meant to include a portion of the anti-sense gene which is effective in interfering with the expression of the sense gene.

The nucleic acid segment to be introduced generally will be substantially identical to at least a portion of the endogenous *REVOLUTA* gene or genes to be repressed. The sequence, however, need not be perfectly identical to inhibit expression. Generally, higher homology can be used to compensate for the use of a shorter *REVOLUTA* sequence. Furthermore, the introduced *REVOLUTA* sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 25 or 40 nucleotides and about the full length *REVOLUTA* gene sequence should be used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of at least about 500 nucleotides is especially preferred. The construct is then transformed into plants and the antisense strand of RNA is produced.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of *REVOLUTA* genes. It is possible to design ribozyme transgenes that encode RNA ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

One class of ribozymes is derived from a number of small circular RNAs which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum

mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. (1988 *Nature*, 334:585-591)(see also U.S. Patent No.: 5,646,023). Tabler et al. (1991, *Gene* 108:175) have greatly simplified the construction of catalytic RNAs by combining the advantages of the anti-sense RNA and the ribozyme technologies in a single construct. Smaller regions of homology are required for ribozyme catalysis, therefore this can promote the repression of different members of a large gene family if the cleavage sites are conserved. Together, these results point to the feasibility of utilizing anti-sense RNA and/or ribozymes as practical means of manipulating the composition of valuable crops.

Another method of suppressing *REVOLUTA* protein expression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been recently shown to be an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli et al. (1990 *Plant Cell* 2:279-289), Hamilton et al. (1999 *Science* 286:950-952), and U.S. Pat. Nos. 5,034,323, 5,231,020, 5,283,184 and 5,942,657.

More recently, a new method of suppressing the expression of a target gene has been developed. This method involves the introduction into a host cell of an inverted repeat transgene that directs the production of a mRNA that self-anneal to form double stranded (ds) RNA structures (Vionnet et al., 1998 *Cell* 95:177-187; Waterhouse et al., 1998 *Proc. Natl. Acad. Sci. USA* 95:13959-13964; Misquitta et al., 1999 *Proc. Natl. Acad. Sci. USA* 96:1451-1456; Baulcombe, 1999 *Current Opinion Plant Biol.* 2:109-113; Sharp, 1999 *Genes and Develop.* 13:139-141). The ds RNA molecules, in a manner not understood, interfere with the post transcriptional expression of endogenous genes that are homologous to the dsRNA. It has been shown that the region of dsRNA homology must contain region that is homologous to an exon portion of the target gene. Thus, the dsRNA may include sequences that are homologous to noncoding portions of the target gene. Alternatively, gene suppressive dsRNA could also be produce by transform a cell with two different transgenes, one expressing a sense RNA and the other a complementary antisense RNA.

A construct containing an inverted repeat of a *REVOLUTA* transcribed sequence is made by following the general example of Waterhouse et al.(1998). The inverted repeat part of the construct comprises about 200 to 1500 bp of transcribed

DNA repeated in a head to head or tail to tail arrangement. The repeats are separated by about 200 to 1500 bp of non-repeated DNA which can also be part of the transcribed *REVOLUTA* region, or can be from a different gene, and perhaps contain an intron. A suitable *REVOLUTA* suppressor transgene construct is made by  
5 attaching in the proper order: a plant promoter; a 3' region from a *REVOLUTA* cDNA oriented in a proper "sense" orientation; a 5' region from the cDNA; the same 3' region of *REVOLUTA* coding sequence from the cDNA but oriented in "anti-sense" orientation; and finally a polyA addition signal. Whatever the order chosen, the transcribed *REVOLUTA* RNA resulting from introduction of the inverted repeat  
10 transgene into a target plant will have the potential of forming an internal dsRNA region containing sequences from the *REVOLUTA* target gene that is to be suppressed. The dsRNA sequences are chosen to suppress a single or perhaps multiple *REVOLUTA* genes. In some cases, the sequences with the potential for dsRNA formation may originate from two or more *REVOLUTA* genes.

15 An additional strategy suitable for suppression of *REVOLUTA* activity entails the sense expression of a mutated or partially deleted form of *REVOLUTA* protein according to general criteria for the production of dominant negative mutations (Herskowitz 1, 1987, *Nature* 329:219-222). The *REV* protein is mutated in the DNA binding motif of the homeodomain, or in such a way to produce a truncated *REV*  
20 protein. Examples of strategies that produced dominant negative mutations are provided (Mizukami, 1996; Emmeler, 1995; Sheen, 1998; and Paz-Ares, 1990).

Generally, where inhibition of expression is desired, some transcription of the introduced sequence occurs. The effect may occur where the introduced sequence contains no coding sequence per se, but only intron or untranslated sequences  
25 homologous to sequences present in the primary transcript of the endogenous sequence. The introduced sequence generally will be substantially identical to the endogenous sequence intended to be repressed. This minimal identity will typically be greater than about 65%, but a higher identity might exert a more effective repression of expression of the endogenous sequences. Substantially greater identity  
30 of more than about 80% is preferred, though about 95% to absolute identity would be most preferred. As with antisense regulation, the effect should apply to any other proteins within a similar family of genes exhibiting homology or substantial homology.

For sense suppression, the introduced sequence, needing less than absolute  
35 identity, also need not be full length, relative to either the primary transcription

product or fully processed mRNA. This may be preferred to avoid concurrent production of some plants which are overexpressers. A higher identity in a shorter than full length sequence may compensate for a longer, less identical sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and identity of non-coding segments will be equally effective. Normally, a sequence of the size ranges noted above for antisense regulation is used.

Wild-type REVOLUTA gene function can also be eliminated or diminished by using DNA regions flanking the REVOLUTA gene to target an insertional disruption of the REVOLUTA coding sequence (Miao et al., 1995; *Plant J.* 7:359-365; Kempin et al., 1997 *Nature* 389:802-803). The targeted gene replacement of REVOLUA is mediated by homologous recombination between sequences in a transformation vector that are from DNA regions flanking the REV gene and the corresponding chromosomal sequences. A selectable marker, such as kanamycin, bar or pat, or a screenable marker, such as beta-glucuronidase (GUS), is included in between the REV flanking regions. These markers facilitate the identification of cells that have undergone REV gene replacement. Plants in which successful REVOLUTA gene replacement has occurred can also be identified because plant tissues have an altered number of cell.

#### Promoters

An illustrative example of a responsive promoter system that can be used in the practice of this invention is the glutathione-S-transferase (GST) system in maize. GSTs are a family of enzymes that can detoxify a number of hydrophobic electrophilic compounds that often are used as pre-emergent herbicides (Weigand et al., 1986 *Plant Molecular Biology* 7:235-243). Studies have shown that the GSTs are directly involved in causing this enhanced herbicide tolerance. This action is primarily mediated through a specific 1.1 kb mRNA transcription product. In short, maize has a naturally occurring quiescent gene already present that can respond to external stimuli and that can be induced to produce a gene product. This gene has previously been identified and cloned. Thus, in one embodiment of this invention, the promoter is removed from the GST responsive gene and attached to a REVOLUTA coding sequence. If the REVOLUTA gene is derived from a genomic DNA source than it is necessary to remove the native promoter during construction of the chimeric gene. This engineered gene is the combination of a promoter that responds to an external chemical stimulus and a gene responsible for successful production of REVOLUTA protein .

An inducible promoter is a promoter that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor, that binds specifically to an inducible promoter to activate transcription, is present in an inactive form which is then directly or indirectly converted to the active form by the inducer. The inducer can be a chemical agent such as a protein, metabolite, a growth regulator, herbicide or a phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible promoter may be exposed to an inducer by externally applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods. If it is desirable to activate the expression of the target gene to a particular time during plant development, the inducer can be so applied at that time.

Examples of such inducible promoters include heat shock promoters, such as the inducible 70KD heat shock promoter of *Drosophila melanogaster* (Freeling et al., *Ann. Rev. of Genetics*, 19:297-323); a cold inducible promoter, such as the cold inducible promoter from *B. napus* (White, et al., 1994 *Plant Physiol.* 106); and the alcohol dehydrogenase promoter which is induced by ethanol (Nagao, R. T. et al., Miflin, B. J., Ed. *Oxford Surveys of Plant Molecular and Cell Biology* 1986 Vol. 3, p 384-438, Oxford University Press, Oxford).

Alternatively, the *REVOLUTA* transgenes of the present invention can be expressed using a promoter such as is the BCE.4 (*B. campestris* embryo) promoter which has been shown to direct high levels of expression in very early seed development (i.e. is transcribed before the napin promoter). This is a period prior to storage product accumulation but of rapid pigment biosynthesis in the *Brassica* seed (derived from Johnson-Flanagan et al., 1989 *J. Plant Physiol.* 136:180; Johnson-Flanagan et al., 1991 *Physiol. Plant* 81:301). Seed storage protein promoters have also been shown to direct a high level of expression in a seed-specific manner (Voelker et al., 1989 *Plant Cell* 1:95; Altenbach et al., 1989 *Plant Mol. Biol.* 13:513; Lee et al., 1991, *Proc. Nat. Acad. Sci. USA* 99:6181; Russell et al., 1997 *Transgenic Res* 6:157-68). The napin promoter has been shown to direct oleosin gene expression in transgenic *Brassica*, such that oleosin accumulates to approximately 1% of the total seed protein (Lee et al., 1991 *Proc. Nat. Acad. Sci. USA* 99:6181). Table 2 lists other embryo specific promoters that can be used to practice the present invention.

Table 2. Embryo Specific Promoters

| Promoter                                 | Embryo                                   | Endosperm                | Timing  | Reference   |
|--|--|--------------------------|---|---|
| oleosin<br>from<br><i>Arabidopsis</i>    | strong,<br>uniform                       | none                     | traces at heart,<br>higher early- to<br>late- cotyledonary<br>stage | Al et al. 1994 <i>Plant Mol. Biol.</i> 25:193-205.        |
| USP from<br><i>Vicia faba</i>            | strong, uniform                          | none                     | early not known,<br>strong in late cot.,                            | Baumlein et al. 1991 <i>Mol. Gen. Genet.</i> 225:459-467. |
| Legumin<br>from <i>Vicia faba</i>        | strong,<br>preferential in<br>cotyledons | aleurone layer<br>(late) | early not known,<br>strong in late cot.,                            | Baumlein et al. 1991.                                     |
| Napin from<br><i>Brassica</i>            |  | ?                        | late  | Kohno-Murase 1994 <i>Plant Mol. Biol.</i> 26:1115-1124    |
| Albumin<br>S1 from<br><i>Arabidopsis</i> | in axis only                             | none                     | early- to late-<br>cotyledonary stage                               | Guerche et al., 1990 <i>Plant Cell</i> 2:469-478.         |
| Albumin<br>S2                            | in axis and<br>cotyledons                | none                     | early- to late-<br>cotyledonary stage                               | Guerche et al., 1990.                                     |

In choosing a promoter it may be desirable to use a tissue-specific or developmentally regulated promoter that allows suppression or overexpression of in certain tissues without affecting expression in other tissues. "Tissue specific promoters" refer to coding region that direct gene expression primarily in specific tissues such as roots, leaves, stems, pistils, anthers, flower petals, seed coat, seed nucellus or epidermal layers. Transcription stimulators, enhancers or activators may be integrated into tissue specific promoters to create a promoter with a high level of activity that retains tissue specificity. For instance, promoters utilized in overexpression will preferably be tissue-specific. Overexpression in the wrong tissue, such as leaves when attempting to overexpress in seed storage areas, could be deleterious. Preferred expression cassettes of the invention will generally include, but are not limited to, a seed-specific promoter. A seed specific promoter is used in order to ensure subsequent expression in the seeds only.

Examples of seed-specific promoters include the 5' regulatory regions of an *Arabidopsis* oleosin gene as described in United States Patent 5,977,436 to Thomas et al issued November 2, 1999 "Oleosin 5' regulatory region for the modification of plant seed lipid composition" (incorporated in its entirety by reference), which when

operably linked to either the coding sequence of a heterologous gene or sequence complementary to a native plant gene, direct expression of the heterologous gene or complementary sequence in a plant seed.

5 Examples also include promoters of seed storage proteins which express these proteins in seeds in a highly regulated manner such as, for dicotyledonous plants, phaseolin (bean cotyledon) (Sengupta-Gopalan, et al., 1985 *Proc. Natl. Acad. Sci. U.S.A.* 82:3320-3324), a napin promoter, a conglycinin promoter, and a soybean lectin promoter, patatin (potato tubers) (Rocha-Sosa, et al., 1989 *EMBO J.* 8:23-29), convicilin, vicilin, and legumin (pea cotyledons) (Rerie, et al., 1991 *Mol. Gen. Genet.* 10 259:148- 157; Newbigin, et al., 1990 *Planta* 180:461-470; Higgins, et al., 1988 *Plant Mol. Biol.* 11:683-695), phytohemagglutinin (bean cotyledon) (Voelker, et al. 1987 *EMBO J.* 6:3571-3577), conglycinin and glycinin (soybean cotyledon)(Chen, et al. 1988 *EMBO J.* 7: 297-302), and sporamin (sweet potato tuberous root) (Hattori, et al., 1990 *Plant Mol. Biol.* 14:595-604). For monocotyledonous plants, promoters 15 useful in the practice of the invention include, but are not limited to, maize zein promoters (Schernthaner, et al., (1988) *EMBO J.* 7:1249-1255), a zein promoter, a waxy promoter, a shrunken-1 promoter, a globulin 1 promoter, and the shrunken-2 promoter, glutelin (rice endosperm), hordein (barley endosperm) (Marris, et al. 1988 *Plant Mol. Biol.* 10:359-366), glutenin and gliadin (wheat endosperm) (United States 20 Patent Number: 5,650,558). Differential screening techniques can be used to isolate promoters expressed at specific (developmental) times, such as during fruit development. However, other promoters useful in the practice of the invention are known to those of skill in the art.

Particularly preferred promoters are those that allow seed-specific expression. 25 This may be especially useful since seeds are a primary organ of interest, and also since seed-specific expression will avoid any potential deleterious effect in non-seed tissues. Examples of seed-specific promoters include, but are not limited to, the promoters of seed storage proteins, which can represent up to 90% of total seed protein in many plants. The seed storage proteins are strictly regulated, being 30 expressed almost exclusively in seeds in a highly tissue-specific and stage-specific manner (Higgins et al., 1984 *Ann. Rev. Plant Physiol.* 35:191-221; Goldberg et al., 1989 *Cell* 56:149-160). Moreover, different seed storage proteins may be expressed at different stages of seed development. Expression of seed-specific genes has been studied in great detail (see reviews by Goldberg et al. (1989) and Higgins et al. 35 (1984). There are currently numerous examples of seed-specific expression of seed

storage protein genes in transgenic dicotyledonous plants. These include genes from dicotyledonous plants for bean  $\beta$ -phaseolin (Sengupta-Gopalan et al., 1985; Hoffman et al., 1988 *Plant Mol. Biol.* 11:717-729), bean lectin (Voelker et al., 1987), soybean lectin (Okamuro et al., 1986 *Proc. Natl. Acad. Sci. USA* 83:8240-8244), soybean Kunitz trypsin inhibitor (Perez-Grau et al., 1989 *Plant Cell* 1:095-1109), soybean  $\beta$ -conglycinin (Beachy et al., 1985 *EMBO J.* 4:3047-3053; pea vicilin (Higgins et al., 1988), pea convicilin (Newbiggin et al., 1990 *Planta* 180:461-470), pea legumin (Shirsat et al., 1989 *Mol. Gen. Genetics* 215:326-331); rapeseed napin (Radke et al., 1988 *Theor. Appl. Genet.* 75:685-694) as well as genes from monocotyledonous plants such as for maize 15 kD zein (Hoffman et al., 1987 *EMBO J.* 6:3213-3221), maize 18 kD oleosin (Lee et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:6181-6185), barley  $\beta$ -hordein (Marris et al., 1988 *Plant Mol. Biol.* 10:359-366) and wheat glutenin (Colot et al., 1987 *EMBO J.* 6:3559-3564). Moreover, promoters of seed-specific genes operably linked to heterologous coding sequences in chimeric gene constructs also maintain their temporal and spatial expression pattern in transgenic plants. Such examples include use of *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in *Arabidopsis* and *B. napus* seeds (Vandekerckhove et al., 1989 *Bio/Technology* 7:929-932), bean lectin and bean  $\beta$ -phaseolin promoters to express luciferase (Riggs et al., 1989 *Plant Sci.* 63:47-57), and wheat glutenin promoters to express chloramphenicol acetyl transferase (Colot et al., 1987).

Also suitable for the expression of the nucleic acid fragment of the invention will be the heterologous promoters from several soybean seed storage protein genes such as those for the Kunitz trypsin inhibitor (Jofuku et al., 1989 *Plant Cell* 1:1079-1093; glycinin (Nielson et al., 1989 *Plant Cell* 1:313-328), and  $\beta$ -conglycinin (Harada et al., 1989 *Plant Cell* 1:415-425); promoters of genes for  $\alpha$ - and  $\beta$ -subunits of soybean  $\beta$ -conglycinin storage protein for expressing the mRNA or the antisense RNA in the cotyledons at mid- to late-stages of seed development (Beachy et al., 1985 *EMBO J.* 4:3047-3053) in transgenic plants; *B. napus* isocitrate lyase and malate synthase (Comai et al., 1989 *Plant Cell* 1:293-300), delta-9 desaturase from safflower (Thompson et al. 1991 *Proc. Natl. Acad. Sci. USA* 88:2578-2582) and castor (Shanklin et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:2510-2514), acyl carrier protein (ACP) from *Arabidopsis* (Post-Beittenmiller et al., 1989 *Nucl. Acids Res.* 17:1777), *B. napus* (Safford et al., 1988 *Eur. J. Biochem.* 174:287-295), and *B. campestris* (Rose et al., 1987 *Nucl. Acids Res.* 15:7197),  $\beta$ -ketoacyl-ACP synthetase

from barley (Siggaard-Andersen et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:4114-4118), and oleosin from *Zea mays* (Lee et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:6181-6185), soybean (Genbank Accession No: X60773) and *B. napus* (Lee et al., 1991 *Plant Physiol.* 96:1395-1397).

5 Attaining the proper level of expression of the nucleic acid fragments of the invention may require the use of different chimeric genes utilizing different promoters. Such chimeric genes can be transferred into host plants either together in a single expression vector or sequentially using more than one vector.

10 On the other hand, pollen specific promoter -- i.e., promoters regulating temporal expression at a time prior to or soon after pollination so that fruit development and maturation is induced without significant seed development -- are usually undesirable. Such undesired promoters include but are not limited to inducible promoters, microspore or megaspore promoters, pollen specific promoters, or maternal tissue promoters such as seed coat promoters or any other promoter  
15 associated with a gene involved in pollination or ovule maturation or development.

In addition, enhancers are often required or helpful to increase expression of the gene of the invention. It is necessary that these elements be operably linked to the sequence that encodes the desired proteins and that the regulatory elements are operable. Enhancers or enhancer-like elements may be either the native or chimeric  
20 nucleic acid fragments. This would include viral enhancers such as that found in the 35S promoter (Odell et al., 1988 *Plant Mol. Biol.* 10:263-272), enhancers from the opine genes (Fromm et al., 1989 *Plant Cell* 1:977-984), or enhancers from any other source that result in increased transcription when placed into a promoter operably linked to the nucleic acid fragment of the invention. For example, a construct may  
25 include the CaMV 35S promoter with dual transcriptional enhancer linked to the Tobacco Etch Virus (TEV) 5' nontranslated leader. The TEV leader acts as a translational enhancer to increase the amount of protein made.

The promoter elements described in Table 2 can be fused to the *REVOLUTA* sequences and a suitable terminator (polyadenylation region) according to well  
30 established procedure. Promoters specific for different tissue types are already available or can be isolated by well-established techniques (see for example U.S. Patents Nos.: 5,792,925; 5,783,393; 5,859,336; 5,866,793; 5,898,096; and 5,929,302).

"Digestion", "cutting" or "cleaving" of DNA refers to catalytic cleavage of the  
35 DNA with an enzyme that acts only at particular locations in the DNA. These

enzymes are called restriction endonucleases, and the site along the DNA sequence where each enzyme cleaves is called a restriction site. The restriction enzymes used in this invention are commercially available and are used according to the instructions supplied by the manufacturers. (See also sections 1.60-1.61 and sections 3.38-3.39 of Sambrook et al., *supra*.)

"Recovery" or "isolation" of a given fragment of DNA from a restriction digest means separation of the resulting DNA fragment on a polyacrylamide or an agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Lawn et al. (1982 *Nucleic Acids Res.* 9:6103-6114), and Goeddel et al. (1980).

The foregoing may be more fully understood in connection with the following representative examples, in which "Plasmids" are designated by a lower case p followed by an alphanumeric designation. The starting plasmids used in this invention are either commercially available, publicly available on an unrestricted basis, or can be constructed from such available plasmids using published procedures. In addition, other equivalent plasmids are known in the art and will be apparent to the ordinary artisan. The following examples are in no way intended to limit the scope of the present invention, but rather only illustrate the many possible ways of practicing the invention.

#### Example 1

##### Identification of *REVOLUTA*

##### Mapping the *REVOLUTA* gene using polymorphic DNA markers

To map a gene using small differences or polymorphisms in the DNA, a segregating population of *Arabidopsis* derived from two different ecotypes was screened. To generate this segregating population, a homozygous plant containing mutations in the *revoluta* gene (*rev-1*) of the Nossen (No) ecotype was crossed to a wild-type plant of the Landsberg erecta (Ler) ecotype. In the resulting F1 progeny, one chromosome of each pair was of the No ecotype, and the other was of the Ler ecotype. All the F1 progeny contained the *rev-1* mutation from the No parent and a wild-type *REVOLUTA* gene from the Ler parent. One of these F1 progeny (called 21A) was allowed to self-fertilize and to produce F2 seeds in which recombination between the No and Ler chromosomes would have occurred. The F2 plants grown

from these seeds were segregating for the different polymorphisms or markers and for the *rev-1* mutation.

In order to detect polymorphisms between the different ecotypes, a technique called simple sequence length polymorphisms (SSLP) was used (Bell et al., 1994  
5 *Genomics* 19:137-144). SSLP markers are a set of two primers that amplify a specific region of genomic DNA in a PCR reaction (polymerase chain reaction). The size of the genomic DNA amplified can vary in specific regions between different *Arabidopsis* ecotypes. This allows a determination of the region as being from the Ler or No ecotypes. Two SSLP markers, nga129 and MBK5, had already been  
10 identified in the region of chromosome 5 determined to contain the *REVOLUTA* gene (Talbert, et al., 1995). The nga129 primers (Table 3) amplify a 179 basepair (bp) fragment from the Ler ecotype and a 165 bp fragment from the No ecotype (Bell et al., 1994). The MBK5 primers were known to amplify an ~180 bp fragment from the Ler ecotype ([http://genome.bio.upenn.edu/SSLP\\_info/coming-soon.html](http://genome.bio.upenn.edu/SSLP_info/coming-soon.html)).  
15 Experiments conducted with these primers on No ecotype DNA demonstrated that a ~207 bp fragment from the No ecotype was amplified with these primers.

Therefore, these SSLP markers were used to screen the segregating population of 21A progeny described above. First, F2 plants homozygous for the *rev-1* mutation were identified by morphology (Talbert et al., 1995). Genome DNA  
20 was then prepared as follows. Approximately 50 mg of leaf material was ground in a microcentrifuge tube for 10 seconds with a blue pestle (Kontes Glass Co., Vineland, NJ). Then, 100  $\mu$ l of PEB (100mM Tris, 8.0, 50mM EDTA, 0.5M NaCl, 0.7% SDS, and 20 mg/ml freshly added proteinase K) was added and leaf material was ground 20 seconds more. Finally, 325  $\mu$ l PEB was added and the material ground  
25 until no leaf chunks remain (15 seconds.) After heating at 65°C for 1 hour, 260  $\mu$ l saturated NaCl was added. The tubes were microfuged for 20 minutes at top speed. The supernatant was transferred to a new tube containing 850  $\mu$ l of 85% isopropanol. This mixture was centrifuged for 10 minutes and the resulting pellet washed in 70% ethanol. The dried pellet was then resuspended in 200  $\mu$ l TE buffer, then 133  $\mu$ l LiCl  
30 was added and the tubes stored overnight at 4°C. RNA was pelleted for 10 min at room temperature. The supernatant was transferred into a new tube to which 2 volumes of ethanol was added. After 10 minutes centrifugation, the pellet was air dried and resuspended in 50  $\mu$ l 10 mM Tris (pH 8.0). For PCR, either 1  $\mu$ l or 1  $\mu$ l of a 1:10 dilution of this DNA was used.

Genomic DNA was amplified in a PCR reaction using either the nga 129 primers or the MBK5 primers (Table 3) in 1X Buffer, 2mM MgCl<sub>2</sub>, 0.2mM dNTPs 0.25mM oligonucleotide, 2U Taq polymerase (Life Technologies, Inc., Rockville, MD). The PCR conditions included a 94°C denaturation step for 3 minutes followed  
5 by 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 40 seconds. Each experiment included control DNA from No, Ler, and 21A plants. Out of the first 372 chromosomes screened (from 186 plants), 60 chromosomes had a Ler marker, indicating that recombination had occurred between the No chromosome and the Ler chromosome. Of the 360 chromosomes analyzed for MBK5, only 15 had the  
10 Ler marker.

Additional *rev-1* plants were screened with an SSLP marker ~3.4 Mb south (towards the telomere) of nga129 F/R called K21L19. This SSLP marker and others were identified using the following protocol. First, a text file of the DNA sequences (FASTA format) was created from the known DNA sequences of chromosome 5 near  
15 the region where *rev-1* was mapped to (<http://www.ncbi.nlm.nih.gov/Entrez/nucleotide.html>). The DNA sequence text file was saved as a text only document in Microsoft Word98, and used as a database for a search engine available through the following webpage: <http://blocks.fhrc.org/~jorja/blastnew.html>. The *Arabidopsis* database was searched  
20 for strings of repetitive DNA such as "GA" or "TA" repeats of at least 12 bp long. Then PCR primers flanking the repetitive region were chosen using a primer program ([http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)). Primer pairs were chosen to amplify regions of about 150-250 bp in size. These primer pairs were then tested on DNA samples extracted from No, Ler, and 21A plants to determine if  
25 any polymorphisms could be detected. Primer pairs that amplified DNA fragments that were polymorphic between the No and Ler ecotypes were used to further map the *REVOLUTA* gene. These new SSLP markers (see Table 3) were named after the bacterial artificial chromosome clone (BAC) in which they were found (the *Arabidopsis* genome has been cloned into ~100 Kb pieces cloned in BACs or other  
30 similar vectors, which have been aligned contiguously along the chromosomes). If more than one marker was identified within a BAC, the primer pairs were given additional identification numbers.

Table 3: Oligonucleotides used for SSLP

| Primer Name<br>(SEQ ID NO.) | Primer Sequence                  |
|-----------------------------|----------------------------------|
| nga129F<br>(SEQ ID NO.:13)  | 5' TCAGGAGGAACTAAAGTGAGGG 3'     |
| nga129R<br>(SEQ ID NO.:14)  | 5' CACTGAAGATGGTCTTGAGG 3'       |
| MBK5-1<br>(SEQ ID NO.:15)   | 5' ATCACTGTTGTTTACCATTA 3'       |
| MBK5-2<br>(SEQ ID NO.:16)   | 5' GAGCATTTCACAGAGACG 3'         |
| K21L19L<br>(SEQ ID NO.:17)  | 5' CTCCTCCTTTCCAGACACA 3'        |
| K21L19R<br>(SEQ ID NO.:18)  | 5' TTCCACCAATTCACACCA 3'         |
| MUP24-1<br>(SEQ ID NO.:19)  | 5' CGTAAAACGTCGTCGTTTCATT 3'     |
| MUP24-2<br>(SEQ ID NO.:20)  | 5' ATCGCTGGATTGTTTTGGAC 3'       |
| MAF19L<br>(SEQ ID NO.:21)   | 5' TTCTAAGAATGTTTTTACCACCAAAA 3' |
| MAF19R<br>(SEQ ID NO.:22)   | 5' CCAACTGCGACTGCCAGATA 3'       |
| MUP24-3<br>(SEQ ID NO.:23)  | 5' TCCGATTGGTCTAAAGTACGA 3'      |
| MUP24-4<br>(SEQ ID NO.:24)  | 5' TGACCAAGGCCAAACATACT 3'       |
| MUP24-13<br>(SEQ ID NO.:25) | 5' GAAATCTCACCGGACACCAT 3'       |

MUP24-14            5' CGAATCCCCATTCGTCATAG 3'  
(SEQ ID NO.:26)

MAE1-1            5' TTTCCAACAACAAAAGAATATGG 3'  
(SEQ ID NO.:27)

MAE1-2            5' TGGTATGCGGATATGATCTTT 3'  
(SEQ ID NO.:28)

MAE1-3            5' CACTCGTAGCATCCATGTTCG 3'  
(SEQ ID NO.:29)

MAE1-4            5' TCAGATTCAATCGAAAACGAAA 3'  
(SEQ ID NO.:30)

MAE1-5            5' CCGTGGAGGCTCTACTGAAG 3'  
(SEQ ID NO.:31)

MAE1-6            5' CGTTACCTTTTGGGTGGAAA 3'  
(SEQ ID NO.:32)

When DNA from the plants containing *ngal29 F/R/rev-1* recombinant chromosomes was screened using K21L19L and K21L19R primers (K21L19 L/R)(Table 3), 6 of the original 60 *ngal29* recombinant chromosomes were also recombinant for K21L19 L/R DNA region. In addition, 350 more chromosomes were analyzed for the K21L19 L/R polymorphic marker in which only 9 were recombinant for K21L19 L/R giving a total of 15 recombinants. The 350 recombinant chromosomes were also analyzed with the MBK5-1 and MBK5-1 PCR primers (MBK5 1/2)(Table 3). Eight new recombinants were identified at the MBK5 locus defined by the MBK5 1/2 primer pair for a total of 23 MBK5 1/2 recombinants. The K21L19 L/R and MBK5 1/2 loci define a region of 1.95 Mb in chromosome 5 of *Arabidopsis*.

Other SSLP primer/markers were generated in this region, of these the most informative were the primers MUP24-1 and 2 (MUP24 1/2) and MAF19 L and R MAF19 L/R (Table 3, FIGURE 1). These markers were used to screen DNA isolated from the K21L19 and MBK5 1/2 recombinant plants. Of the 15 K21L19 L/R recombinants, 3 were recombinant for the MUP24 1/2 marker, and none for the MAF19L/R marker. Of the 23 MBK5 1/2 recombinants, 6 were recombinant for

MAF19 L/R, and none for MUP24 1/2. As shown in FIGURE 1, these results placed the *REVOLUTA* gene in between MUP24 1/2 and MAF19 L/R in a region encompassing ~340 Kb.

5 New SSLP markers were generated in this region, and used to further define where recombination occurred between the Ler and No (*rev-1* containing) chromosomes. The markers are listed in FIGURE 2 and include: MUP24 3/4, MUP24 13/14, MAE1 1/2, MAE1 3/4, and MAE1 5/6.

REVOLUTA is encoded by MUP24.4 and is a new HD-Zip III subfamily member

10 From the above - described mapping results, the smallest chromosome region containing the *REVOLUTA* gene was approximately 68,000 bp long. An examination of the translated open reading frames in this region revealed about 11 potential genes that could encode *REVOLUTA*. The MUP24.4 - a homeodomain leucine zipper containing protein (HD-Zip) was determined to be the gene of interest. The DNA sequence encoding this HD-Zip protein was determined in DNA isolated  
15 from six different *revoluta* alleles (*rev1-6*). Genomic DNA from leaves of the different *rev* alleles was prepared as described above. The MUP24.4 gene was amplified using long distance PCR with the primers in Table 4 and the conditions described in (Henikoff et al., 1998, *Genetics* 149:307-318) except that denaturation steps were carried out at 94°C and 20 second extensions were added to each cycle  
20 after 10 cycles for a total of 40 cycles of PCR amplification.

Table 4: Primers used to amplify MUP24.4 using LD-PCR

| Primer Name<br>(SEQ ID NO.) | Primer Sequence                   |
|-----------------------------|-----------------------------------|
| HDAL<br>(SEQ ID NO:33)      | 5' AAAATGGAGATGGCGGTGGCTAAC 3'    |
| HDAR<br>(SEQ ID NO:34)      | 5' TGTC AATCGAATCACACAAAAGACCA 3' |

The resulting PCR products from each *revoluta* mutant and wild-type *REVOLUTA* genes were cloned into a TOPO II vector (Invitrogen, Carlsbad, CA) according to manufacturers instructions except that half the amount suggested for the TOPO  
25 vector and PCR products were used.

Plasmids containing inserts were purified using a spin miniprep kit (QIAGEN Inc., Valencia, CA), and sequenced using the oligonucleotides listed in Table 5 with

the ABI PRISM Big Dye kit (Applied Biosystems, now PE Biosystems, Foster City, CA) according to manufacturer's instructions.

Table 5. Primers used to sequence the HD-Zip protein MUP24.4

| Primer Name<br>(SEQ ID NO.) | Primer Sequence                       |
|-----------------------------|---------------------------------------|
| Rev-1<br>(SEQ ID NO:35)     | 5' CAG ACT TTG ATC TGC TTA GGA TC 3'  |
| Rev-2<br>(SEQ ID NO:36)     | 5' TGA GCC TAA GCA GAT CAA AGT C 3'   |
| Rev-3<br>(SEQ ID NO:37)     | 5' ACC GGA AGC TCT CTG CGA TG 3'      |
| Rev-4<br>(SEQ ID NO:38)     | 5' TCG CAG AGG AGA CTT TGG CAG 3'     |
| Rev-5<br>(SEQ ID NO:39)     | 5' GGA GCC TTG AAG TTT TCA CTA TG 3'  |
| Rev-6<br>(SEQ ID NO:40)     | 5' GGT ATT TAA TAA GGC CTT GTG ATG 3' |
| Rev-7<br>(SEQ ID NO:41)     | 5' AGA ACC TTT AGC CAA AGA TTA AGC 3' |
| Rev-8<br>(SEQ ID NO:42)     | 5' AGC ATC GAT CTG AGT GGG CTG 3'     |
| Rev-9<br>(SEQ ID NO:43)     | 5' GTA CCG GGA TTG ACG AGA ATG 3'     |
| Rev-10<br>(SEQ ID NO:44)    | 5' TGA GGA GCG TGA TCT CAT CAG 3'     |
| Rev-11<br>(SEQ ID NO:45)    | 5' GCC AGT GTT CAT GTT TGC GAA C 3'   |
| Rev-12<br>(SEQ ID NO:46)    | 5' ATG GCG GTG GCT AAC CAC CGT GAG 3' |

M13 Forward            5' GTA AAA CGA CGG CCA G 3'  
(SEQ ID NO:47)

M13 Reverse            5' CAG GAA ACA GCT ATG AC 3'  
(SEQ ID NO:48)

Sequence analysis of two independently generated clones per *revoluta* allele indicate that the *REVOLUTA* gene sequence [SEQ ID NO:1] is mutated in each of these six *revoluta* alleles. The observed mutations are found in both putative gene coding sequences (*rev-3* [SEQ ID NO:5] and *rev-5* [SEQ ID NO:9]) and at putative intron/exon splice junctions (*rev-1* [SEQ ID NO:3], *rev-2,4* [SEQ ID NO:7] and *rev-6* [SEQ ID NO:11]) (See FIGURE 3). Thus, DNA sequence analysis identified open reading frames in all six *revoluta* mutant genes that are capable of expressing a *REV* HD-Zip protein but the *revoluta* protein made in each cases has an altered amino acid sequence. The amino acid sequence predicted for the wild-type *REVOLUTA* protein is shown in FIGURE 3 [SEQ ID NO:2] with the mutant amino acids and splice sites indicated. Translation of the *rev-4* mutant DNA [SEQ ID NO:7] indicates that the mutation causes a translation frame shift at the beginning of exon 10 that results in a novel eight amino acid carboxy terminal sequence. The *rev-4* protein terminates at an out of frame stop codon, thus translation of the *rev-4* allele produces a truncated *rev-4* polypeptide [SEQ ID NO:8]. SEQ ID NO:1 lists the complete wild-type DNA sequence for the genomic DNA region encoding the *REVOLUTA* gene. TABLE 6 lists the nucleotide positions mutated in each of the *revoluta* alleles and the nucleotide change associated with each mutant allele.

20            Table 6: *Arabidopsis* No-ecotype changes present in *revoluta* mutant alleles

| <i>revoluta</i><br>mutant | SEQ ID No.:   | Base Change | Location        |
|---------------------------|---------------|-------------|-----------------|
| <i>rev-1</i>              | SEQ ID No.:3  | G → A       | nucleotide 2819 |
| <i>rev-2</i>              | SEQ ID No.:7  | G → A       | nucleotide 2093 |
| <i>rev-3</i>              | SEQ ID No.:5  | C → T       | nucleotide 3252 |
| <i>rev-4</i>              | SEQ ID No.:7  | G → A       | nucleotide 2093 |
| <i>rev-5</i>              | SEQ ID No.:9  | T → C       | nucleotide 2651 |
| <i>rev-6</i>              | SEQ ID No.:11 | C → T       | nucleotide 1962 |

An alignment of the 842 amino acid *REV* protein sequence with previously identified members of the HD-Zip class III family is shown in Figure 4. There was extensive homology between *REV* and the other four proteins over their entire lengths. *REV* had 66% identity (78% similarity) to *ATHB-9* and *ATHB-14*, and 61% identity (75% similarity) to *ATHB-8*. Comparison of *REV* to F5F19.21 (AAD12689.1), a putative new member of the family identified based on sequence similarity, yielded 64% identity and 77% similarity. F5F19.21 was expressed when analyzed using RT-PCR (not shown) and was represented by multiple Genbank EST database entries. When the N-terminal region of the protein, containing the homeobox and leucine zipper domains, was removed prior to alignment (leaving residues 114-832), the homology between the proteins was still quite high: *REV* showed 64% identity with *ATHB-9* and *ATHB-14*, 61% with *F5F19*, and 58% with *ATHB-8*. Further analysis of the *REV* protein sequence indicated that it contained a second leucine zipper motif at residues 432 to 453. Amongst the *Arabidopsis* HD-ZipIII family members known, *REV* is the only protein that contained a second predicted leucine zipper.

#### Example 2

##### *REVOLUTA* Clones and Expression Vectors

A variety of recombinant DNA clones have been made that contain the wild-type *REVOLUTA* gene isolated from genomic DNA obtained from *Arabidopsis* ecotypes Columbia (Co) and Nossen (No) ecotypes. In addition, genomic DNA clones have been obtained from *revoluta* mutants: *rev-1*, *rev-2*, *rev-3*, *rev-4*, *rev-5* and *rev-6*. *Revoluta* mutants *rev-1*, *rev-2* and *rev-4* are in the No ecotype background and the *rev-3*, *rev-5* and *rev-6* mutants are in Columbia. Overlapping regions of wild-type Columbia *Revoluta* cDNA were cloned separately into a vector for sequencing. The cDNA sequenced included approximately 350 nucleotides of untranslated 5' sequence, the entire *Revoluta* coding region and approximately 400 nucleotides of untranslated 3' sequence. The wild-type Columbia *REV* cDNA sequence was in agreement with the predicted spliced nucleotide sequence available on the Kazusa database site [[www.kazusa.or.jp/arabi/chr5/clone/MUP24/index.html](http://www.kazusa.or.jp/arabi/chr5/clone/MUP24/index.html)].

##### Expression of *REVOLUTA* from an endogenous promoter

A region of genomic DNA running from approximately 2.8 kb upstream of the *Revoluta* coding sequence (5' untranslated DNA) through 200 bp downstream of the initiating Methionine was amplified by PCR from Columbia genomic DNA and cloned into the pCRII-TOPO vector from Invitrogen (PCR primers used: forward

primer (includes BamHI restriction site):  
5' TTGGATCCGGGAACACTTAAAGTATAGTGCAATTG 3' [SEQ ID NO:49],  
reverse primer: 5' CAGACTTTGATCTGCTTAGGCTC 3', [SEQ ID NO:50]).  
Clones from independent PCR reactions were sequenced to verify the accuracy of the  
5 PCR amplification. A clone whose sequence matched that in the *Arabidopsis*  
database, except for the apparent deletion of 1 T bp from a stretch of 12 Ts  
approximately 1.2 kb 5' of the *Revoluta* coding sequence, was chosen for use in  
cloning the endogenous *Revoluta* promoter region (nucleotides 1-2848 of SEQ ID  
NO:1). A clone, pNO84, containing the genomic DNA sequence of *REVOLUTA* was  
10 isolated from a No-ecotype plant. A 2.8 kb BamHI-SalI restriction digest DNA  
fragment, including approximately 2.6 kb of promoter and upstream sequence and  
0.2kb of *REV* coding sequence, was cloned into the BamHI and SalI sites of clone  
pNO84 to generate a *REVOLUTA* gene from ecotype No linked to its endogenous  
promoter. To clone a 3' polyadenylation signal onto the 3' end of the pNO84  
15 *Revoluta* gene, approximately 0.7 kb of the 3' end of a *Revoluta* Co gene, starting  
immediately downstream of the *REV* stop codon was amplified using the polymerase  
chain reaction with the following oligonucleotides (5' primer includes a NotI site:  
5' TTGCGCCGCTTCGATTGACAGAAAAAGACTAATTT 3' [SEQ ID NO:51];  
3' primer includes ApaI and KpnI sites:  
20 5' TTGGGCCCGGTACCCTCAACCAACCACATGGAC 3' [SEQ ID NO:52]). The  
amplified polyA addition site DNA fragment was cloned into the NotI and ApaI sites  
of pNO84 3' of the *REVOLUTA* coding sequence. *REVOLUTA* expression  
transgenes containing the expected 3' polyA addition sequence were verified by DNA  
sequencing. The resulting *REVOLUTA* transgene containing the *REV* promoter,  
25 coding, and 3' regions was cloned out of the original vector using KpnI and ligated  
into the pCGN1547 T-DNA binary vector (McBride et al., 1990 *Plant Mol. Biol.*  
14:269-276).

REVOLUTA expressed from the 35S cauliflower mosaic virus promoter

A DNA fragment encoding approximately 900 bp of the 35S cauliflower  
30 mosaic viral promoter (35S CaMV) was amplified from the pHomer 102 plasmid by  
PCR using primers 5' AAGGTACCAAGTTCGACGGAGAAGGTGA 3' [SEQ ID  
No.:53] and 5' AAGGATCCTGTAGAGAGAGACTGGTGATTCAG 3' [SEQ ID  
No.:54]. Clones containing amplified DNA fragments from independent PCR  
reactions were sequenced to verify the accuracy of the PCR amplification. KpnI and  
35 BamHI restriction sites were included in the PCR primers to allow for the isolation of

a 900 bp KpnI-BamHI fragment that includes the amplified 35S CaMV promoter. This KpnI-BamHI 35S CaMV fragment was inserted 5' of the REV genomic sequence in clone pNO84 at the KpnI and BamHI sites to generate a No *Revolvata* transgene linked approximately 70 bp downstream of the 35S CaMV promoter transcription start site. The 3' end of the REV gene was placed downstream of the REV coding region following the same procedure described above. The entire 35S CaMV *Revolvata* transgene was cloned into T-DNA binary vector pCGN1547 using KpnI.

#### REV Inverted Repeat Constructs

REV cDNA was amplified using the following primers: REVIR-1 TTATCGATAGCTTTGCTTATCCGGGAAT [SEQ ID NO:138] and REVIR-2 TTGCGGCCGCCTG-ACAAGCCATACCAGCAA [SEQ ID NO:139]; REVIR-3 TTGCGGCCGCAGTTCAACGTGTTGC-AATGG [SEQ ID NO:140] and REVIR-4 TTGCATGCGCTAGCGTCGTCGCTTCCAAGTGAAT [SEQ ID NO:141]; and REVIR-5 TTGTCGACCCGCGGAGCTTTGCTTATCCGGGAAT [SEQ ID NO:142] and REVIR-6 TTGATGCGCTAGCCTGACAAGCCATACCAGCAA [SEQ ID NO:143]. These PCR products were cloned behind the CaMV 35S promoter in the order 1/2 then 3/4 then 5/6, and then cloned into pCGN1547. All these IR primers have restriction sites on the end. REVIR-1 and REVIR-5 correspond to bp 5496-5515 (in exon 12) and REVIR-2 and REVIR-6 correspond to 6226-6245 (in exon 15). The linker sequence is made from the product of REVIR-3 corresponding to 6268-6288 (in exon 15) and REVIR-4 corresponding to 6509-6528 (in exon 16). The construct therefore consists of: CLAI restriction site 5496-5582; 5668-5748; 5834-5968; 6051-6245 NOTI restriction site 6268-6388; 6477-6528 NHEI SPHI restriction sites 6245-6051; 5968-5834; 5748-5668; 5582-5496 SAC II restriction site (SEQ ID NO:144).

Additional inverted repeat constructs are made essentially as described above, and include the following: An inverted repeat construct is made from At REV comprising Exons 3-7, 3670 to 3743; 3822 to 3912; 4004 to 4099; 4187 to 4300; and 4383-4466 (SEQ ID NO:145); a linker of exon 15 (SEQ ID NO:146) and SEQ ID NO:147. Similarly, an inverted repeat construct is made from tomato REV comprising SEQ ID NO:148 and SEQ ID NO:150, with a linker of SEQ ID NO:149. Inverted repeat constructs are made from rice, including an inverted repeat construct from rice Rev1, comprising SEQ ID NO:151 and SEQ ID NO:153, with a linker of

SEQ ID NO:152 and an inverted repeat construct from rice Rev2, comprising SEQ ID NO:154 and SEQ ID NO:156, with a linker of SEQ ID NO:155.

### Example 3

#### Complementation of revoluta mutants using REVOLUTA transgenes

5 *Agrobacterium* strain At503 was transformed with the above constructs and cocultivated with root explants from *rev-1* and wild-type Nossen 2-3 week old seedlings (Valvekens et al., 1988 *Proc. Nat. Acad. Sci. USA* 85:5536-5540). Regenerated plants from this tissue are analyzed for complementation of the *rev* phenotype by comparing the transformed plants to the nontransformed *rev* mutant  
10 plants. Alternatively, *Arabidopsis* plants expressing a *Revoluta* transgene can be made using *in planta* transformation (Bechtold et al., 1998 *Methods Mol. Biol.* 82:259-266). Gene expression of the transgenes is determined by performing Northern blot hybridization assays using *Revoluta* transgene specific hybridization probes that do not hybridize significantly to endogenous *Revoluta* mRNA.  
15 Alternatively, *Revoluta* gene expression is measured by performing reverse transcriptase reactions on isolated mRNA samples and than using copy DNA from the reverse transcriptase reaction as substrate for PCR (see "PCR Strategies", M.A. Innis, D.H. Gelfand and J.J. Sninsky, eds., 1995, Academic Press, San Diego, CA (Chapter 14); "PCR Protocols: A Guide to Methods and Applications", M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds., Academic Press, NY (1990)). The amount of PCR amplification product reflects the level of *Revoluta* gene expression in the plants at the time the tissue was collected for preparation of the mRNA sample.

#### Partial Complementation of the *rev-1* mutant

We confirmed that the HD-Zip protein encoded by *MUP24.4* was the *REV*  
25 gene product, by transforming constructs containing the wild-type coding region into homozygous *rev-1* plants. Partial complementation was seen in one out of six fertile T2 lines transformed with the *5'REV* construct. Figure 5A shows two T2 *rev-1* plants, one transformed with the vector alone (left) and one transformed with the *5'REV* construct (right). Plants transformed with the *5'REV* construct had an  
30 increased number of lateral shoots in the axils of the cauline leaves on the main inflorescence, relative to the *rev-1* control plant transformed with the vector. They also had narrower leaf stalks, and smaller, less revolute leaves compared to the *rev-1* control. Additionally the flowers in this transformed line, like wild-type flowers, are smaller than those on the *rev-1* control plants (Figure 5B and 5C). Together these  
35 results supported the conclusion that the HD-Zip coding region was the *REV* gene,

but suggested that a specific expression pattern may be necessary to complement the *rev-1* mutation since no plants were complemented with a Cauliflower Mosaic Virus 35S promoter- *Revoluta* construct.

#### Suppression of *REV* with an inverted repeat transgene

5 Introduction of antisense RNA and inverted gene repeats into wild-type organisms has been shown to interfere with normal gene function in a variety of systems (reviewed in Sharp and Zamore, 2000). More recently, Waterhouse et al. (1998) showed that transformation of wild-type plants with a construct containing an inverted repeat of a wild-type gene under the control of a ubiquitous promoter, 10 induces silencing of the endogenous gene. These results have been confirmed by Chuang and Meyerowitz (2000). Therefore, to further substantiate the conclusion that the HD-Zip protein identified was the *REV* gene, we transformed an inverted repeat construct of this ORF under the control of the CaMV 35S promoter into wild-type Columbia plants and determined the induction of a *Rev* phenotype. Of 16 15 independent transformants examined, five showed a *Rev* phenotype with similar or lesser intensity to that conferred by the weak alleles, *rev-3* and *-5*. In particular, these plants, like *rev* mutant plants, had a large number of empty axils (Figure 5E-H), compared to the wild-type Columbia plants (Figure 5D and H). Figure 5H shows a control Columbia plant (right) and a transgenic *Rev*-like plant containing the inverted 20 repeat construct (left).

#### Example 4

##### *REV* mRNA is expressed in proliferating and in non-dividing tissue

##### In situ hybridization

Non-radioactive in situ hybridization was performed as described in 25 [http://www.arabidopsis.org/cshl-course/5-in\\_situ.html/](http://www.arabidopsis.org/cshl-course/5-in_situ.html/). Either a 455 bp central portion of *REV*, or a 779 bp 3' portion of *REV* was amplified from cDNA as described above using the primers REVcentral-1 GGAGCCTTGAAGTTTCACTATG [SEQ ID NO:175] and REVcentral-2 AGGCTGCCTTCCTAATCCAT [SEQ ID NO:176]; or the primers REV3'-1 30 TGAGGAGCGTGATCTCATCAG [SEQ ID NO:177] and REV 3'-2 CAAAATTATCACATCATTCCCTTT [SEQ ID NO:178] and cloned into the Topo II vector (Invitrogen, Carlsbad, CA). The central *REV* probe was used for Figure 7 A-L, N-O, and the 3' *REV* probe for P-Q. A 662 bp of *FIL* was amplified from cDNA using primers FIL-1 CGTCTATGTCCTCCCCTTCC [SEQ ID NO:179] and 35 FIL-2 AACGTTAGCAGCTGCAGGA [SEQ ID NO:180] and cloned into the TopoII

vector. *Histone H4* was amplified from cDNA using primers H4-1 TGGAAAGGGAGGAAAAGGTT [SEQ ID NO:181] and H4-2 GCCGAATCCGTAAAGAGTCC [SEQ ID NO:182] and cloned into the TOPOII vector. Sense and antisense probes were generated as described in the protocol using  
5 a kit (Roche Biochemicals, Indianapolis, IN). Pictures were taken on a Nikon Microphot using a Nikon Coolpix digital camera and imported in Adobe Photoshop 4.01.

To determine the level of *REV* expression in different tissues, semi-quantitative RT-PCR was performed on RNA isolated from 3-4 week old plants  
10 (young cauline leaves, young rosette leaves) and 6-7 week old plants (buds, flowers, stems, older cauline leaves, older rosette leaves) using primers from the *REV* gene. Control reactions were performed simultaneously using primers from the actin gene (*ACT2*; Accession ATU41998).

*REV* and *ACT2* were simultaneously amplified using RT-PCR on cDNA  
15 prepared from various plant tissues. The resulting products were blotted and probed with the respective genes. The blots were quantified using a Phosphorimager detection system and analyzed with NIH Image (1.60). The *REV* levels were corrected for loading differences using the *ACT2* levels. For both genes, amplification of the genomic sequence yields a larger fragment than that derived  
20 from the cDNA with the same primers, as expected due to the absence of intronic sequences. Similar results were obtained from duplicate experiments. The tissue source for each lane are as follows: (A) bud, (B) flower, (C) stem, (D) young cauline leaves from 3-4 week plant, (E) older cauline leaves from 6-7 week plant, (F) young rosette leaves from 3-4 week plant, (G) older rosette leaves from 6-7 week plant, (H)  
25 no cDNA control, (I) 0.005 ng genomic DNA, (J) 0.05 ng genomic DNA, (K) 0.5 ng genomic DNA, (L) 5 ng genomic DNA.

After PCR amplification, the reaction products were blotted and probed with the respective genes. Quantitation of the *REV* signal intensity, after normalization to the *ACT2* signal, indicated that *REV* mRNA was detected in all tissues tested, as  
30 shown in Figure 6. It was, however, most abundant in flowers and buds (Figure 6, lanes A and B). The amount of *REV* mRNA dropped about twofold in stems and young cauline leaves (lanes C and D) and was further reduced in older cauline leaves and rosette leaves (lanes E, F and G).

In situ hybridization experiments with *REV* antisense probes showed results  
35 consistent with the RT-PCR experiments and are shown in Figure 7. The *REV*

mRNA was most abundant in apices and in regions of active cell division throughout the plant (Figure 7A-L). *REV* was expressed in wild-type inflorescence meristems and its expression increased in floral primordia (Figure 7A-C). In the floral meristem, an increased concentration of *REV* mRNA was apparent in sepals, stamen and carpel primordia, relative to the surrounding floral tissue (Figures 7C-D and 7F-I). However, *REV* expression decreased in the sepals of later stage flowers while expression remained strong in developing carpels and stamens at this stage (Figure 7A, C, F). *REV* mRNA was also abundant in axillary meristems (Figure 7A). In the cauline leaves, expression was detected in two gradients simultaneously, one decreasing from the proximal to distal direction in the leaf, and the other decreasing in the adaxial to abaxial direction in the leaf (Figure 7E). *REV* mRNA was detected in early embryos (Figure 7K) and continued at high levels throughout the cell division phase of embryogenesis and in the endosperm (Figure 7L and J). Finally, *REV* mRNA was detected in mature non-dividing tissue of the stem, particularly in the cortical and vascular regions (Figure 7P). This expression pattern correlates with the increased numbers of cell layers seen in the cortex of *rev-1* stems (Figure 7R) compared to wild-type stems (Figure 7S).

*Rev-1* mutant plants display an altered pattern of the S-phase cells

The histone H4 gene is transcribed only in actively dividing cells and cells undergoing endoreduplication. Consequently, histone H4 mRNA can be used as a marker of cell cycle activity. To better understand how the *REV* gene influences cell division patterns in developing plants, histone H4 mRNA in situ hybridizations were performed on wild-type and *rev-1* mutant plants. The number of cells expressing the H4 mRNA appeared increased in *rev-1* mutant plants relative to wild type as shown in Figures 8A and 8B. This was particularly noticeable in the adaxial regions of cauline leaves and in the stem, both of which are regions that undergo excess growth in *rev* mutants. The striking localization of cell divisions to the adaxial compartment of *rev* cauline leaves affected the entire length of the leaf. In the thickened region proximal to the axil, clusters of cell divisions were common in the *rev-1* mutant (Figure 8D) compared to wild type (Figure 8C).

### Example 5

#### REV double mutants

##### FIL is properly expressed in rev mutants

###### REV double mutants

5 *lfy* REV double mutants were obtained as described in Talbert et al., (1995). Briefly, REV F2 individuals from a *rev-1* X *lfy-6/+* cross were progeny tested for segregation of *lfy* rev F3 double mutants. The putative *lfy-6 rev-1* plants were tested using PCR to verify the presence of the *lfy* mutation because *rev* and *lfy* are tightly linked. The *lfy-6* CAPS markers used were designed to take advantage of the single  
10 base pair change giving rise to the *lfy-6* mutation: a CAA to UAA change at codon 32. The primers are AACGAGAGCATTGGTTCAAG [SEQ ID NO:183] and CAACGAAAGATATGAGAGAG [SEQ ID NO:184]. Cutting the resulting PCR product with MaeIII distinguishes the *lfy-6* mutant from the wild-type gene. For the  
15 *rev fil* mutant, pollen from homozygous *rev-1* plants were crossed to homozygous *fil-1* plants. The heterozygous progeny was crossed to *rev-1* pollen and plants homozygous for both *rev-1* and *fil-1* identified.

###### Scanning Electron Microscopy

Samples were fixed in 3% glutaraldehyde in 0.02M sodium phosphate pH 7.0, and vacuum infiltrated for 15-30 minutes, then stored at 4°C for 16 hours or greater.  
20 Samples were placed in 1% osmium tetroxide (Polysciences, Warrington,PA) for 2-4 hours before dehydration in an ethanol series. The samples were dried using a Denton DCP-1 Critical Point Drying Apparatus (Denton Vacuum Inc., Moorestown, NJ). Samples were mounted on carbon conductive pads fixed to SEM specimen mounts and coated with Au/Pd. A Jeol JSM-840A scanning microscope was used.  
25 The images were taken using Polaroid Type 55 film, then scanned and imported into Adobe Photoshop 4.01

In *fil* mutants, flowers form earlier than in wild-type plants, tertiary shoots fail to form due to an apparent lack of meristem formation at the base of cauline leaves, and flowers show aberrant number, shape and arrangement of organs. Additionally  
30 severe *fil* alleles sometimes form flowerless pedicels or pedicels with single sepal structures on their distal end which resemble the filaments formed in *rev* plants (Chen et al., 1999; Sawa et al., 1999). In *fil rev* double mutants the primary inflorescence is severely shortened, and all floral primordia appear to terminate as flowerless pedicels. These structures, like pedicels on wild-type flowers, are smooth. However,  
35 because all floral primordia become flowerless pedicels, it has been suggested that

*REV* and *FIL* have partially redundant functions to promote flower formation in floral primordia (Chen et al., 1999).

Given this strong double-mutant phenotype, it was helpful to determine the expression of *FIL* mRNA in *rev* plants. In wild-type plants, *FIL* mRNA expression occurs weakly throughout the SAM, but as the floral meristem becomes distinct from the SAM, *FIL* expression increases on the abaxial side of the meristem (Siegfried et al., 1999). *FIL* in situ on *rev-1* tissue were indistinguishable from the wild-type controls, indicating that disruption of the *rev* gene product does not influence *FIL* expression (Figure 7E-I).

#### 10 Interactions of *rev* with other mutations

In order to better understand *REV* activity in floral meristems, we created *rev-1 lfy-6* double mutant plants. *LFY* function is required for proper specification of floral meristem identity. As shown in Figure 9, *lfy rev* double mutants, like *rev fil* double mutants, were short plants with a single inflorescence terminating in a bundle of filamentous structures. Unlike the filamentous structures formed in *rev fil* double mutants, which are usually smooth and resemble flowerless pedicels (Figure 9 B, F and G), the filamentous structures formed on *rev lfy* double mutants ranged from smooth to hairy acropetally, and were leaf-like in that they had stellate trichomes, although some were carpelloid (Figure 9 A, E, H-I). A scale-like structure was visible at the base of each filament (Figure 9H and I) and may represent a rudimentary subtending organ (Long and Barton, 2000). Additionally *rev lfy* double mutants had one or more filamentous stem appendages, usually on the opposite side as the first cauline leaf (Figure 9 A and C). The appendages resembled the structures often detected in the axil of *rev* single mutants (Figure 9D). As with the *fil rev* double mutant, the *lfy rev* double mutant indicates that *REV* has a role in floral meristem maintenance.

In concert with *LFY*, *APETALA 1* is required to establish the floral meristem. Ectopic expression of either *LFY* or *API* during vegetative development can result in precocious flower formation (Weigel and Nilsson (1995) Nature 377, 495; Mandel and Yanofsky (1995) Nature 377: 522). *API* plays an additional role in determining the identity of sepal and petal organs in the first and second whorl. Specifically, in *apl-1* mutants, sepals are converted into cauline leaf or bract-like structures, and petals are absent having failed to be initiated. In addition, floral meristems are converted partially or completely into inflorescence meristems in the axil of the cauline leaf-like sepals, leading to the production of highly branched structures

(Bowman 1993 Dev 119, 721-743 Figure 9). Unlike the *rev lfy* double mutant, *rev-1 apl-1* double mutants produce normal size inflorescences with floral defects expected from the respective single mutant phenotypes (Figure 9). The *rev-1 apl-1* mutant flowers resembled single *apl-1* mutant flowers, except that they had longer bract-like sepals than the *apl-1* mutant flowers (Figure 9 I). Although this phenotype of the *rev-1 apl-1* mutant is additive, the lack of axillary meristems of the *rev-1* mutant is epistatic to the increased number of axillary meristems of the *apl-1* mutant which results in a non-branched structure (Figure 9).

*AGAMOUS* is another multifunctional gene that regulates floral organ identity and is required for determinate growth of the flower. In *ag-1* mutants, petals develop in place of the stamens in the third whorl, and a new flower is initiated in place of the carpels in the fourth whorl. The phenotype of the *rev-1 ag-1* mutant is additive with the double mutant flowers producing enlarged petals as in *rev-1* plants, but with petals in the third whorl as in *ag-1* plants (Figure 9). Also, *rev-1 ag-1* double mutant flowers reiterate flower development in the fourth whorl as *ag-1* single mutants.

The *CLAVATA* genes control the size of the apical meristem. Loss of function *clv* mutants have enlarged apical meristems due to the accumulation of undifferentiated stem cells in the central zone of the apical meristem. The strong *clv1-4* mutant also has club shaped seed pods due to the presence of additional carpels. The double *clv1-4 rev-1* mutant phenotype is synergistic because they have massive overgrowth of structures from within the floral bud. These callus-like tissues actually burst through the seed pod as they continue growing. This result is consistent with a role for REV in limiting cell divisions in the floral tissues that is partially redundant with CLV1 as revealed by the double mutant phenotype.

#### 25 Example 6

##### Identification and Isolation of REVOLUTA From Other Plant Species

In one embodiment the present invention provides a method to identify and use *REVOLUTA* genes and proteins that function as modulators of cell division in the plant species from which they are isolated. *REVOLUTA* orthologs to the *Arabidopsis* *REVOLUTA* gene are isolated using a combination of a "sequence similar test" and a *REVOLUTA* "gene function test." First, a candidate *REVOLUTA* gene sequence is isolated from target plant DNA, such as for example, genomic DNA or DNA maintained in a gene library, by the polymerase chain reaction using "CODEHOP" PCR primers (Rose et al., 1998 *Nucleic Acids Res.* 26:1628-1635) that amplify subfamily III HD-Zip polynucleotides. The amplified DNA is then sequenced to

determine that the PCR product encodes a region of protein that is at least about 70% identical, more preferably at least about 75% identical, and most preferably at least about 80% identical to the *Arabidopsis REVOLUTA* protein sequence corresponding to the PCR amplified region. The "gene function test" is then performed using a polynucleotide region from the candidate *REVOLUTA* coding sequence that has been transferred into a plant transformation vector and transformed back into the plant species from which the candidate *REVOLUTA* gene was derived. Actual *REVOLUTA* genes are those that modulate plant cell division when the *REVOLUTA* transgene is expressed in the transformed plant.

10        Identification of HD-Zip Subfamily III PCR primers

To generate HD-Zip subfamily III CODEHOP primers, the known HD-Zip III amino acid sequences were entered into the blockmaker program located at the Fred Hutchinson Cancer Research Center website: <http://blocks.fhcrc.org>. The program compares the sequences and generates blocks of homology conserved between the different proteins. Table 8 lists the HD-Zip III amino acid block made from six HDZip III family members.

Table 8

HD-Zip III amino block identified using the the Fred Hutchinson Cancer Research Center "blocks" computer program

| HD-Zip Block                    | Amino Acid Sequence   |
|---------------------------------|---|
| HD-Zip Protein                  |   |
| <u>HD-ZipIII: Block A</u>       |   |
| (block width = 43)              |   |
| <i>REVOLUTA</i><br>SEQ ID No.:2 | <sup>1</sup> 24 GKYVRYTAEQVEALERVYAECFKPSSLRRQQLIRECSILANIE |
| Athb-14<br>SEQ ID No.:55        | 24 GKYVRYTPEQVEALERVYTECPKPSLRRQQLIRECPILSNIE               |
| Athb-8<br>SEQ ID No.:56         | 14 GKYVRYTPEQVEALERLYNDCPKPSSMRRQQLIRECPILSNIE              |
| Athb-9<br>SEQ ID No.:57         | 20 GKYVRYTPEQVEALERVYAECFKPSSLRRQQLIRECPILCNIE              |
| CRHB1<br>SEQ ID No.:58          | 19 GKYVRYTSEQVALEKLYCECPKPTLLQRQQLIRECSILRNVD               |

F5F19.21 16 GKYVRYTPEQVEALERLYHDCPKPSSIRRQQLIRECPILSNIE  
 SEQ ID No.:59

HD-ZipIII: Block B

(block width = 42)

*REVOLUTA* 70 IKVWFQNRRCRDQQRKEASRLQSVNRKLSAMNKLLMEENDRL  
 SEQ ID No.:2

Athb-14 70 IKVWFQNRRCREKQRKEAARLQTVNRKLSAMNKLLMEENDRL  
 SEQ ID No.:55

Athb-8 60 IKVWFQNRRCREKQRKEASRLQAVNRKLTAMNKLLMEENDRL  
 SEQ ID No.:56

Athb-9 66 IKVWFQNRRCREKQRKESARLQTVNRKLSAMNKLLMEENDRL  
 SEQ ID No.:57

CRHB1 65 IKVWFQNRRCREKQRKEWCRLQSLNGKLTPIINTMLMEENVQL  
 SEQ ID No.:58

F5F19.21 62 IKVWFQNRRCREKQRKEASRLQAVNRKLTAMNKLLMEENDRL  
 SEQ ID No.:59

HD-ZipIII: Block C

(block width = 29)

*REVOLUTA* 154 SPAGLLSIAEETLAEFLSKATGTAVDWVQ  
 SEQ ID No.:2

Athb-14 167 NPAGLLSIAEEALAEFLSKATGTAVDWVQ  
 SEQ ID No.:55

Athb-8 153 SPAGLLSIADETLTEFISKATGTAVEWVQ  
 SEQ ID No.:56

Athb-9 163 NPANLLSIAEETLAEFLCKATGTAVDWVQ  
 SEQ ID No.:57

CRHB1 109 HVAQLVTINHALRRQLSSTPSHFRFPTVS  
 SEQ ID No.:58

F5F19.21 154 SPAGLLSIAEETLAEFLSKATGTAVEWVQ  
 SEQ ID No.:59

---

**HD-ZipIII: Block D**

(block width = 31)

|                 |     |  |
|-----------------|-----|--|
| <i>REVOLUTA</i> | 446 | VLCASMLLQNVPPAVLIRFLREHRSEWAD                  |
| SEQ ID No.:2    |     |  |
| Athb-14         | 464 | VLCASMLLQNVPPAVLVRFLREHRSEWAD                  |
| SEQ ID No.:55   |     |  |
| Athb-8          | 452 | VLCASMLLQNVPPSILLRFLREHRQEWAD                  |
| SEQ ID No.:56   |     |  |
| Athb-9          | 460 | VLCASMLLQNVPPPLVLRFLREHRAEWAD                  |
| SEQ ID No.:57   |     |  |
| CRHB1           | 138 | LMNIYAIVRLQHVPIPECRS <sup>2</sup> XXXXXXXXXXXX |
| SEQ ID No.:58   |     |  |
| F5F19.21        | 453 | VLCASMLLQNVPPAILLRFLREHRSEWAD                  |
| SEQ ID No.:59   |     |  |

---

<sup>1</sup>The number denotes the amino acid position of the first amino acid in each block using the amino acid numbers in each protein sequence disclosed in the referenced SEQ ID Number.

<sup>2</sup>X means that no corresponding amino acid is found in the optimized computer alignment.

5 HD-Zip class III PCR primers were designed with the HD-Zip III "block " amino acid sequence data, presented in Table 8, by inputting the "block" sequence data into the CODEHOP program using either the gibbs algorithm or the motif algorithm. The PCR primer output was further refined by selecting a particular plant species, in this example rice, to which the PCR primer sequence was biased based upon the preferred codon usage compiled for rice (other plant codon biases can also be selected using the CODEHOP program). Table 9 presents a set of possible CODEHOP HD-Zip III PCR primers that can be compiled using the CODEHOP program to amplify HD-Zip genes from rice, barley and corn.

Table 9

HD-Zip III PCR primer designed using the Fred Hutchinson Cancer Research Center "CODEHOP" computer program.

---

|              |                                       |
|--------------|---------------------------------------|
| HD-Zip Block | Oligonucleotide Sequence <sup>1</sup> |
|--------------|---------------------------------------|

---

HD-ZipIII: Block A**Forward**

|               |                                 |
|---------------|---------------------------------|
| Rice A1F      | 5'-GGCGGCAGCAGCTGatbmnggartg-3' |
| SEQ ID No.:60 | R Q Q L I R E C                 |

Degen<sup>2</sup>=48, temp<sup>3</sup>=62.4

|               |                                      |
|---------------|--------------------------------------|
| Rice A2F      | 5'-GGAGAGGGTGTACTGCGAGtgyccnaarcc-3' |
| SEQ ID No.:61 | E R V Y C E C P K P                  |

Degen=16, temp=62.2 rice A2

|               |                                |
|---------------|--------------------------------|
| Rice A3F      | 5'-TGCGGTACACCCCGarcargtnea-3' |
| SEQ ID No.:62 | R Y T P E Q V E                |

Degen=32, temp=63.3

|               |                                |
|---------------|--------------------------------|
| Rice A4F      | 5'-TGCGGTACACCCCGArcargtnea-3' |
| SEQ ID No.:63 | R Y T P E Q V E A              |

Degen=64, temp=63.3

|               |                              |
|---------------|------------------------------|
| Rice A5F      | 5'-CGGTACACCCCGAGcargtnea-3' |
| SEQ ID No.:64 | R Y T P E Q V E A            |

Degen=32, temp=64.2

|               |                                    |
|---------------|------------------------------------|
| Rice A6F      | 5'-TCGACAGGCTCTACTGcgantgyccnaa-3' |
| SEQ ID No.:65 | E R V Y C E C P K                  |

Degen=32, temp=60.4

Rice A7F 5'-TGGAGAGGGTGTACTGCGAntgyccnaarc-3'  
 SEQ ID No.:66 E R V Y C E C P K P

Degen=64, temp=60.4

Rice A8F 5'-COGACCTCCATGCGGmgncarcaryt-3'  
 SEQ ID No.:67 P S S L R R Q Q L

Degen=64 temp=60.8

Rice A9F 5'-CCATGCGGGCGGcArcarytnat-3'  
 SEQ ID No.:68 L R R Q Q L I

Degen=32, temp=62.4

HD-ZipIII: Block A

**Reverse**

Rice A1R 5'-CGGGGGTGTACCGCaertayttnc-3'  
 SEQ ID No.:69

Degen=16, temp=62.1

Complementary to:

G K Y V R Y T P E  
 ccnttyatreaCGCCATGTGGGGGC

Rice A2R 5'-CTGCTCGGGGGTGTACcknaertaytt-3'  
 SEQ ID No.:70

Degen=32, temp=60.1

Complementary to:

K Y V R Y T P E Q  
 ttyatrcankcCATGTGGGGGCTCGTC

Rice A3R 5'-ACCTGCTCGGGGGTgtancknaerta-3'  
 SEQ ID No.:71

-68-

Degen=64, temp=60.1

Complementary to:

Y V R Y T P E Q V

atrcankcnatGTGGGGCTCGTCCA

Rice A4R  
SEQ ID No.:72

Degen=64, temp=63.2

Complementary to:

V R Y T P E Q V E

cankcnatrtgGGGGCTCGTCCACC

Rice A5R  
SEQ ID No.:73

5' -CACCTCTCCAGGGCctsnacytgytc-3'

Degen=32, temp=61.2

Complementary to:

E Q V E A L E R V

ctygtycanstCCGGGACCTCTCCAC

Rice A6R  
SEQ ID No.:74

5' -CAGTACACCTCTCCAGGGcytsnacytgyt-3'

Degen=64 temp=62.0

Complementary to:

Q V E A L E R V Y C

tygtycanstycGGGACCTCTCCACATGAC

Rice A7R  
SEQ ID No.:75

5' -CAGTACACCTCTCCAGGGcytsnacytg-3'

Degen=32, temp=62.0

Complementary to:

Q V E A L E R V Y C

gtycanstycGGGACCTCTCCACATGAC

---

**HD-ZipIII: Block B****Forward**Rice B1F  
SEQ ID No.:76

5' -CCATGAACAAGATGCTGatggargaraa-3'

M N K M L M E E N

Degen=4, temp=63.3

Rice B2F  
SEQ ID No.:77

5' -CGGCTGCAGACCGTgaayvgnaryt-3'  
R L Q S V N R K L

Degen=96, temp=63.3

Rice B3F  
SEQ ID No.:78

5' -GACCGCCATGAACAAGATGytnatggarga-3'  
T A M N K M L M E E

Degen=16, temp=60.1

Rice B4F  
SEQ ID No.:79

5' -CGCCATGAACAAGATGCTnatggargara-3'  
A M N K M L M E E N

Degen=16, temp=62.2

Rice B5F  
SEQ ID No.:80

5' -CCATGAACAAGATGCTGATggargaraayg-3'  
M N K M L M E E N D

Degen=8, temp=63.3

HD-ZipIII: Block B

Reverse

Rice B1R  
SEQ ID No.:81

5' -CGGCACCGCCGGttytgraacca-3'

Degen=4, temp=64.2

Complementary to:

W P Q N R R C R  
accaargtyttGGCCGCCACGGC

Rice B2R  
SEQ ID No.:82

5' -ATCTGGTTCATGGGGTCaryttncbrtt-3'

Degen=96, temp=60.1

Complementary to:

N R K L T A M N K M

ttrbenttyraCTGGCGGTACTTGTCTA

Rice B3R  
SEQ ID No.:83

5' -CGCCGGTCTCTGGaaccanacytt-3'

degen=8, temp=64.3

Complementary to:

K V W F Q N R R

ttycanaccaaggTCTTGGCCGC

Rice B4R  
SEQ ID No.:84

5' -GCACCGCCGGTCTtgraaccanac-3'

degen=8, temp=61.0

Complementary to:

V W F Q N R R C

canaccaargtCTTGGCCGCCACG

HD-ZipIII: Block D

**Forward**

Rice D1F  
SEQ ID No.:85

5' -CCAAGGCCACCATGCTGytnarmaygt-3'

K A S M L L Q N V

Degen=64, temp=62.3

Rice D2F  
SEQ ID No.:86

5' -AAGGCCACCATGCTGCTncarmaygtnc-3'

K A S M L L Q N V P

Degen=128, temp=60.4

Rice D3F  
SEQ ID No.:87

5' -CCACCATGCTGCTGcarmaygtacc-3'

S M L L Q N V P

Degen=32, temp=60.1

Rice D4F  
SEQ ID No.:88

5' -CCCGTCTGCATCCGGttytnmgnga-3'

A V C I R F L R E

Degen=128, temp=63.1

Rice D5F 5'-CCGTCTGCATCCGGTTCytumngarca-3'  
 SEQ ID No.:89 V C I R F L R E H

Degen=128, temp=61.2

Rice D6F 5'-GTCTGCATCCGGTTCCTGmngarcaymg-3'  
 SEQ ID No.:90 V C I R F L R E H R

Degen=64, temp=60.2

Rice D7F 5'-TGCGGAGCACCGGnvngartgggc-3'  
 SEQ ID No.:91 R E H R S E W A

Degen=96, temp=62.9

Rice D8F 5'-GCGGGAGCACCGGTcngartgggcng-3'  
 SEQ ID No.:92 R E H R S E W A D

Degen=32, temp=62.6

Rice D9F 5'-GGAGCACCGGTCCgartgggcnga-3'  
 SEQ ID No.:93 R E H R S E W A D

Degen=8, temp=60.3

HD-ZipIII: Block D

Reverse

Rice D1R 5'-GACGGGCGGCggnacrtskytg-3'  
 SEQ ID No.:94

Degen=32, temp=60.4

Complementary to:

Q N V P P A V  
 gtyktrcanggCGGCGGGCAG

Rice D2R 5'-GACGGGCGGCggnacrtskytgn-3'

SEQ ID No.:95

Degen=128, temp=60.4

Complementary to:

Q N V P P A V

angtyktrcangGCGGCGGGCAG

Rice D3R

5' - CACTCCGACCGGTGCTcncknarraa-3'

SEQ ID No.:96

Degen=128, temp=61.5

Complementary to:

F L R E H R S E W

aarrankcnctCGTGGCCAGCCTCAC

<sup>1</sup>First line shows the oligonucleotide sequence of the CODEHOP designed primer. The degenerate nucleotide alphabet used by CODEHOP is: A → A, C → C, G → G, T → T, R → AG, Y → CT, M → AC, K → GT, W → AT, S → CG, B → CGT, D → AGT, H → ACT, V → ACG; and N → ACGT. The second line shows the amino acid sequence encoded by all of the redundant primers.

<sup>2</sup>"Degen" means the number of degenerate oligonucleotides within the primer pool that encode the designated amino acid sequence.

<sup>3</sup>"Temp." means the mean melting temperature of the degenerate oligonucleotide primer pool.

<sup>4</sup>"Complementary to" refers to the HD-Zip amino acid block that the designated reverse oligonucleotide is complementary to, i.e. the peptide encoding strand sequence region of the HD-Zip block.

Other HD-Zip III PCR primers can be selected by changing the primers sequences listed in Table 9 to reflect the appropriate codon usage bias of the target plant species. However, as shown below the CODEHOP HD-Zip III primers designed specifically for rice were also capable of amplifying HD-Zip fragments from the monocot plants  
5 maize and barley.

Isolation of Monocot HD-Zip Clones

Monocot HD-Zip III homologs were identified using CODEHOP primers Rice A2F [SEQ ID NO:71] and Rice B2R [SEQ ID NO:81] or Rice A2F and Rice B2R [SEQ ID No:82] selected from Table 9. These primers were used in a 20 μL  
10 PCR reaction using 2 Units AmpliTaq Gold (Perkin Elmer), the supplied buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, and 0.5 μM each primer. The template DNAs for PCR used were: 1.5 μL of a rice (*Oryza sativa* L. *indicam* var. IR36) cDNA library

(Stratagene FL1041b); 1.5  $\mu$ L of purified genomic rice (*Oryza sativa*) DNA (about 400 ng); 1.5  $\mu$ L of purified genomic barley (*Hordeum vulgare*) DNA (about 400 ng); 1.5  $\mu$ L of purified genomic maize (*Zea mays*) DNA (about 400 ng). PCR conditions included a 95°C incubation for 9 minutes, followed by 5 cycles of 95°C (30 seconds); 5 60°C to 55°C (30 seconds) decreasing by 1°C each cycle; 72°C (2 minutes); then 35 cycles of 95°C (30 seconds); 55°C (30 seconds); and 72°C (2 minutes). The resulting PCR DNA products were analyzed by gel electrophoresis, then cut out from a 0.8% low melt agarose gel (SeaPlaque, FMC Bioproducts, Rockland, ME) in TAE buffer, and purified using a PCR clean up kit (Promega). The DNA fragments were cloned 10 into a TOPO II vector kit (Invitrogen). DNA was purified from bacterial cells using a spin miniprep kit (Qiagen) and sequenced using BIG dye (Applied Biosystems). The rice, maize and barley DNA and protein sequences are disclosed in SEQ ID Nos:97-126, respectively.

The BLAST2 computer program of Altschul et al. (1997) was used to 15 compare the amplified monocot sequences with the corresponding protein region in *Arabidopsis REVOLUTA*. Computer aided sequence comparisons were performed using version BLAST2.0.9 at the National Institutes of Health webpage site: <http://www.ncbi.nlm.nih.gov/gorf/wblast2.cgi>. Each of the amplified sequences had a high degree of amino acid sequence identity or similarity to the *Arabidopsis* 20 *REVOLUTA* protein (about 79% to 88% amino acid identity and about 87% to 97% amino acid similarity). These data demonstrate that genes can be isolated from distantly related monocot plant species using the HD-Zip CODEHOP primers disclosed in Table 9, that encode peptides regions that have a high degree of sequence homology to the corresponding amino acid region of the *Arabidopsis* 25 *Revoluta* protein [SEQ ID No.:2].

#### REVOLUTA Function Test

Plant genes isolated using the above - described methods are then tested for *Revoluta* function. Functional testing to identify actual *Revoluta* genes is done by cloning the polynucleotide sequences amplified using various combinations of the 30 forward and reverse HD-Zip block PCR primers listed in Table 9 into plant transformation vectors. The putative *Revoluta* sequence is oriented in the plant transformation vector using one of the gene suppression strategies previously outlined, such as by making an inverted repeat transgene or an antisense transgene. Regenerated transgenic plants are examined and the number of cells contained in 35 various tissues is compared to the number of cells in the corresponding tissues of

untransformed plants. Plants that have been transformed with suppressor transgenes comprising *Revoluta* genes of the present invention have a statistically significant change in the number of cells within a representative cross sectional area of the tissue. Alternatively, the size of various plant organs such as leaves and shoots are significantly different as described for *Arabidopsis* by Taylor et al.(1995).

Alternatively, labeled DNA sequences are amplified using the forward and reverse HD-Zip CODEHOP PCR primers listed in Table 9, by using, for example, biotin or radiolabeled nucleotides in the PCR. The labeled HD-Zip III sequences are then used to screen a cDNA or genomic plant clone library via nucleic acid hybridization. Clones that positively hybridize to the labeled PCR amplified HD-Zip sequences are then isolated and the DNA inserts characterized by DNA sequencing to identify HD-Zip III coding and noncoding sequences. Regions of the isolated HD-Zip III genes are then manipulated *in vitro* to construct gene suppressive transgenes that are then tested in transgenic plants to identify HD-Zip III genes that have the same function as *REVOLUTA*, i.e. they modulate cell division.

#### Identification and Isolation of REVOLUTA From Tomato

The Tomato REV gene is identified using primers generated using the Codehops program as described above. Genomic DNA from 50 mg of young *Lycopersicon esculentum* leaves is isolated as described in Example 1 above. One microliter of DNA is PCR amplified using the conditions described in Example 3. The following primers are used: rice A2F; GGAGAGGGTGTACTGCGAGTGYCCNAARCC [SEQ ID NO:61] and tomato J1R; CAGCAGAATAAGCATCAACATTATAATCNGCCCAYT [SEQ ID NO:162]. The product is sequenced and a genomic clone (SEQ ID NO:163), encoding a protein having 84% identity and 90% similarity to the Rev-1 protein is identified. The coding region and amino acid sequence of the tomato Rev protein are presented as SEQ ID NO:164 and SEQ ID NO: 165, respectively.

Further analysis shows that there is significant identity at the amino acid level between the tomato REV and the other *Arabidopsis* HD-ZipIII family members (See Table 10).

Table 10

TomatoREV

|          |     |
|----------|-----|
| REV      | 84% |
| Athb-9   | 71% |
| Athb-8   | 66% |
| Athb-14  | 73% |
| F5F19.21 | 68% |

5

The tomato REV is then tested and shown to have *REVOLUTA* function, essentially as described above.

10

Identification and Isolation of REVOLUTA From Rice

Rice REV1:

Rice *Oryza sativa* leaf DNA, isolated essentially as described above, or cDNA from the library (Stratagene FL1041b) is used as a template for PCR essentially as described. The following primers are used for the rice genomic DNA REV1 clone:

15

TG-cDNA; GTRAGTGCCCCATACTTGCT (SEQ ID NO:165)  
R25AS-J; GCCGTTACGGCSTCRTRAANCC (SEQ ID NO:166)

To pull out the 5' end of the REV1cDNA, the following primers are used: one specific to the cloning vector, R22S; CGACGACTCCTGGAGTCCGTCAG (SEQ ID NO:167) and the other in the coding region of the gene, TGTATCATTTGCCAGCGGAG (SEQ ID NO:168). The nucleic acid sequence of rice Rev1 gene is found in SEQ ID NO:169 (genomic) and SEQ ID NO:170 (cDNA).

20

The amino acid sequence of Rice Rev1 is set forth in SEQ ID NO:171. The rice Rev1 is then tested and shown to have *REVOLUTA* function, essentially as described above.

25

Rice REV2:

Rice cDNA as described above was used as a template for PCR using the following oligos:

Rice A2f [SEQ ID No:71]:

GGAGAGGGTGTACTGCGAGTGYCCNAARCC

30

R10AS-K [SEQ ID NO: ]: GCAGCAGCATGGAGGCYTTNGCRCA

PCR is performed, essentially as described above, to isolate the cDNA of rice Rev2. The nucleic acid sequence of rice Rev2 is set forth in SEQ ID NO:172. The amino acid sequence of rice Rev2 is set forth in SEQ ID NO:173. The rice Rev2 is then tested and shown to have *REVOLUTA* function, essentially as described above.

### Example 7

#### Modulation of Cell Division in Maize

Zygotic immature embryos of about 0.5 to 1 mm are isolated from developing seeds of *Zea mays* using the methods disclosed in U.S. Patent 5,712,135. The freshly  
5 isolated embryos are enzymatically treated for 1-2 minutes with an enzyme solution II (0.3% macerozyme (Kinki Yakult, Nishinomiya, Japan) in CPW salts (Powell et al., 1985 "Plant Cell Culture, A Practical Approach", R. A. Dixon ed., Chapter 3) with 10% mannitol and 5 mM 2-N-Morpholino-ethane sulfonic acid (MES), pH 5.6). After 1-2 minutes incubation in this enzyme solution, the embryos are carefully  
10 washed with N6aph solution (macro- and micro-elements of N6 medium (Chu et al., 1975 *Sci. Sin. Peking* 18:659) supplemented with 6 mM asparagine, 12 mM proline, 1 mg/l thiamine-HCl, 0.5 mg/l nicotinic acid, 100 mg/l casein hydrolysate, 100 mg/l inositol, 30 g/l sucrose and 54 g/l mannitol).

After washing, the embryos are incubated in the maize electroporation buffer,  
15 EPM-NaCl (150 mM NaCl, 5 mM CaCl<sub>2</sub>, 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and 0.425M mannitol, pH 7.2). Approximately 100 embryos in 200 µl EPM-NaCl are loaded in each cuvette. About 20 µg of linearized maize HD-Zip protein-3 plasmid DNA, is added per cuvette. The maize HD-Zip protein-3 plasmid contains an inverted repeat of the entire maize protein-3 polynucleotide sequence as set forth in SEQ ID No.:107. Transcription of  
20 the inverted repeat maize protein-3 transgene is under the control of a maize 18 kD oleosin promoter (Qu et al., 1990 *J. Biol. Chem.* 265:2238-2243). In addition, the maize HD-Zip protein-3 plasmid also contains a chimaeric gene comprising the kanamycin resistance gene (neo) and 3' polyA addition region under the control of  
25 the CaMV 35S3 promoter (EP 359617).

After 1 hour DNA incubation with the explants, the cuvettes are transferred to an ice bath. After 10 minutes incubation on ice, the electroporation is carried out as follows: one pulse with a field strength of 375 V/cm is discharged from a 900 µF capacitor. The electroporation apparatus is as described by Dekeyser et al., (1990  
30 *Plant Cell* 2:591). Immediately after electroporation, fresh liquid N6aph substrate is added to the explants in the cuvette, after which the explants are incubated for a further 10 minute period on ice.

Afterwards, the embryos are transferred to Mahl VII substrate (macro- and micro-elements and vitamins of N6 medium supplemented with 100 mg/l casein hydrolysate, 6 mM proline, 0.5 g/l MES, 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-  
35

D) and 2% sucrose solidified with 0.75 g/l  $MgCl_2$  and 1.6 g/l Phytigel (Sigma Chemical Company, St Louis, Mo.), pH 5.8) and supplemented with 0.2M mannitol. After 2-3 days the embryos are transferred to the same substrate supplemented with 200 mg/l kanamycin. After approximately 14 days, the embryos are transferred to  
5 Mah1 VII substrate without mannitol, supplemented with kanamycin (200 mg/l). The embryos are further subcultured on this selective substrate for approximately two months with subculturing intervals of about 3 weeks. The induced embryogenic tissue is then carefully isolated and transferred to MS medium (Murashige et al., 1962 *Physiol. Plant* 15:473-497) supplemented with 5 mg/l 6-benzylaminopurine or  
10 5 mg/l zeatin. The embryogenic tissue is maintained on this medium for approximately 14 days and subsequently transferred to MS medium without hormones and 3-6% sucrose. Developing shoots are transferred to 1/2 MS medium with 1.5% sucrose for further development to normal plantlets. These plantlets are transferred to soil and cultivated in the greenhouse.

15 Modulation of plant cell division is determined by comparing the size of corn kernels in the transformed plants as compared to kernels obtained from untransformed control plants. More specifically, the embryos within the transgenic kernels are increased in size due to an increased number of cells. The size and development of maize embryos with specific attention to the number of cells,  
20 determined by reference to methods of Scott et al. (1998 *Development* 125:3329-41) and Ingram et al. (1999 *Plant Mol. Biol.* 40:343-54

#### Example 8

##### Modulation of Cell Division in Maize Shoots

An embryogenic maize callus line is prepared as described in U.S. Patent  
25 5,780,708. Maize callus is subcultured 7 to 12 days prior to microprojectile bombardment. Maize callus is prepared for bombardment as follows. Five clumps of callus, each approximately 50 mg in wet weight are arranged in a cross pattern in the center of a sterile 60X15 mm petri plate (Falcon 1007). Plates are stored in a closed container with moist paper towels, throughout the bombardment process.

30 Maize callus is transformed with a mixture of two plasmid DNA molecules. pHD-Zip-invp-1 contains a rice actin promoter and a 3' nos polyadenylation addition sequence region. An inverted repeat of polynucleotide sequence SEQ ID No.:103 is inserted in between the rice actin promoter (Wang et al., 1992 *Mol. Cell. Biol.* 12:3399-3406) and the nopoline synthase (nos) polyA sequence (Chilton et al.,  
35 1983). pHYG11 is a plasmid that contains the hygromycin coding sequence (Grütz

et al. 1983 *Gene* 25:179-188) and a maize Adh1S intron sequence that enhances protein expression of transgenes in transgenic plants (U.S. Patent 5,780,708).

5 PHD-Zip-invp-1 plasmid DNA and pHYG11 is coated onto M-10 tungsten particles (Biolistics) exactly as described by Klein et al. (1988 *Bio/Technology* 6:559-563) except that, (i) twice the recommended quantity of DNA is used, (ii) the DNA precipitation onto the particles is performed at 0°C., and (iii) the tubes containing the DNA-coated tungsten particles are stored on ice throughout the bombardment process.

10 All of the tubes contain 25 µl of 50 mg/ml M-10 tungsten in water, 25 µl of 2.5M CaCl<sub>2</sub>, and 10 µl of 100 mM spermidine along with a total of 5 µl of 1 mg/ml plasmid DNA. Each of the above plasmid DNAs are present in an amount of 2.5 µl.

15 All tubes are incubated on ice for 10 min., the particles are pelleted by centrifugation in an Eppendorf centrifuge at room temperature for 5 seconds, 25 µl of the supernatant is discarded. The tubes are stored on ice throughout the bombardment process. Each ballistic preparation is used for no more than 5 bombardments.

Macroprojectiles and stopping plates are obtained from Biolistics, Inc. (Ithaca, N.Y.). They are sterilized as described by the supplier. The microprojectile bombardment instrument is obtained from Biolistics, Inc.

20 The sample plate tray is placed 5 cm below the bottom of the stopping plate tray of the microprojectile instrument, with the stopping plate in the slot nearest to the barrel. Plates of callus tissue prepared as described above are centered on the sample plate tray and the petri dish lid removed. A 7 X 7 cm square rigid wire mesh with 3 X 3 mm mesh and made of galvanized steel is placed over the open dish in order to retain the tissue during the bombardment. Tungsten/DNA preparations are sonicated as described by Biolistics, Inc. and 2.5 µl of the suspensions is pipetted onto the top of the macroprojectiles for each bombardment. The instrument is operated as described by the manufacturer.

30 Immediately after all samples are bombarded, callus from all of the plates treated with the pHYG11 and pHD-Zip-invp-1 plasmid DNAs are transferred plate for plate onto F-medium containing 15 mg/l hygromycin B, (ten pieces of callus per plate). These are referred to as round 1 selection plates. Callus from the T.E. treated plate are transferred to F-medium without hygromycin. This tissue is subcultured every 2-3 weeks onto nonselective medium and is referred to as unselected control callus.

35

After about 14 days of selection, tissue appear essentially identical on both selective and nonselective media. All callus from plates of the pHYGII/pHD-Zip-invp-1 bombardment and one T.E. treated plate are transferred from round 1 selection plates to round 2 selection plates that contain 60 mg/l hygromycin. The round 2  
5 selection plates each contained ten 30 mg pieces of callus per plate, resulting in an expansion of the total number of plates.

After about 21 days on the round 2 selection plates, all of the material is transferred to round 3 selection plates containing 60 mg/l hygromycin. After about 79 days post-bombardment, the round 3 sets of selection plates are checked for viable  
10 sectors of callus. Viable sectors of callus are dissected from a background of necrotic tissue on the plates treated with pHYGII/pHD-Zip-invp-1 and transferred to F-medium without hygromycin.

After about 20 days on F-medium without hygromycin, the transformed callus is transferred to F-medium containing 60 mg/l hygromycin. The transformed  
15 callus is capable of sustained growth through multiple subcultures in the presence of 60 mg/l hygromycin.

#### Confirmation of transformed callus

To show that the pHYGII/pHD-Zip-invp-1 treated callus has acquired the hygromycin resistance gene, genomic DNA is isolated from the callus that has  
20 sustained capacity to grow on 60 mg/l hygromycin and unselected control callus and analyzed by Southern blotting. DNA is isolated from callus tissue by freezing 2 g of callus in liquid nitrogen and grinding it to a fine powder which is transferred to a 30 ml Oak Ridge tube containing 6 ml extraction buffer (7M urea, 250 mM NaCl, 50 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1% sarcosine). To this is added 7 ml of  
25 phenol:chloroform 1:1, the tubes shaken and incubated at 37°C. for 15 min. Samples are centrifuged at 8K for 10 min. at 4°C. The supernatant is pipetted through miracloth (Calbiochem 475855) into a disposable 15 ml tube (American Scientific Products, C3920-15A) containing 1 ml 4.4M ammonium acetate, pH 5.2. Isopropanol, 6 ml is added, the tubes shaken, and the samples incubated at -20°C. for  
30 15 min. The DNA is pelleted in a Beckman TJ-6 centrifuge at the maximum speed for 5 min. at 4°C. The supernatant is discarded and the pellet is dissolved in 500 µl TE-10 (10 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0) 15 min. at room temperature. The samples are transferred to a 1.5 ml Eppendorf tube and 100 µl 4.4M ammonium acetate, pH 5.2 and 700 µl isopropanol is added. This is incubated  
35 at -20°C. for 15 min. and the DNA pelleted 5 min. in an Eppendorf microcentrifuge

(12,000 rpm). The pellet is washed with 70% ethanol, dried, and resuspended in TE-1 (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

Ten  $\mu$ g of isolated DNA is digested with restriction endonuclease and analyzed by Southern Blot hybridization using a probes from both the pHD-Zip-invp-1 plasmid and pHYG11 plasmid. Digested DNA is electrophoresed in a 0.8% w/v agarose gel at 15 V for 16 hrs in TAE buffer (40 mM Tris-acetate, pH 7.6, 1 mM EDTA). The DNA bands in the gel are transferred to a Nytran membrane (Schleicher and Schuell). Transfer, hybridization and washing conditions are carried out as per the manufacturer's recommendations. DNA samples extracted from the hygromycin resistant callus tissue that are transformed with the maize HD-Zip-invp-1 transgene have DNA fragments that hybridize specifically with the HD-Zip-invp-1 hybridization probe. No hybridization signal is observed in DNA samples from control callus.

#### Plant regeneration and production of seed

Portions of the transformed callus are transferred directly from plates containing 60 mg/l hygromycin to RM5 medium which consists of MS basal salts (Murashige et al. 1962) supplemented with thiamine-HCl 0.5 mg/l, 2,4-D 0.75 mg/l, sucrose 50 g/l, asparagine 150 mg/l, and Gelrite 2.5 g/l (Kelco Inc., San Diego).

After about 14 days on RM5 medium, the majority of transformed callus and unselected control callus are transferred to R5 medium (RM5 medium, except that 2,4-D is omitted). The plates are cultured in the dark for about 7 days at 26°C. and transferred to a light regime of 14 hrs light and 10 hrs dark for about 14 days at 26°C. At this point, plantlets that have formed are transferred to one quart canning jars (Ball) containing 100 ml of R5 medium. Plants are transferred from jars to vermiculite for about 7 or 8 days before transplanting them into soil and growing them to maturity. About 40 plants are produced from the transformed callus and about 10 plants are produced from control callus (untransformed hygromycin sensitive callus).

Controlled pollinations of mature transformed plants is conducted by standard techniques with inbred *Zea mays* lines MBS501 (Mike Brayton Seeds), and FR4326 (Illinois Foundation Research). Seed is harvested about 45 days post-pollination and allowed to dry further for 1-2 weeks.

#### Analysis of the R1 progeny

R1 plants are tested for the presence of the HPT and pHD-Zip-invp-1 transgene sequences by PCR analysis. To conduct the PCR assay, 0.1 g samples are

taken from plant tissues and frozen in liquid nitrogen. Samples are then ground with 120 grit carborundum in 200  $\mu$ l 0.1M Tris-HCl, 0.1M NaCl, 20 mM EDTA, 1% Sarkosyl pH 8.5) at 40°C. Following phenol/chloroform extraction and ethanol and isopropanol precipitations, samples are suspended in T.E. and analyzed by  
5 polymerase chain reaction (K. B. Mullis, U.S. Patent 4,683,202).

PCR is carried out in 100  $\mu$ l volumes in 50 mM KCl, 10 mM Tris-HCl pH 8.4, 3 mM MgCl<sub>2</sub>, 100  $\mu$ g/ml gelatin, 0.25  $\mu$ M each of the appropriate primers, 0.2 mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 2.5 Units of Tag DNA polymerase (Cetus), and 10  $\mu$ l of the DNA preparation. The mixture is  
10 overlaid with mineral oil, heated to 94°C. for 3 min, and amplified for 35 cycles of 55°C. for 1 min, 72°C. for 1 min, 94°C. for 1 min. The mixture is then incubated at 50°C. for 2 min and 72°C. for 5 min. 10  $\mu$ l of the PCR product is electrophoresed in agarose gels and visualized by staining with ethidium bromide.

For analysis of the presence of the hygromycin-B phosphotransferase (HPT)  
15 gene, a PCR primer complementary to the CaMV 35S promoter, and one complementary to the HPT coding sequence is employed. Thus, in order to generate the appropriately sized PCR product, the HPT template DNA must contain contiguous CaMV 35S promoter region, Adhl intron, and 5' protein HPT coding sequence region. For analysis of the presence of the maize HD-Zip protein-1  
20 inverted repeat transgene, PCR primers complementary to sequences within the HD-Zip protein-1 inverted repeat region are employed.

R<sub>1</sub> plants that are homozygous for the maize HD-Zip protein-1 inverted repeat transgene exhibit a plant morphology phenotype that is caused by a transgene suppression of endogenous HD-Zip protein-1 function. Loss of wild-type HD-Zip  
25 protein-1 function causes modulation of maize cell division. The modulated cell division phenotype results in transformed plants whose leaves are longer and contain more cells. Cell numbers in stem and leaves are determined as explained by Talbert et al. 1995 *Development* 121:2723-35.

#### Example 9

##### Modulation of Cell Division in Rice Seed

30 Dehusked mature seeds of the rice cultivar Nipponbare are surfaced-sterilized, placed on solid 2N6 medium (N6 medium (Chu et. al. 1975 *Sci. Sin. Peking* 18:659), supplemented with 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 1.0 mg/l thiamine-HCl, 2.0 mg/l 2,4-D, 30 g/l sucrose, and 2.0 g/l Phytigel, pH 5.8), and  
35 cultured at 27°C. in the dark. Callus develops from the scutella of the embryos

within 3-4 weeks. Embryogenic portions of primary callus are transferred to N67 medium (N6 medium (Chu et al. 1975), supplemented with 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 1.0 mg/l thiamine-HCl, 2.0 g/l casamino acids (vitamin assay, Difco), 1.0 mg/l 2,4-D, 0.5 mg/l 6-benzylaminopurine, 20 g/l sucrose, 30g/l  
5 sorbitol, and 2.0 g/l Phytigel, pH 5.8) for propagation into compact embryogenic callus.

About three to four weeks after subculture, the embryogenic callus are used for transformation with rice genomic HD-Zip gp-1 plasmid DNA. The callus is cut into fragments with a maximum length of about 1.5 to 2 mm. The callus pieces are  
10 washed twice in EPM (5 mM CaCl<sub>2</sub>, 10 mM HEPES and 0.425 M mannitol) and then preplasmolyzed in this buffer for 30 minutes to 3 hours at room temperature (25°C). Then, the callus fragments are washed twice with EPM-KCl (EPM buffer with 80 mM KCl) and transferred to electroporation cuvettes. Each cuvette is loaded with about 150 to 200 mg of callus fragments in 100 to 200 µl EPM-KCl. 10 to 20 µg of a  
15 plasmid DNA, either circular pHD-Zip-asgp-1 or linearized pHD-Zip-asgp-1, are added per cuvette. pHD-Zip-asgp-1 is a plasmid that contains an antisense HD-Zip protein-1 transgene that is under the transcriptional control of a rice actin promoter (Wang et al., 1992). The antisense HD-Zip-gp-1 transgene comprises a polynucleotide sequence cloned from rice genomic DNA (Example 3) that is set forth  
20 in SEQ ID No.:115. The pHD-Zip-asgp-1 plasmid also contains a chimaeric gene comprising the bar gene under the control of the CaMV 35S3 promoter (see European patent publication ("EP") 359617). The bar gene (see EP 242236) encodes phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin. The chimeric bar transgene comprises a phosphinothricin acetyl  
25 transferase coding sequence and a chloroplast targeting transit sequence.

The DNA is incubated with the callus fragments for about 1 hour at room temperature. Electroporation is then carried out as described in Example 4. After electroporation, liquid N67 medium without casamino acids is added to the callus fragments. The callus fragments are then plated on solid N67 medium without  
30 casamino acids but supplemented with 5, 10 or 20 mg/l phosphinothricin (PPT) and are cultured on this selective medium at 27 °C. under a light/dark regime of 16/8 hours for about 4 weeks. Developing PPT-resistant calli are isolated and subcultured for about two to three weeks onto fresh N67 medium without casamino acids but containing 5 mg/l PPT. Thereafter, selected PPT-resistant calli are transferred to plant  
35 regeneration medium N6M25 (N6 medium (Chu et al. 1975), supplemented with 0.5

mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 1.0 mg/l thiamine-HCl, 288 mg/l aspartic acid, 174 mg/l arginine, 7.0 mg/l glycine, 1.0 mg/l O-naphthalenacetic acid (NAA), 5.0 mg/l kinetin, 20 g/l sucrose and 2.0 g/l Phytigel, pH 5.8) supplemented with 5 mg/l PPT. Plantlets develop within approximately 1 month and are then transferred to hormone-free N6 medium (Chu et al. 1975), supplemented with 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxin-HCl, 1.0 mg/l thiamine-HCl, 1.0 g/l casamino acids, 20 g/l sucrose, and 2.0 g/l Phytogel, pH 5.8) on which they are kept for another 2 to 3 weeks, after which they are transferred to soil and cultivated in the greenhouse.

#### 10 Characterization of the Transformed Rice Plants

The above transformed rice plants are cultivated in soil until seed set and seed maturation. Seeds of the progeny of the transformants are sown in aqueous 400-fold Homai hydrate (Kumiai Kagaku Inc.) solution containing 70 mg/l of hygromycin and incubated therein at 25°C. for 10 days, thereby selecting for the resistance to hygromycin. Twenty seeds of each plant of the progeny of the transformants are sown and cultured for about 3 weeks. Leaves are collected from the hygromycin resistant R<sub>1</sub> plants and compared to leaves collected from rice plants that were regenerated using the above described methods but do not contain an antisense HD-Zip-asgp-1 transgene. Leaves collected from the pH<sub>1</sub>D-Zip-asgp-1 transformed plants exhibit modulated cell division as indicated by the increased size of the rice leaves.

Transformed and non transformed plants are analyzed by means of a Southern hybridization in which plant genomic DNA, is digested and probed with pRiceHD-Zip-gp-1 DNA. The hybridization data shows that the transgenic plants exhibiting modulation of cell division contain at least part of one copy of pRiceHD-Zip-gp-3 plasmid DNA that is integrated into the rice genome.

#### 25 Example 10

##### Modulation of Cell Division in Rice Stems

##### Preparation of Sample Cultured Tissues

Scutellum callus from variety Koshihikari of japonica rice (*Oryza sativa* L.) is prepared for *Agrobacterium tumefaciens* mediated transformation using the method of Hiei et al. (1994; U.S. Patent 5,591,616). Mature seeds of rice are sterilized by being immersed in 70% ethanol for 1 minute and then in 1% sodium hypochlorite solution for 30 minutes. The seeds are then placed on 2N6 solid medium (inorganic salts and vitamins of N6 (Chu, 1978 *Proc. Symp. Plant Tissue Culture*, Science Press Peking, pp. 43-50), 1 g/l of casamino acid, 2 mg/l of 2,4-D, 30 g/l of sucrose, 2 g/l of Gelrite).

After culturing the mature seeds for about 3 weeks, callus growth forms that originates from scutella. This scutella callus is transferred to 2N6 medium and cultured therein for 4-7 days. The resulting calli are used as "scutellum callus" samples.

5           The calli originated from scutella are transferred to AA liquid medium (major inorganic salts of AA, amino acids of AA and vitamins of AA (Toriyama et al., 1985 *Plant Science* 41:179-183), MS minor salts (Murashige et al., 1962 *Physiol. Plant.* 15:473-497), 0.5 g/l of casamino acid, 1 mg/l of 2,4-D, 0.2 mg/l of kinetin, 0.1 mg/l of gibberellin and 20 g/l of sucrose) and the cells are cultured therein at 25°C in the  
10       dark under shaking of 120 rpm to obtain suspended cultured cells. The medium is replaced with fresh medium every week.

#### Ti Plasmid (Binary Vector)

The T-DNA region of pTOK232 (Hiei et al., 1994) is altered by replacement of the CaMV 35S promoter/Gus/nos polyA transgene with the following inverted  
15       repeat rice HD-Zip protein-1 transgene construction. SEQ ID NO:121 discloses the DNA sequence of a rice cDNA insert obtained in Example 3. An inverted repeat of SEQ ID No.:121 is inserted between a rice actin promoter region and a nopaline synthase polyA addition sequence. This altered pTOK232 plasmid is a binary transformation vector called pTOKivr-HD-Zip-p1.

20           *Agrobacterium* strain LBA4404 has a Ti-plasmid from which the T-DNA region was deleted is used as the host bacteria for the rice transformation. Strain LBA4404 has a helper plasmid PAL4404 (having a complete vir region), and is available from American Type Culture Collection (ATCC 37349). Binary vector pTOKivr-HD-Zip-p1 is introduced into LBA4404 by the triple cross method of Ditta  
25       et al. (1980 *Proc. Natl. Acad. Sci. USA* 77:7347-7351).

Colonies obtained by culturing the pTOKivr-HD-Zip-p1 *Agrobacterium* strains on AB medium (Drlica et al., 1974 *Proc. Natl. Acad. Sci. USA* 71:3677-3681) containing hygromycin (50 µg/ml) and kanamycin (50 µg/ml) for about 3-10 days are collected with a platinum loop and suspended in modified AA medium (same as the  
30       composition of the above-described AA medium except that concentrations of sucrose and glucose are changed to 0.2 M and 0.2 M, respectively, and that 100 µM of acetosyringone is added, pH 5.2). The cell population is adjusted to  $3 \times 10^9$  -  $5 \times 10^9$  cells/ml and the suspensions are used for inoculation of rice callus.

#### Inoculation Conditions

Rice scutellum callus tissues is washed with sterilized water and immersed in the above-described suspension of *Agrobacterium* for 3-10 minutes. Tissue is also incubated with LBA4404 that does not contain a binary vector as a negative control.

5 The co-cultivating scutellum callus samples are cultured at 25°C. in the dark for 2-5 days on 2N6 solid medium containing acetosyringone, glucose and sucrose in the same concentrations as mentioned above. The resulting inoculated tissues are then washed with sterilized water containing 250 mg/l of cefotaxime and then continued to be cultured on the aforementioned 2N6 solid media containing 250 mg/l  
10 cefotaxime.

#### Selection of Transformed Cells and Tissues

Scutellum callus that has been cultured with the *Agrobacterium* strains for 3 days are cultured on 2N6 medium containing 250 mg/l of cefotaxime for about 1 week. Hygromycin-resistant cultured tissues are selected by culturing the cultured  
15 tissues on 2N6 medium containing 50 mg/l of hygromycin for 3 weeks (primary selection). The obtained resistant tissues are further cultured on N6-12 medium (N6 inorganic salts, N6 vitamins, 2 g/l of casamino acid, 0.2 mg/l of 2,4-D, 0.5 mg/l of 6BA, 5 mg/l of ABA, 30 g/l of sorbitol, 20 g/l of sucrose and 2 g/l of Gelrite) containing 50 mg/l of hygromycin for about 2-3 weeks (secondary selection), and the  
20 calli grown on this medium are transferred to a plant regeneration medium N6S3 containing 0, 20 or 50 mg/l of hygromycin. In all of the media used after the co-cultivation with *Agrobacterium*, cefotaxime is added to 250 mg/l. Calli are incubated on N6S3 medium at 25°C under continuous illumination (about 2000 lux). Regenerated plants ( $R_0$  generation) are eventually transferred to soil in pots and  
25 grown to maturity in a greenhouse.

Seeds of the progeny of the transformants are sown in aqueous 400-fold Homai hydrate (Kumiai Kagaku Inc.) solution containing 70 mg/l of hygromycin and incubated therein at 25°C. for 10 days, thereby selecting for the resistance to  
30 hygromycin. Twenty seeds of each plant of the progeny of the transformants are sown and cultured for about 3 weeks. Leaves are collected from seedlings transformed with the HD-Zip protein-1 inverted repeat transgene and untransformed control plants. The transformed plants have an increased number of cells in their leaves due to transgene induced modulation of cell division. Cell numbers in leaves are determined as explained by Talbert et al. 1995 *Development* 121:2723-35.

Transformed and non transformed plants are also analyzed by means of a Southern blot hybridization method in which plant genomic DNA, is digested and probed with pRiceHD-Zip-p-1 DNA. The hybridization data shows that the transgenic plants exhibiting modulation of cell division contain at least part of one copy of pRiceHD-Zip-p-1 plasmid DNA that is integrated into the rice genome.

#### Example 8

##### Sense expression of the REVOLUTA gene

##### driven from the 35S Cauliflower Mosaic Virus promoter

A DNA fragment encoding approximately 900 bp of the 35S Cauliflower Mosaic Virus promoter was amplified from the pHomer102 plasmid by PCR using primers AAGGTACCAAGTTCGACGGAGAAGGTGA [SEQ ID NO:53] and AAGGATCCTGTAGAGAGAGACTGGTGATTTCAG [SEQ ID NO:54]. Clones from independent PCR reactions were sequenced to verify the accuracy of the PCR amplification. Kpn I and BamHI restriction sites were included in the PCR primers to allow for the isolation of a 900 bp KpnI-BamHI fragment that includes the amplified 35S promoter. This KpnI-BamHI fragment was inserted 5' of the REV genomic sequence in clone NO84 at KpnI and Bam HI sites to generate a NO REV gene linked approximately 70 bp downstream of the 35S promoter transcription start site. The 3' end of the REV gene was placed downstream of the REV coding region as described below.

As described above, NO84 is a clone containing the genomic DNA sequence of REVOLUTA isolated from a NO-ecotype plant. The REV NO84 gene was amplified using long distance PCR with the primers HDAL: AAAATGGAGATGGCGGTGGCTAAC [SEQ ID NO:33] and HDAR: TGTC AATCGAATCACACAAAAGACCA [SEQ ID NO:34] and essentially the conditions described above, except that denaturation steps were carried out at 94°C and 20 second extensions were added to each cycle after 10 cycles for a total of 40 cycles.

To clone the 3' polyadenylation signal onto the end of the gene, approximately 0.7 kb of the 3' end of Columbia REV starting immediately downstream of the stop codon was amplified using PCR using the following oligonucleotides (5' primer includes a NotI site: TTGCGCCCGCTTCGATTGACAGAAAAGACTAATTT [SEQ ID NO:51]; 3' primer includes ApaI and KpnI sites: TTGGGCCCGGTACCCTCAACCAACCACATGGAC [SEQ ID NO:52]). Clones

were verified by sequencing, and the 3' region of REV placed downstream of the NO84REV coding region in the NotI and ApaI sites of the vector. The resulting gene containing the 35S promoter, REV coding, and REV 3' regions was cloned out of the original vector using KpnI and ligated to the pCGN1547 T-DNA binary vector.

5        Transformation of 35S-REV gene

Agrobacterium strain At503 was transformed with the above constructs and used to transform wild-type No plants using in planta transformation.

Five independently transformed lines were characterized for their growth phenotype. We found increases in leaf, stem and seed size (see Figures 10-12, and  
10 Table 11). Increased size was displayed to different extent by the different lines. The line that showed the largest increase in seed size produced seed that was nearly twice as heavy as the control seed.

The production of independent transgenic lines displaying large organs and seeds indicate that expression of the CaMV35S-REV gene in plants results in  
15 increased growth. Notably, the phenotype of CaMV35S-REV plants does not show any of the abnormalities displayed by rev mutants: abnormal flower, empty axils and contorted leaves. Thus, sense expression of REV is useful in obtaining crop plants with valuable characteristics.

20        All publications and patents mentioned in the above specification are herein incorporated by reference. While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

**EDITORIAL NOTE**

**APPLICATION NUMBER – 14787/01**

**The following Sequence Listing pages 1 to 40 are part of the description. The claims pages follow on pages "88" to "92".**

DNA AND PROTEIN SEQUENCES

*Arabidopsis REVOLUTA* Gene SEQ ID NO:1

First/ last position of the 18 exons based upon ATG at 2849.

|      |      |
|------|------|
| 2849 | 3081 |
| 3390 | 3552 |
| 3670 | 3743 |
| 3822 | 3912 |
| 4004 | 4099 |
| 4187 | 4300 |
| 4383 | 4466 |
| 4542 | 4697 |
| 4786 | 4860 |
| 4942 | 5048 |
| 5132 | 5306 |
| 5394 | 5582 |
| 5668 | 5748 |
| 5834 | 5968 |
| 6051 | 6388 |
| 6477 | 6585 |
| 6663 | 6812 |
| 6890 | 7045 |

Start of promoter is +1

GGGAACACTTAAAGTATAGTGCATTGTATTCAAAGTACAAATTAATCATTAAATTGTATTAGAAAAT  
 GATATATTGTCATCGTACTAATTTGTC'TTTTGAATTCAATTGTAAGTACTACTACTGGTTTTTCATT  
 TTTTCAGATTATTTCTCGGTTCTTAAAAAGAAGAAAACACATACCTAATATCGTGTGTATCAAAAAGCC  
 ATGTGCGAGCAACCATCTTCTTTGTAAATTTGACCCTTTTGTGTATCATTATATATCGAGTGTGTTGTA  
 ATTGTTGGTTGATTTTGTGTTATTTGAGAGGCTAGTCATTTACGCAATTTCTAAATTTATCTTTTAGT  
 ACGTATCAAGATGTTGGAGCAACTGTGTCCAACATACATGACATGTGAAATTATAATTGTTAAACAA  
 AAGGACATGTGAAATTACTATCTACTTAAAGAAAAAAACCAGAAAAGAAAAAAGGTATTAATTTGGA  
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 ATCATGATATGTAAAAAATTGTCGAAGAGGTTTAGTCGTATCACTTTGTTTACTGATCACTGTCTTC  
 TGATTCATTTTTTCAGTTTTTCTTTTTCAAATGTAGCTCACAAATTAAGTTATTCAGTCTTTAAT  
 CAGATAGTTTAATACTAGTAACTAGCTCATTTAGGCTTTAAACACCTCTTTCTGATTACTAGCCCACT

CTTTGGTGGTTCTTACATATCACACCTAACTATACTGTGTATCCTTGAAGTGAAAATCAAATTTACCA  
TTCGTATCTTACTTACATACACTATTATTTTCCCTTTTTTTTTTCACTCAAGGTCTACTCTTTGATA  
CCATAGCTATAATTTGGAAATAACTATTTACAGTGTATTAATTATACCTAAAACAGTTTAATCTGGACT  
AAATATTTAGATAGATGTTACAAATTTGGTTCGTCTAATAAATGAAGACAAGACATGCTAACAAATAA  
AACACTACCACAAAGGGATAGTGAGAGAATGTGTTTTGCAACAAGACATAACTTTGATTGCTTGATGT  
GTTAAAATGATTATGTCAGAGACAGAGAGCGATCATAGGCTTTCTTTATTTCTAATAACGTCGATTTT  
CTTCTCTTTTTCTGGCGTTCAATAATGTCGAATTTTAATTCTTGATTTTTGCAACTCTAAATATCCTT  
AACGACATTGAAGCATGGTGCATGTCGATCGTTAATAATAAAGTTGAACAAAAATCTTGTTGAATTA  
TTACAAGCACAGCTTCAATAGCATAACTTTACGAGACGACCAGATCTTATAGACGAGTTTCGCTTTTA  
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CATAACTTCATTTTTTAAAAACATCAATAATTAATATACGATTACAACCTAAAAAATTAGTCACCCTAT  
AGTACATAACAATGATGATAGTTTTTTCTTTTTGGTGAATGTTAAAATAAAATGTTAGCCATATTAA  
ACGATAGTTCTTTAACTTAGCCATTGTAAGATATTTCTTACTTTAGTTTTTCCGTAGAAGATATTCA  
TTATGGTATGGATAGTATATACCTTAACTGAGTTTAAATATTGGATCAATACCATCTAATAACACATA  
TCTGATGTTCAATACTTAATAATTTTGCATAAATGTTAAGCGTGACAACTTAAAAAAAAACACATCAA  
CAGAGTAAAAACATATCTGTTAAATAAGAAAAATGTCATTTTTATAACACTTAAAAAAAAATGCATGA  
AGCGTTTCATAGTTTTTTTTTTTTCAAAGTAATGTAGGCGTTAGATATTTCTTACAATTTTTTGAAA  
AATATTTTTTATGTTGTGATTGGCTGATACAGGTAACATAAATCTTTTAAAGAATTGAAGAAAAAT  
TGAAAAGTAAATAGATGGATTCCTATATTGTCATTTAGAAAAACAGTAGGGACAACCTCGTAAATGA  
TAGCCGATTTATTAACAAATAAATTTAAATTAGAAAAAGGAAAAAACGCACCACCTTTCTTTTTTC  
GCTGATGCACAGCTTGTCCGTTTGCCTGCAAACTCTCTGTTTCACAATTTTTTCTTCTCTTTCTC  
TCTCTTCTCTTTTATTCCTCTGTTCCAAAGTTCAGCAGAAGCAACACACACATCACTTACTATCTC  
TCTCTCCTTCTTCACTTTCTCACATAACCAAATCTCTCTTTCTCTTTTTTTTTGAAGTCTCCTTTG  
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GGGTTTTCTTCTTATTGTTCTTAAGGTTTCTGTGCTCTTCAATCTTCTGATATAATGCTTCTTTAAG  
AGAGTTGACATCATCACTTTCTTGGGTACTCTTCTCTGTTTCTCCCAGAAAATCCAATCTGTAAT  
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 AATCTGTCTGGTAGATTTGGTTATTCAATAGCTTGGTGTAAC TGTCTTGTGACGATATGGTTACTGT  
 CTGATCTGGTGTCTAATCTTAGGAGTTTTGTTGATTCTGTTTTGTTGTGTGGTTTTCAGGTGTGAGATA  
 AGCAGAGGAAAGAGGCGTCGAGGCTCCAGAGCGTAAACCGGAAGCTCTCTGCGATGAATAAACTGTTG  
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 GCAGCTAACTACTGTTGTATGTAAC TTAACATTTCTTTTTGTCAAATGTGTTCTTAAAGAATCATTTG  
 TTACTCCTATCAGTTCAACATGTAGCTTGAGTTATAAAGTTACTGACTTGTGTTTTAACTTCAGGTT  
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 GTTTTGGTCTTAGATTGCTCTCAATCGCAGAGGAGACTTTGGCAGAGTTCTATCCAAGGCTACAGGA  
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 TGGTTCGGTGA CTGTAGGAGCCTTGAAGTTTTCACTATGTTCCCGGCTGGTAATGGTGGCACAATCGA  
 GCTTGTTFATATGCAGGTGAATCCTTTAGCCTCTTCTGGTTAGTTTTCTATCTCTAACACTTGAAGA  
 TGAATGAATAAAGTTGTGACATTTGTTTCAGACGTATGCACCAACGACTCTGGCTCCTGCCCGGATTT  
 CTGGACCCTGAGATACACAACGAGCCTCGACAATGGGAGTTTTGTGGTATGCAGCTCTCATAATGTCT  
 AGTGTTTACAGAAAACTCTGGGATCTTGATGTTTTTCATATGTCTTTAAAAGGTTTGTGAGAGGTCG  
 CTATCTGGCTCTGGAGCTGGGCCTAATGCTGCTTCAGCTTCTCAGTTTGTGAGAGCAGAAATGCTTTC  
 TAGTGGGTATTTAATAAGGCCTTGTGATGGTGGTGGTTCTATTATTCAATTGTCGATCACCTTAATC  
 TTGAGGTACTTAAATCTTACATGTGGCATT TTTGTGTGTGTTTTCAGGAATTTCTAGAAGAATTGATT  
 ATAAACATTTGTTCTTGCATTGTAGGCTTGGAGTGTCCGGATGTGCTTCGACCCCTTTATGAGTCAT  
 CCAAAGTCGTTGCACAAAAAATGACCATTTCCGTGAGTGTATACATATAATAACCTTAAGCTTTGATT  
 GATTCATATAACATATCTAACGGTTGGAGGTGCTTCATGTTTTAGGCGTTGCGGTATATCAGGCAATT  
 AGCCCAAGAGTCTAATGGTGAAGTAGTGTATGGATTAGGAAGGCAGCCTGCTGTTCTTAGAACCTTTA  
 GCCAAAGATTAAGCAGGTACTTCGATCTTGAGCTAAAACCTAATGTTCTTTGCTCTGTTTGTCTATT  
 GTCATTTTTCTGTTCTTGGTTTTCTTGAAGGGCTTCAATGATGCGGTTAATGGGTTTGGTGACGAC  
 GGGTGGTCTACGATGCATTGTGATGGAGCGGAAGATATTATCGTTGCTATTAACTCTACAAAGCATTT  
 GAATAATATTTCTAATCTCTTTCTGTTCCTTGGAGGCGTCTGCTGTTGCAAGGCTTCAATGCTTCTCC  
 AAGTAAGTTAGTGTGCCAGTATTGGTACTTTGTGTTCTTTTGACAGTTTTCTATGGCTGAAATTTGT  
 GTTATCTATTGCTTCTGTAGAATGTTCTCTGCGGTTTTGATCCGGTTCCTTAGAGAGCATCGATC  
 TGAGTGGGCTGATTTCAATGTTGATGCATATTCGCTGCTACACTTAAAGCTGGTAGCTTTGCTTATC

CGGGAATGAGACCAACAAGATTCACTGGGAGTCAGATCATAATGCCACTAGGACATACAATTGAACAC  
GAAGAAGTAAGGCTTCAAAGTCTTACCTGCCGACAAAACATCATTTTTATGTCTCTCTCTTACATAT  
ATATTTGGTTTTGTTATGTTTAGATGCTAGAAGTTGTTAGACTGGAAGGTCATTCTCTTGCTCAAGAA  
GATGCATTTATGTACGGGATGTCATCTCCTTCAGGTATATCACTTCTAAGTTCTAACCCAATGGAT  
CTTGAAATTTTTACCATTTCAAAGTTAAAATTGACCTTAATGATTTATGGTAGATTTGTACCGGGATT  
GACGAGAATGCCGTTGGAGCTTGTTCTGAACTGATATTTGCTCCGATTAATGAGATGTTCCCGGATGA  
TGCTCCACTTGTTCCCTCTGGATTCGGAGTCATACCCGTTGATGCTAAAACGGTACTCTTCTTTGCTG  
TACCACTGATTTTTCTTTTACTTAGAGATGTTTTGTTTCAAGGCTCATTTTTTCTTACTCATAACAGGG  
AGATGTACAAGATCTGTTAACCCTAATCACCGTACACTAGACTTAACTTCTAGCCTTGAAGTCGGTC  
CATCACCTGAGAATGCTTCTGGAAACTCTTTTTCTAGCTCAAGCTCGAGATGTATTCTCACTATCGCG  
TTTTCAATTCCTTTTTGAAAACAACCTTGCAAGAAAATGTTGCTGGTATGGCTTGTGAGTATGTGAGGAG  
CGTGATCTCATCAGTTCAACGTGTGCAATGGCGATCTACCGTCTGGGATAAGCCCGAGTCTGGGCT  
CCAAATTGTCCCAGGATCTCCTGAAGCTGTTACTCTTGCTCAGTGGATCTCTCAAAGTTACAGGTGG  
GGGTGTAATGTTTACTCTCGTCTCTTTCTTATAATCCTCGAACTTATCGATGATGCCTTATGCTGAT  
ATGTTTGTTTTTCCAGTCATCACTTAGGCTCGGAGTTGCTGACGATGATTCACTTGGAAAGCGACGAC  
TCGGTACTAAAACCTTCTATGGGATCACCAAGATGCCATCCTGTGTGCTCATTAAAGGTATGTGTCT  
ACACCAAACAAAAGCAGAATACACCTGTAGTTTTAGACGTATAATATGGTCTGGATATGTTGCAGCC  
ACAGCCAGTGTTTCATGTTTGCGAACCAAGCTGGTCTAGACATGCTAGAGACAACACTTGTAGCCTTAC  
AAGATATAACACTCGAAAAGATATTCGATGAATCGGGTCGTAAGGCTATCTGTTCCGACTTCGCCAAG  
CTAATGCAACAGGTAAAGAACCAAAAACAAAACATCTGCAGATAAATGGTTTTGATTCAATTTGTCTGA  
GAACTATCTTTGCGTCTACAGGGATTTGCTTGCTTGCTTCCAGGAATCTGTGTGTCAACGATGGGAAG  
ACATGTGAGTTATGAACAAGCTGTTGCTTGGAAAGTGTGTTGCTGCATCTGAAGAAAACAACAACAATC  
TGCAATGTCTTGCTTCTCCTTTGTAAACTGGTCTTTTGTGTGATTCGATTTGACAGAAAAGACTAAT  
TTAAATTTACGTTAGAGAACTCAAATTTTTGGTTGTTGTTTAGGTGTCTCTGTTTTGTTTTTAAAT  
TATTTTGATCAAATGTTACTCACTTTCTTCTTTCAACGTATTTGGTTTTAATGTTTTGGGGAAAAA  
AGCAGAGTTGATCAATCTCTATATATAAAGGAATGATGTGATAATTTGTTAAAACCTAAGCTTACAA  
CATTTTTCTATCGCATTGACAGTTTCATTTTCACATCTCTCGCTATATATTAGTAATATAAACTAT  
TTCAAAAACAAAGAATCAACAAGAATCCACAGATGTAAGAAAGAAAAATCACAGCCAAATAACTTTT  
TTATTTATTTGGCCGTTAGATAAAACTACCTTCAAGATTTTCATGCATCTAGCCGGTAAACCTGTCTGA  
TGATTTGACGGCGACAATCTCAGAGACATTGTTGCAACGAAGAACATCTTGACCAAGCTTAGCTCCTGC  
AGCTTTAAGACCTTTAAGCGAAAACCGCATGTTGAAGAGATTGAGTGATGGAAGCTTAGAAAAGCGGAG  
GGAGTTTCTGGTTCGGTAAGAAGTTTCTGAAATCATCCATAAGCAATGGAACCTCGAAATCGGTTTTG  
TTGTGTTTACCACATGTTCTTTAGCTGCGATGTGAGCTCCTGGTTCATGTGGTTGGTTGAG

**REVOLUTA spliced nucleotide sequence**

NO-ecotype changes present in revs-1,2 and 4 affect non-coding sequences.

## rev mutant changes:

|       |      |     |              |
|-------|------|-----|--------------|
| rev-1 | 2819 | G→A | SEQ ID NO:3  |
| rev-2 | 2093 | G→A | SEQ ID NO:7  |
| rev-3 | 3252 | C→T | SEQ ID NO:5  |
| rev-4 | 2093 | G→A | SEQ ID NO:7  |
| rev-5 | 2651 | T→C | SEQ ID NO:9  |
| rev-6 | 1962 | C→T | SEQ ID NO:11 |

1 31  
 ATG GAG ATG GCG GTG GCT AAC CAC CGT GAG AGA AGC AGT GAC AGT ATG AAT  
 AGA CAT TTA

611 91  
 GAT AGT AGC GGT AAG TAC GTT AGG TAC ACA GCT GAG CAA GTC GAG GCT CTT  
 GAG CGT GTC

121 151  
 TAC GCT GAG TGT CCT AAG CCT AGC TCT CTC CGT CGA CAA CAA TTG ATC CGT  
 GAA TGT TCC

181 211  
 ATT TTG GCC AAT ATT GAG CCT AAG CAG ATC AAA GTC TGG TTT CAG AAC CGC  
 AGG TAT TGC

241 271  
 TTC TCT TTA ATA TGG CCA GGA TTA ATT TTT AAT TAA GGA TTT TGA ATT TGA  
 TTC TAT TGG

301 331

ATT TAG TGT GTT ATA TTC AAT GGA TAT GAA GGA CCA CTT TTG TTG TTA TTT  
CAA GAT TTG

361

391

ATG CTT CAA TTC AAT TCT CCG ACA CAA TTT CCT GTT TTT ACA AAA GGG TTC  
CTT TGA ATC

421

451

TGT CTG GTA GAT TTG GTT ATT CAA TAG CTT GGT GTA ACT GTT CTT GTG ACG  
ATA TGG TTA

481

511

CTG TCT GAT CTG GTG TCT AAT CTT AGG AGT TTT GTT GAT TCG TTT TGT TGT  
GTG GTT TCA

541

571

GGT GTC GAG ATA AGC AGA GGA AAG AGG CGT CGA GGC TCC AGA GCG TAA ACC  
GGA AGC TCT

601

631

CTG CGA TGA ATA AAC TGT TGA TGG AGG AGA ATG ATA GGT TGC AGA AGC AGG  
TTT CTC AGC

661

691

TTG TCT GCG AAA ATG GAT ATA TGA AAC AGC AGC TAA CTA CTG TTG TAT GTA  
ACT TAA CAT

721

751

TTC CTT TTG TCA AAT GTG TTC TTA AAG AAT CAT TTG TTA CTC CTA TCA GTT  
CAA CAT GTA

781

811

GCT TGA GTT ATA AAG TTA CTG ACT TGT TGT TTT AAC TTC AGG TTA ACG ATC  
CAA GCT GTG

841

AAT CTG TGG TCA CAA CTC CTC AGC ATT CGC TTA GAG ATG CGA ATA GTC CTG  
CTG GGT AAA

871

901

GTT TCA TTT TTG GTT TTG AAG TAA CCT TTT TCT AAT CTT TTT TCT TTG CCT  
AAT TGC TTG

931

961

GTT TTG GTC TTA GAT TGC TCT CAA TCG CAG AGG AGA CTT TGG CAG AGT TCC  
TAT CCA AGG

991

1021

CTA CAG GAA CTG CTG TTG ATT GGG TTC AGA TGC CTG GGA TGA AGG TTA TAC  
GCA TCT CGT

1051

1081 1111  
 ATC ATT ACT TAA GTG TTA TTT TAT CTG TTG ATA TCT ATG GCA ATA TGT GAA  
 ATA TTG AAA  
 NO-ecotypes  
 C

1141 1171  
 TGT TGT GTG TTG TAG CCT GGT CCG GAT TCG GTT GGC ATC TTT GCC ATT TCG  
 CAA AGA TGC

1201 1231  
 AAT GGA GTG GCA GCT CGA GCC TGT GGT CTT GTT AGC TTA GAA CCT ATG AAG  
 GTA AGA AAG

1261 1291  
 GGA CAC TCT TTT CGT TGC TAA AGA TAC AAG TCA TAA TGT TTC ATT TTC AAC  
 CAG TTT GGG  
 NO-ecotypes T

1321 1351  
 TTT TTT GTG TTC TTA CAG ATT GCA GAG ATC CTC AAA GAT CGG CCA TCT TGG  
 TTC CGT GAC  
 NO-ecotypes  
 A

1381 1411  
 TGT AGG AGC CTT GAA GTT TTC ACT ATG TTC CCG GCT GGT AAT GGT GGC ACA  
 ATC GAG CTT

1441 1471  
 GTT TAT ATG CAG GTG AAT CCT TTA GCC TCT TCT GGT TTA GTT TTC TAT CTC  
 TAA CAC TTG  
 NO-ecotypes T

1501

AAG ATG AAT GAA TAA AGT TGT GAC ATT TGT TCA GAC GTA TGC ACC AAC GAC  
TCT GGC TCC

1531

1561

TGC CCG CGA TTT CTG GAC CCT GAG ATA CAC AAC GAG CCT CGA CAA TGG GAG  
TTT TGT GGT

1591

1621

ATG CAG CTC TCA TAA TGT CTA GTG TTT ACA GAA AAA CTC TGG GAT CTT GAT  
GTT TTT CAT

1651

1681

ATG TCT TTA AAA GGT TTG TGA GAG GTC GCT ATC TGG CTC TGG AGC TGG GCC  
TAA TGC TGC

1711

1741

TTC AGC TTC TCA GTT TGT GAG AGC AGA AAT GCT TTC TAG TGG GTA TTT AAT  
AAG GCC TTG

1771

1801

TGA TGG TGG TGG TTC TAT TAT TCA CAT TGT CGA TCA CCT TAA TCT TGA GGT  
ACT TAA ATC

1831

1861

TTC ACA TGT GGC ATT TTG TGT GTG TTT TCA GGA ATT TCT AGA AGA ATT GAT  
TAT AAA CAT

1891

1921

TTG TTC TTG CAT TGT AGG CTT GGA GTG TTC CGG ATG TGC TTC GAC CCC TTT  
ATG AGT CAT

1951

rev-6

T

1981

2011

CCA AAG TCG TTG CAC AAA AAA TGA CCA TTT CCG TGA GTG TAT ACA TAT AAT  
 AAC CTT AAG

2041 2071  
 CTT TGA TTG ATT CAT ATA ACA TAT CTA ACG GTT GGA GGT GCT TCA TGT TTT  
 AGG CGT TGC

rev-2 and rev-4

A  
 NO-ecotypes T

2101 2131  
 GGT ATA TCA GGC AAT TAG CCC AAG AGT CTA ATG GTG AAG TAG TGT ATG GAT  
 TAG GAA GGC

2161 2191  
 AGC CTG CTG TTC TTA GAA CCT TTA GCC AAA GAT TAA GCA GGT ACT TCG ATC  
 TTG AGC TAA

2221 2251  
 AAC CTA ATT GTT CTT TGC TCT GTT TGC TCA TTG TCA TTT TTT CTG TTC TTG  
 GTT TTC TTG

2281 2311  
 AAG GGG CTT CAA TGA TGC GGT TAA TGG GTT TGG TGA CGA CGG GTG GTC TAC  
 GAT GCA TTG

2341 2371  
 TGA TGG AGC GGA AGA TAT TAT CGT TGC TAT TAA CTC TAC AAA GCA TTT GAA  
 TAA TAT TTC

2401 2431  
 TAA TTC TCT TTC GTT CCT TGG AGG CGT GCT CTG TGC CAA GGC TTC AAT GCT  
 TCT CCA AGT

2461

AAG TTA GTG TGT CCA GTA TTG GTA CTT TGT GTT CTT TTG ACA GTT TTC TAT  
GGC TGA AAT

2491

2521

TTG TGT TAT CTA TTG TCT TCT GTA GAA TGT TCC TCC TGC GGT TTT GAT CCG  
GTT CCT TAG

2551

2581

AGA GCA TCG ATC TGA GTG GGC TGA TTT CAA TGT TGA TGC ATA TTC CGC TGC  
TAC ACT TAA

2611

2641

AGC TGG TAG CTT TGC TTA TCC GGG AAT GAG ACC AAC AAG ATT CAC TGG GAG  
TCA GAT CAT  
rev-5 C

2671

2701

AAT GCC ACT AGG ACA TAC AAT TGA ACA CGA AGA AGT AAG GCT TCA AAG TCT  
TTA CCT GCC

2731

2761

GAC AAA ACA TCA TTT TTA TGT CTC TCT CTT ACA TAT ATA TTT GGT TTT GTT  
ATG TTT AGA  
rev-1

2791

A

2821

TGC TAG AAG TTG TTA GAC TGG AAG GTC ATT CTC TTG CTC AAG AAG ATG CAT  
TTA TGT CAC

2851

2881

GGG ATG TCC ATC TCC TTC AGG TAT ATC ACT TCT AAG TTC TAA CCC AAT GGA  
TCT TGA AAT

2911



AGT TCA ACG TGT TGC AAT GGC GAT CTC ACC GTC TGG GAT AAG CCC GAG TCT  
GGG CTC CAA

3481 3511  
ATT GTC CCC AGG ATC TCC TGA AGC TGT TAC TCT TGC TCA GTG GAT CTC TCA  
AAG TTA CAG

3541 3571  
GTG GGG GTG TAA ATG TTT ACT CTC GTC TCT TTC TTA TAA TCC TCG AAC TTA  
TCG ATG ATG

3601 3631  
CCT TAT GCT GAT ATG TTT GTT TTT CCA GTC ATC ACT TAG GCT CGG AGT TGC  
TGA CGA TTG

3661 3691  
ATT CAC TTG GAA GCG ACG ACT CGG TAC TAA AAC TTC TAT GGG ATC ACC AAG  
ATG CCA TCC

3721 3751  
TGT GTT GCT CAT TAA AGG TAT GTG TCC TAC ACC AAA CAA AAA GCA GAA TAC  
ACC TGT AGT

3781 3811  
TTT AGA CGT ATA ATA TGG TCT GGA TAT GTT GCA GCC ACA GCC AGT GTT CAT  
GTT TGC GAA

3841 3871  
CCA AGC TGG TCT AGA CAT GCT AGA GAC AAC ACT TGT AGC CTT ACA AGA TAT  
AAC ACT CGA

3901 3931  
AAA GAT ATT CGA TGA ATC GGG TCG TAA GGC TAT CTG TTC GGA CTT CGC CAA  
GCT AAT GCA

3961 3991  
 ACA GGT AAA GAA CCA AAA CAA AAA CAT CTG CAG ATA AAT GGT TTT GAT TCA  
 TTT GTC TGA

4021 4051  
 GAA CTA TCT TTG CGT CTA CAG GGA TTT GCT TGC TTG CCT TCA GGA ATC TGT  
 GTG TCA ACG

4081 4111  
 ATG GGA AGA CAT GTG AGT TAT GAA CAA GCT GTT GCT TGG AAA GTG TTT GCT  
 GCA TCT GAA

4141 4171  
 GAA AAC AAC AAC AAT CTG CAT TGT CTT GCC TTC TCC TTT GTA AAC TGG TCT  
 TTT GTG

*Arabidopsis* REVOLUTA Protein SEQ ID NO:2

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQQLIRECS  
 ILANIEPKQI KWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQLVC  
 ENGYMKQQLT TVVNDPSCES VVTTPOHSLR DANSPAGLLS IAETLAEFL SKATGTAVDW  
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF  
 TMFPAGNGGT IELVYMQTYA PTTLAPARDF WTLRYTTS LD NGSFVVCERS LSGSGAGPNA  
 ASASQFVRAE MLSSGYLIRP CDGGGSIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT  
 ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA  
 EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNV D  
 AYS AATLKAG SFAYPGMRPT RFTG SQIIMP LGHTIEHEM LEVVRLEGHS LAQEDAFMSR  
 DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA  
 NHRTLDTSS LEVGPSPENA SGNSFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS  
 VISSVQRVAM AISPSGISPS LGSKLSPGSP BAVTLAQWIS QSYSHHLGSE LLTIDSLGSD  
 DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI  
 CSDFAKLMQQ GFACLPSGIC VSTMGRHVS Y EQAVAWKVFA ASEENNNNLH CLAFSFWNS  
 FV

*Arabidopsis* rev-3 Protein SEQ ID NO:6

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQLIRECS  
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQLVC  
 ENGYMKQQLT TVVNDPSCES VVTTPOHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW  
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF  
 TMFPAGNGGT IELVYMQTYA PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA  
 ASASQFVRAE MLSSGYLIRP CDGGGSIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT  
 ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA  
 EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNVD  
 AYSAAATLKAG SFAYPGMRPT RFTGSQIIMP LGHTIEHEEM LEVVRLEGHS LAQEDAFMSR  
 DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA  
 NHRTLDLISS LEVGPSPENA SGNSFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS  
 VISSVQRVAM AISPSPGISPS LGSKLSPGSP BAVTLAQWIS QSYSHHLGSE LLTIDSLGSD  
 DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI  
 CSDFAKLMOO GFACLPSGIC VSTMGRHVSY EOAVAWKVFA ASEENNNNLH CLAFSFFVNS  
 FV

*Arabidopsis* rev-4 Protein SEQ ID NO:8

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQLIRECS  
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQLVC  
 ENGYMKQQLT TVVNDPSCES VVTTPOHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW  
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF  
 TMFPAGNGGT IELVYMQTYA PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA  
 ASASQFVRAE MLSSGYLIRP CDGGGSIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT  
 ISRCGISGN

*Arabidopsis* REV-5 Protein SEQ ID NO:10

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQLIRECS  
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQLVC  
 ENGYMKQQLT TVVNDPSCES VVTTPOHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW  
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF  
 TMFPAGNGGT IELVYMQTYV PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA  
 ASASQFVRAE MLSSGYLIRP CDGGGSIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT  
 ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA

EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNVD  
 AYSAAATLKAG SFAYPGMRPT RFTGSIIMP LGHTIEHEEM LEVVRLEGHS LAQEDAFMSR  
 DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA  
 NHRTLDLTSS LEVGPSPEA SGNFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS  
 VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSYSHHLGSE LLTIDSLGSD  
 DSVLKLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI  
 CSDFAKLMQQ GFACLPSTGIC VSTMGRHVSY EQAVAWKVFA ASEENNNNLH CLAFSFWNWS  
 FV

*Arabidopsis* REV-6 Protein SEQ ID NO:12

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKSSL RRQLIRECS  
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQVLC  
 ENGYMKQQLT TVVNDPSCES VVTTPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW  
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF  
 TMFPAANGGT IELVYMQTYA PTTLAPARDF WTLRYTTS LD NGSFVVCERS LSGSGAGPNA  
 ASASQFVRAE MLSSGYLIRP CDGGGSIHI VDHLNLEAWS VPDVL

Barley Genomic Clones:

>Barley 2 SEQ ID NO:97

AGCTCTATCCACCGTCAGCAGTTGATCAGAGAGTGCCTATTCTCTCCAACATTGAGCCTAACAGAT  
 CAAAGTATGGTTTCAGAACCGAAGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT  
 CTTTGATCAAAATCTTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT  
 CAAGCGGTG

>Barley 9 SEQ ID NO:99

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGCCTATTCTCTCCAACATTGAGCCTAACAGAT  
 CAAAGTATGGTTTCAGAACCGAAGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT  
 CTTTGATCAAAATCTTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT  
 CGAGCGGTG

>Barley 10 SEQ ID NO:101

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGCCTATTCTCTCCAACATTGAGCCTAACAGAT  
 CAAAGTATGGTTTCAGAACCGAAGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT

CTTTGATCAAATCTTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT  
CAAGCGGTG

>Barley 2 intron spliced out SEQ ID NO:127

AGCTCTATCCACCGTCAGCAGTTGATCAGAGAGTGTCTATTCTCTCCAACATTGAGCCTAACAGAT  
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTTCAAGCGG  
TG

>Barley 9 intron spliced out SEQ ID NO:128

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTATTCTCTCCAACATTGAGCCTAACAGAT  
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTTCGAGCGG  
TG

>Barley 10 intron spliced out SEQ ID NO:129

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTATTCTCTCCAACATTGAGCCTAACAGAT  
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGGGCTTCACGGCTTCAAGCGGT  
G

>Barley 2 protein SEQ ID NO:98

SSIHRQQLIRECPILSNIEPKQIKVWFQNRRCREKQRKEASRLQAV

>Barley 9 protein SEQ ID NO:100

SSIRRQQLIRECPILSNIEPKQIKVWFQNRRCREKQRKEASRLRAV

>Barley 10 protein SEQ ID NO:102

SSIRRQQLIRECPILSNIEPKQIKVWFQNRRCREKQRKEASRLQAV

Corn Genomic Clones:

>Corn 1 SEQ ID NO:103

AGCTCCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCATCCTCTCAAACATCGAGGCCAAGCAGAT  
TAAAGTC

>Corn 2 SEQ ID NO:105

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT  
TAAAGTC

>Corn 3 SEQ ID NO:107

ACCTCCTCCCGCAGGCAGCAATTGCTGCGTGAGTGCCCCACACTTGCTAACATTGAGCCCAAGCAGAT  
CAAGGTC

>Corn 7 SEQ ID NO:109

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT  
TAAAGTC

>Corn 8 SEQ ID NO:111

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT  
TAAAGTC

>Corn 9 SEQ ID NO:113

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT  
TAAAGTC

>Corn Protein 1 SEQ ID NO:104

SSARRQQLRECPILSNIEAKQIKV

>Corn Protein 2 SEQ ID NO:106

SSARRQQLRECPILSNIEAKQIKV

>Corn Protein 3 SEQ ID NO:108

TSSRRQQLRECPILSNIEAKQIKV

>Corn Protein 7 SEQ ID NO:110

SSARRQQLRECPILSNIEAKQIKV

Corn Protein 8 SEQ ID NO:112

SSARRQQLRECPILSNIEAKQIKV

Corn Protein 9 SEQ ID NO:114

SSARRQQLLRECPILSNIEAKQIKV

Rice Genomic Clones

Rice Genomic 1 SEQ ID NO:115

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT  
CAAGGTGTGGTTCCAGAACC GCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTGCGGGCTGCAGGCGC  
TC

Rice Genomic 4 SEQ ID NO:117

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT  
CAAGGTGTGGTTCCAGAACC GCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTGCGGGCTGCAGGCGC  
TC

Rice Genomic 10 SEQ ID NO:119

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT  
CAAGGTGTGGTTCCAGAACC GCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTGCGGGCTGCAGGCGC  
TC

Rice Genomic protein 1 SEQ ID NO:116

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQRKESSRLQAL

Rice Genomic protein 4 SEQ ID NO:118

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQRKESSRLQAL

Rice Genomic protein 10 SEQ ID NO:120

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQRKESSRLQAL

Rice cDNA Clones:

rice cDNA 1 SEQ ID NO:121

TCCTCCCGCAGGCAGCAATTGCTGCGTGAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA  
GGTCTGGTTCCAGAACAGAAAGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

rice cDNA 4 SEQ ID NO:123

TCCTCCCGCAGGCAGCAATTGCTGCGTAAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA  
GGTCTGGTTCCAGAACAGAAAGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

rice cDNA 8 SEQ ID NO:125

TCCTCCCGCAGGCAGCAATTGCTGCGTAAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA  
GGTCTGGTTCCAGAACAGAAAGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

rice cDNA protein 1 SEQ ID NO:122

SSRRQQLLRECPILANIEPKQIKVWFQNRKCRDKQRKESSRLQAV

rice cDNA protein 4 SEQ ID NO:124

SSRRQQLLRKCPILANIEPKQIKVWFQNRRCRDKQRKESSRLQAV

rice cDNA protein 8 SEQ ID NO:126

SSRRQQLLRKCPILANIEPKQIKVWFQNRRCRDKQRKESSRLQAV

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**Key Interior Regions of REV gene products**

arabidopsis REV sequence (amino acids 123-146 of SEQ ID NO:2):

GYMKQQLTTVVNDPSCESVVTTPQ (SEQ ID NO:130)

Corresponding tomato REV sequence

GYMRQQLQSVTMDVSCESGVVTTPQ (SEQ ID NO:131)

Corresponding rice REV1 sequence

AHMRQQLQNTPLANDTSCESNVVTTPQ (SEQ ID NO:132)

Corresponding rice REV2 sequence

AYMKQQLQNPXLGNDTSXESNVTPQ (SEQ ID NO:133)

arabidopsis REV sequence (amino acids 234-246 of SEQ ID NO:2):

CRSLEVFTMFPAG (SEQ ID NO:134)

tomato REV sequence

CRNVEVITMFPAG (SEQ ID NO:135)

rice REV1 sequence

CRNLEVFTMIPAG (SEQ ID NO:136)

rice REV2 sequence

CRSLEVFTMFPAG (SEQ ID NO:137)

#### Primers for Example Inverted Repeat Constructs

|         |                                    |                 |
|---------|------------------------------------|-----------------|
| REVIR-1 | TTATCGATAGCTTTGCTTATCCGGGAAT       | (SEQ ID NO:138) |
| REVIR-2 | TTGCGGCCGCCTGACAAGCCATACCAGCAA     | (SEQ ID NO:139) |
| REVIR-3 | TTGCGGCCGCAGTTCAACGTGTTGCAATGG     | (SEQ ID NO:140) |
| REVIR-4 | TTGCATGCGCTAGCGTCGTCGCTTCCAAGTGAAT | (SEQ ID NO:141) |
| REVIR-5 | TTGTCGACCCGCGGAGCTTTGCTTATCCGGGAAT | (SEQ ID NO:142) |
| REVIR-6 | TTGATGCGCTAGCCTGACAAGCCATACCAGCAA  | (SEQ ID NO:143) |

Example Inverted Repeat (SEQ ID NO:144):

ATCGATAGCTTTGCTTATCCGGGAATGAGACCAACAAGATTCAGTGGGAGTCAGATCATA

ATGCCACTAGGACATACAATTGAACACGAAGAAATGCTAGAAAGTTGTTAGACTGGAAGGT

CATTCTCTTGCTCAAGAAGATGCATTTATGTACGGGATGTCCATCTCCTTCAGATTTGT

ACCGGGATTGACGAGAATGCCGTTGGAGCTTGTCTGAACTGATATTTGCTCCGATTAAT

GAGATGTTCCCGGATGATGCTCCACTTGTCCCTCTGGATCCGAGTCATACCCGTTGAT  
GCTAAAACGGGAGATGTACAAGATCTGTTAACCGCTAATCACCGTACACTAGACTTAACT  
TCTAGCCTTGAAGTCGGTCCATCACCTGAGAATGCTTCTGGAAACTCTTTTTCTAGCTCA  
AGCTCGAGATGTATTCTCACTATCGCGTTTCAATTCCCTTTTGAAAACAACTTGCAAGAA  
AATGTTGCTGGTATGGCTTGC GCGGCCGAGTTCAACGTGTTGCAATGGCGATCTCACCG  
TCTGGGATAAGCCCGAGTCTGGGCTCCAAATTGTCCCAGGATCTCCTGAAGCTGTTACT  
CTTGCTCAGTGGATCTCTCAAAGTTACAGTCATCACTTAGGCTCGGAGTTGCTGACGATT  
GATTCACCTTGGGAGCGACGACGCTAGCGCATGCCAAGCCATACCAGCAACATTTCTTGC  
AAGTTGTTTTCAAAGGGAATTGAAACCGGATAGTGAGAATACATCTCGAGCTTGAGCTA  
GAAAAAGAGTTTCCAGAAGCATTCTCAGGTGATGGACCGACTTCAAGGCTAGAAGTTAAG  
TCTAGTGACGGTGATTAGCGGTTAACAGATCTTGACATCTCCCGTTTTAGCATCAACG  
GGTATGACTCGGAATCCAGAGGGAACAAGTGGAGCATCATCCGGGAACATCTCATTAATC  
GGAGCAAATATCAGTTCAGAACAAAGCTCCAACGGCATTCTCGTCAATCCCGGTACAAATC  
TGAAGGAGATGGACATCCCGTGACATAAATGCATCTTCTTGAGCAAGAGAATGACCTTCC  
AGTCTAACAACTTCTAGCATTTCTTCGTGTTCAATTGTATGTCCTAGTGGCATTATGATC  
TGACTCCCAGTGAATCTTGTTGGTCTCATTCCCAGGATAAGCAAAGCTCCGCGG

**Example II of an inverted repeat from Arabidopsis REV**

Exons 3-7 (positions 3670 to 3743 of SEQ ID NO:1; positions 3822 to 3912 of SEQ ID NO:1; positions 4004 to 4099 of SEQ ID NO:1; positions 4187 to 4300 of SEQ ID NO:1; and positions 4383-4466 of SEQ ID NO:1)

(SEQ ID NO:145):

ATCGATGTTAACGATCCAAGCTGTGAATCTGTGGTCACAACCTCCTCAGCATTGCTTAGA  
GATGCGAATAGTCTCTGCTGGATTGCTCTCAATCGCAGAGGAGACTTTGGCAGAGTTCCTA  
TCCAAGGCTACAGGAACTGCTGTTGATTGGGTTTCAGATGCCTGGGATGAAGCCTGGTCCG  
GATTCGGTTGGCATCTTTGCCATTCGCAAAGATGCAATGGAGTGGCAGCTCGAGCCTGT  
GGTCTTGTAGCTTAGAACCTATGAAGATTGCAGAGATCCTCAAAGATCGGCCATCTTGG  
TTCCGTGACTGTAGGAGCCTTGAAGTTTTCACTATGTTCCCGGCTGGTAATGGTGGCACA  
ATCGAGCTTGTATTATATGCAGACGTATGCACCAACGACTCTGGCTCCTGCCCGGATTTC  
TGGACCCTGAGATACACAACGAGCCTCGACAATGGGAGTTTTGTGCGCGGCCGC

Linker (exon15) (SEQ ID NO:146):

GGAGATGTACAAGATCTGTAAACCGCTAATCACCGTACACTAGACTTAACTTCTAGCCTT  
GAAGTCGGTCCATCACCTGAGAATGCTTCTGGAACTCTTTTTCTAGCTCAAGCTCGAGA  
TGTATTCTCACTATCGCGTTTCAATTCCCTTTTGAAAACAACCTGCAAGAAAATGTTGCT  
GGTATGGCTTGTGAGTATGTGAGGAGCGTGATCTCATCAGTTCAACGTGTTGCAATGGCG

ATCTCACCGTCTGGGATAAGCCCGAGTCTGGGCTCCAAATTGTCCCAGGATCTCCTGAA  
GCTGTACTCTTGCTCAGTGGATCTCTCAAAGTTACAGGCTAGCCATGCCTGCCCGCGA  
TTTCTGGACCCTGAGATACACAACGAGCCTCGACAATGGGAGTTTGTGCGCGGCCG

(SEQ ID NO:147):

CACAAAACCTCCCATTTGTCGAGGCTCGTTGTGTATCTCAGGGTCCAGAAATCGCGGGCAGG  
AGCCAGAGTCGTTGGTGCATACGTCTGCATATAAACAAGCTCGATTGTGCCACCATTACC  
AGCCGGGAACATAGTGAAAACTTCAAGGCTCCTACAGTCACGGAAACCAAGATGGCCGATC  
TTTGAGGATCTCTGCAATCTTCATAGGTTCTAAGCTAACAAGACCACAGGCTCGAGCTGC  
CACTCCATTGCATCTTTGCGAAATGGCAAAGATGCCAACCGAATCCGGACCAGGCTTCAT  
CCCAGGCATCTGAACCAATCAACAGCAGTTCTGTAGCCTTGGATAGGAACTCTGCCAA  
AGTCTCCTCTGCGATTGAGAGCAATCCAGCAGGACTATTGCGATCTCTAAGCGAATGCTG  
AGGAGTTGTGACCACAGATTCACAGCTTGGATCGTTAACCCGCGG

Example III. IR construct from tomato clone (SEQ ID NO:148):

ATCGATATTGCTGATATCCTCAAAGATCGACCTTCTTGGTTCCGCGACTGCCGGAATGTTGAAGTTAT  
CACAATGTTTCCTGCTGG  
    AAATGGTGGTACAGTTGAGCTTTTGTATACCCAGATATATGCTCCCACAACTCTGGCTCCCG  
CGCGTGATTTTTGGACGC  
    TGAGATACACAACAACCCCTAGACAATGGTAGTCTCGTGGTTTGTGAAAGATCCCTATCTGGT  
AATGGGCCTGGCCCAAAT  
    CCTACTGCTGCTTCCCAGTTTGTAAAGAGCTCAAATGCTTCCATCTGGATATCTGATCCGACC  
GTGTGATGGTGGAGGATC

AATCATACATATTGTTGATCACCTGAATCTTGAGGCATGGAGTGCCCTGAGATTTTGCCTC  
CACTCTATGAATCGTCTGA

AAGTTGTGGCACAGAAAATGACTATTGCAGCACTGCGATATGCAAGGCAACTAGCTCAAGAG  
ACTAGCGGCGAGCGCGGCCGC

Linker (SEQ ID NO:149):

GTAGTATATGGTCTAGGAAGGCAACCTGCTGTTCTCGAACATTAGCCAGAGATTATGCAGAGGGTT  
CAATGATGCCATCAATGGATTCCGGTGACGATGGCTGGTCAATGTTAAGTTCAGATGGTGCTGAAGATG  
TCATAGTTGCTGTCAATTCAAGGAAGAACCTCGCAACCACCTCCATTCTTTCCCCGCTTGGTGGC  
GTCCTTTGTACCAAAGCATCAATGCTACTCCAGAATGTCCCCCTGCCGACTGGTTCGGTTTCTGAG  
GGAGCACCGTTCAGAATGGGCCGATTATGCTAGCGCATGC

(SEQ ID NO:150):

CTCGCCGCTAGTCTCTTGAGCTAGTTGCCTTGCCATATCGCAGTGCTGCAATAGTCATTTTCT  
GTGCCACAACITTCGACGATTTCATAGAGTGGACGAAAATCTCAGGGGCACTCCATGCCTCAAGATTC  
AGGTGATCAACAATATGTATGATTGATCCTCCACCATCACACGGTCCGATCAGATATCCAGATGGAAG  
CATTTGAGCTCTTACAAACTGGGAAGCAGCAGTAGGATTTGGGCCAGGCCCATACCAGATAGGGATC  
TTTCACAAACCACGAGACTACCATTGTCTAGGGTGTGTGTATCTCAGCGTCCAAAAATCACGCGCG  
GGAGCCAGAGTTGTGGGAGCATATATCTGGGTATACAAAAGCTCAACTGTACCACCATTTCCAGCAGG  
AAACATTTGTATAACTTCAACATTCGGGCAGTCGCGGAACCAAGAAGGTCGATCTTTGAGGATATCAG  
CAATCCGCGG

Example of an IR for riceREV1 (SEQ ID NO:151):

ATCGATtggttcatgagaatgccacatgacagcagctgcagaatactccgctggcaaat  
gatacaagctgtgaatcaaatgtgactaccctcaaaacccttaagggatgcaagtaaccctctgg  
gtcctttcaattgcagaggagaccttgacagagttcctctcaaaggctactggtacagctattgatt  
gggtccagatgcctgggatgaagcctggccgattcggttggtattgtggcatttcacatggtgc  
ccgtggtgtgctgccgtgcctgtggttggtgaacctagaaccaaaaaagtggtagagatattgaa  
agatcgtccatcttggttccgtgatgtcgaaacctggaagtctttacaatgattccagcaggaaatg  
gaggaacggttgaacttgtctacacacagttgtatgctccaacaacttagttcctgcaCGCGGCCGC

linker(SEQ ID NO:152):

atgctagggagtagcagtgatggaggtggctatgataaggttccgggatggactccggtaaatagt  
 gcgctacacgcctgagcaggtggaggcgcttgagcgggtgtacgccgattgccccagccaacctcct  
 cccgcaggcagcaattgctgcgtgagtgccccatacttgctaacattgagcccaagcagatcaaGCTA  
GCGCATGC

(SEQ ID NO:153):

tgcaggaactaaagttggtggagcatacaactgtgtgtagacaagttcaaccgttcctccat  
 ttctgctggaatcattgtaaagacttccaggttccgacaatcacggaaccaagatggacgatctttc  
 aatatctctaccacttttgttggttctaggttccacaaaccacaggcacggcagcaacaccacgggca  
 accatgtgaaatggccacaataccaaccgaatccggaccaggttcatcccaggcatctggacceat  
 caatagctgtaccagtagcctttgagaggaactctgtcaaggtctcctctgcaattgaaaggagccca  
 gaggggttacttgcacccctaaagggttttgaggggtagtacatttgattcacagcttgatcatt  
 tgccagcggagatttctgcagctgctgtcgcatgtgggcatttctcatgaaccaCCGCGG

Example for RiceREV2 (SEQ ID NO:154):

ATCGAT gaatcaaatg tgaccactcc tcagaacct ctgagagatg caagtaacc  
 gtctggactc cttacaattg cggaggagac cctgacagag ttctctcca aggtacag  
 gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggat  
 tgtggccgtt tcacatggtt gccgtggtg tgctgcccg gctgtggtt tggatgatc  
 agaaccaaca aagatcgtgg agatcttaa agaccgcca tcttggttcc gtgattgctg  
 aagtcttgaa gtcttcacaa tgtttccagc tggaaatggg ggcacgatcg aacttgttta  
 catgcagatg tatgctcta ctacttggg tctgacag gatttttggg cacttagata  
 cacaactaca atggatgatg gcagccttgt ggtctgtgagCGCGGCCGC

Linker (SEQ ID NO:155):

ccgcacagca atttgtaaga gctgagatgc ttcttagcgg  
 ctatctagtg cgcccatgcg aggggtggtg ctcctcgtg catattgtgg accatctgga  
 tcttgaggct tggagtgttc cagaagtgtc tcggccactc tacgagtcac ctagggtagt  
 tgctcagaaa atgactgctg cagcGCTAGCGCATGC

(SEQ ID NO:156):

ctcacagaccacaaggctgccatcatccattgtagttgtgtatctaagtgccccaaatctcgtgcag  
 gaaccaagttagtaggagcatacatctgcatgtaaacaaagttcgatcgtgccaccatttccagctgga  
 aacattgtgaagacttcaagacttcgacaatcacggaaccaagatgggcggtcttttaagatctccac  
 gatcttgggttctagattcaccacacaggcacgggcagcaacaccacggcaaccatgtgaaa

cggccacaatacctcaaacgaatccggaccaggettcaccccaggcattggcacccaatcaacagcagtc  
cctgtagccttgagaggaactctgtcagggctcctccgcaattgtaaggagtcacagacgggttact  
tgcattctcagaggggtctgaggagtggtcacatttgattcCCGCGG

Rice REV1 (SEQ ID NO:157):

atg ctaggagta gcagtgatgg aggtggctat gataaggttt ccgggatgga  
ctccggtaaa  
tatgtgcgct acacgcctga gcaggtggag ggccttgagc ggggtgtacgc cgattgcccc  
aagccaacct cctcccgcag gcagcaattg ctgcgtgagt gccccatact tgctaacatt  
gagcccaagc agatcaaggt ctggttccag aacagaaggt gccgggataa gcagcgggaag  
gagtcttcac ggcttcaggc tgtcaacagg aaattgacgg caatgaacaa gctacttatg  
gaagagaatg agcgactcca gaagcaggtc tcccaattgg ttcattgagaa tgccccatg  
cgacagcagc tgcagaatac tccgctggca aatgatacaa gctgtgaatc aatgtgact  
accctcaaa accctttaag ggatgcaagt aaccctctg ggctccttc aattgcagag  
gagacctga cagagttcct ctcaaaggct actggtacag ctattgattg ggtccagatg  
cctgggatga agcctggtec ggattcgggt ggtattgtgg ccatttcaca tgggtgcccc  
tggtgttgcct gccgtgcctg tggtttggtg aacctagaac caacaaaagt ggtagagata  
ttgaaagatc gtccatcttg gttccgtgat tgtcgaaacc tggaaagtctt tacaatgatt  
ccagcaggaa atggaggaac ggttgaactt gtctacacac agttgtatgc tccaacaact  
ttagttcctg cacgagattt ttggacgta cggtagacaa ccacaatgga agatggcagt  
cttgtggctc gtgagagatc ttaagtgggt tcagggggcg gtccaagtgc tgctctgct  
cagcaatatg tgagagcggga aatgctcca agtgatacc tggttcgccc atgtgaagg  
gggggatcaa ttgtgcacat agtgaccat ctggatcttg aggcattggag tgttcctgag  
gtgcttcggc cactctatga atcttcaagg gtatgctc agaaaatgac tactgcccga  
ctccggcaca tcagacaaat tgcctcaagaa acaagtgggg aagtgggtga tgcttgggg  
aggcaaccag cagtgtacg gacttttagt caaaggctga gcagaggctt taacgatgcc  
attagtgggt tcaatgatga tgggtggctc ataatgggtg gagacgggtg tgaagatgta  
gttattgctt gcaactcaac taagaaagt aggagtagca gcaatgcngn catcgcttt

RICE REV2 (SEQ ID NO:158)

cccaaaacc cagctcctcc cgccgccagc agntgctccg  
 ngactgcccc atcctcgcca acategagcc caagcagatc aaggctctggt tccagaacag  
 aagggtgccga gataagcagc ggaaggaggc atcaaggctt caggccngna accgaaaatt  
 gacggcgatg aataagcttn tcatggagga gaatgagcgt cttcagaagc aggnctccca  
 gctggtccat gagaacgcgt acatgaagca gcaacttcag aatccgncat tgggcaatga  
 tacaagctgn gaatcaaatg tgaccactcc tcagaaccct ctgagagatg caagtaacce  
 gtctggactc cttacaattg cggaggagac cctgacagag ttcctctcca aggctacagg  
 gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggtat  
 tgtggccgtt tcacatggtt gccgtggtg tgctgcccgt gcctgtggtt tggggaatct  
 agaaccaaca aagatcgtgg agatcttaa agaccgcca tcttggttcc gtgattgtcg  
 aagtcttgaa gtcttcacaa tgtttccagc tggaaatggt ggacagatcg aacttgttta  
 catgcagatg tatgctccta ctactttggt tcctgcacga gatttttggga cacttagata  
 cacaactaca atggatgatg gcagccttg ggtctgtgag agatcattga gtggttctgg  
 aggtggtnc aagncagcct ccgcacagca atttgtaaga gctgagatgc ttcctagcgg  
 ctatctagtg cccccatgag aggggtggtg ctcctgctg catattgtgg accatctgga  
 tcttgaggct tggagtgttc cagaagtgt tcggccactc tacgagtcac ctagggtagt  
 tgctcagaaa atgactgctg cagcngtgcg gcacatcaga caaattgctc aagagacaag  
 cggggagggt gtatacgctt tggggaggca acctgctggt ttgaggacat ttagtcagag  
 gttgagtaga ggcttcaatg atgctattag tggtttcaac gatgatggtt ggtctgtcat  
 ggggtgggat ggcatcgaag atgtgatcat tgcttgcaat gcaaagaggg ttaggaatac  
 tagcncttcg gccaatgctt tt

Rice REV1 protein (SEQ ID NO:159):

maaavamlgsssdgggydkvsgmdsgkyvrytpeqvealervyadcpkptssrrqllrecp  
 ilaniekqikvwfqnrccrdkqrkessrlqavnrlktamnkllmeenerlqkqvsqlvhenahrqg  
 lqntplandtscesnvtppnplrdasnpagllsiaeetlteflskatgtaidwvqmpgmkpgpdsfv  
 ivaishgcpwcccracglvnleptkveilkdrpswfrdcnlevftmipagnggtvelvytqlyapt  
 tlvpardfwtlrytttmedgslvcerslsgsgggsaasaqyvraemlpsgylvrpcegggsivhi  
 vdhdleawsvpevlrplyessrvvaqkmttaalrhirqiaqetsgevvialgrqpavlrtsqrlsr  
 gfndaisgfnddgwsimggdgvedvviacnstkkvrsssnaxiafgaggii

Rice REV2 protein (SEQ ID NO:160):

ervycecpkpssrrqqlrdcpilaniepkqikvwfqnrccrdkqrkeasrlqaxnrklta  
 mnklxmeenerlqkqvsqlvhenaymkqqlqnpxlgnstdsxesnvtppnplrdasnpaglltiaet  
 lteflskatgtavdwvmpgmkpgpdsfgivavshgcrvvaaracglvnleptkiveilkdrpswfrd

crslevftmpagnggtielvymqmyapttlvpardfwlryttmddgalvvcerslsgsgggxsxa  
saqqfvraemlpsgylvrpceggsvvhivdhldleawsvpevlrplyessrvvaqkmtaaavrhirq  
iaqetsgevvyalgrqpavlrtfsqrlsrgfndaisgfnddgwsvmggdiedviiacnakrvrntsx  
sanafvtppgvi

tomato J1R (SEQ ID NO:162):

CAGCAGAATAAGCATCAACATTATAATCNGCCCAYT

Tomato REV Genomic (exons underlined) (SEQ ID NO:163)

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCATATTCTGTGCAATATCGAGCCTAAGCAGAT  
CAAAGTTTGGTTTCAGAACAGAAGGTATACTTCCATTGTTCAATTTTGCCCAAATTTGGTTTATGTT  
TTGTTGTTAATTGCATACATTTTTATATGTCTATTGTGTACGATTGATCTGCACTTTACTTTGTTTAG  
TACTGCTCGAATCTTGTATTAGTTAGATCAGTGATGATAAACTGAATGTATCACTGTAGTTCTCCTTG  
CCTAGGCTTGTTGGTTGAGTGGTAGGGTATGTGTTAACCTTAGGTGATTGGGAAATTGAGCTTAGAGT  
TTGGTATGGAGGTAACGTTGTATCATTTCAGGTGTCGAGAGAAGCAAAGGAAAGAGTCTTCTCGAT  
TGCAGACTGTGAACAGAAAGTTGTCTGCGATGAATAAACTGTTGATGGAGGAGAATGACCGCTTGCA  
AAACAAGTCTCGCAGCTTGTATGTGAAAATGGCTATATGCGGCAACAATTGCAAAATGTAAGCTAACT  
TAACTCTTCTGTTATTTTTTATGTCCAAAAGCTCCATGTGTGCTTACTATATAGTAGATTAATGTCA  
AACATATCTTGTCTTTTTTGTTCACCTGATCTATGCTGCTGAAATGGCTACTCACTGTGTAGTCTAGA  
TTATACAATATCCACCGCTATTGAGTCCATGATTTAATCAGTCAGTCTTATAATTCTGGAATGCGT  
TACTTTATATATGGGACTAAATTGGCATGGCATTATTTTTGTGTAGTAGTACAAGAAACATTTAAGGT  
CCTGTGACTTCAAATTTGTAAGATGACAGATATCACCAGTCATTTGTGGATCAAGAGGACTTAATTTA  
AGCTTACTTAAGACTCTAATTGTGTTTGTCTGCAGGTATCGGCGGCCACTACTGATGTAAGTTGTGAAT  
CAGGGGTAACCACCTCCTCAGCATTCCCTTAGAGATGCTAACAACCCTGCTGGGTAATAAATTTAAAACA  
GCTATTTCTTCACTCCTTACTTATATGATGTTAATTTAAAACGTGTTTACTACTGTATCTTTGGAGG  
AAGTAAATAGCAAATTTCACAATTTAAGGGACTGATTATTTATCTCTAAGTCATGTTTATTCTCTATG  
CAGACTACTACCAATTGCAGAAGAAACCTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTGCG  
ATTGGGTCCCGATGCCTGGGATGAAGGTTGAACTCTAGTCAATCACCTTTTATTTTTTAAAATTCAGT  
ATTTCCATCTGTATCATTGACCAGACGGCTAAAAGGCAATATTATCATTCAATTTGTCAGCCTGGTCCG  
GATTCAGTTGGGATTTTTGCCATCTCACACAGTTGCAGTGGAGTGGCAGCCGAGCATGTGGTCTTGT  
TAGTTTAGAGCCAAACAAGGTAACAATTTGGAAGTCTATTGAGAAATTTACTGCTGCTCCATTGCTA  
GTTTTAGTCCATTAATGATTGTAGATGTTGTGAGCTTTTTCTTACTAAAACATTTTACAGATTGCTGA  
TATCCTCAAAGATCGACCTTCTTGGTCCGCGACTGCCGGAATGTTGAAGTTATCACAATGTTTCCCTG  
CTGGAAATGGTGGTACAGTTGAGCTTTTGTATACCCAGGTGAATACCTTCTCCTCAATCTCTATGTAC

ACTTCTGATTTGATTAGATACAGCATTGAGGGGATCAATGAATCATTTCTTTCAGATATATGCTCCCA  
CAACTCTGGCTCCCGCGGTGATTTTTGGACGCTGAGATACACAACAACCCTAGACAATGGTAGTCTC  
GTGTAAGCAATCCTTCACATTTAAGTGAGCTTGTGTTGGCGACCTGGCCACTTTTATACTTAGTTCT  
GGCATTCCCTGGTTTAACTAGTCTTTTAAACATCTCAACCTTTCAATCCTTGGATTGAACAGAAGTCTT  
GAAATGTAATATTTTTGGGTCATATTTAACCAAATGCTGCATTATAATCCCCGTCTAGACCTTTGAGT  
ATCTTGCTACTTCAGTATAATACTTGGCTCCATTATTTGTGATTCTTAATAGTGAATTTCTATTAGCTG  
CGTCATTTGGTAGATGTTGCTCACAGTTTCTTTTTGTGTGGCATCAATTTATCCTCCTCACCAAGTT  
TGTGAAAGATCCCTATCTGGTAATGGGCCCTGGCCCAAATCCTACTGCTGCTTCCCAGTTTGTAAAGAGC  
TCAAATGCTTCCATCTGGATATCTGATCCGACCGTGTGATGGTGGAGGATCAATCATACATATTGTTG  
ATCACCTGAATCTTGAGGTAAGATTTTGTAAAGTACTGCTTACCTTTGTGATGAACCTGTTTTGCATG  
GTAGCTGCAATTCACTTCATATATTTTTCAGGCATGGAGTGCCCTGAGATTTTGCCTCCACTCTATG  
AATCGTCGAAAGTTGTGGCACAGAAAATGACTATTGCAGTGAGTTCAACCCTTCGTTATCATTTAATA  
CGGCATATAGATTTATATGTTTGTGAGTTTAAAGTACTTGTGCAGTATCACACTTCCCATAGCTTAC  
TGCCACAGAAGAAGAACCATGATTTTCTGCTTTACTTTCTTTTCTGTGAAGGCCACTGCGATATGCAAG  
GCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGGCAACCTGCTGTTCTTCGAA  
CATTCAGCCAGAGATTATGCAGGTGATGCTTATTTCTGATTTTTGTTATGTGGCTTTGAGATGATGAA  
AATTTATGCACTTCTGAGATGCCAATTCTGAAGTACATATACAAGTACCTTATTAGGCCATTTCTATA  
TTGCAGAGGGTTCAATGATGCCATCAATGGATTCCGGTGACGATGGCTGGTCAATGTTAAGTTGAGATG  
GTGCTGAAGATGTCATAGTTGCTGTCAATCAAGGAAGAACCTCGCAACCACCTCCATTCTCTTTCC  
CCGCTTGGTGGCGTCTTTGTACCAAAGCATCAATGCTACTCCAGGTGAATAGTGGATCTTTCTTGAA  
CTGAATAGAATTTTTTCAATTCGACAACTACCTTGCTCTTGTTAATACACAACAAACAGAAGTTCACAAG  
TTCATATTTGCATCCTCTTTTACGATACCAACTGAGAGACTGGTCCATATCAGCAATAGATGGAGTTA  
ATTGTTAAGACAAGTGAACTGGATAAATGAGAATAATTTGACTCTTTTGTTCCTGGCAGAATGTCC  
CCCCTGCCGTACTGGTTCCGGTTTCTGAGGGAGCACCGTTCAGAA

Tomato REV coding (SEQ ID NO:164):

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCATATTTCTGTGGAATATCGAGCCTAAGCAGAT  
CAAAGTTTGGTTTTCAGAACAGAAGGTGTGAGAGAGAAGCAAAGGAAAGAGTCTTCTCGATTGCAGACTG  
TGAACAGAAAGTTGTCTGCGATGAATAAACTGTTGATGGAGGAGAATGACCGCTTGCAGAAACAAGTC  
TCGCAGCTTGTATGTGAAAATGGCTATATGCGGCAACAATTGCAAAATGTATCGGCGGCCACTACTGA  
TGTAAGTTGTGAATCAGGGGTAACCACTCCTCAGCATTCCCTTAGAGATGCTAACAACCCTGCTGGAC  
TACTACCAATTCAGAAAGAACTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTGCGATTGG  
GTCCCAGTGCCTGGGATGAAGCCTGGTCCGATTGAGTTGGGATTTTTGCCATCTCACACAGTTGCAG  
TGGAGTGGCAGCCCAGCATGTGGTCTTGTAGTTTAGAGCCAACAAGATTGCTGATATCCTCAAAG

ATCGACCTTCTTGGTTCCGCGACTGCCGGAATGTTGAAGTTATCACAATGTTTCCTGCTGGAAATGGT  
 GGTACAGTTGAGCTTTTGTATACCCAGATATATGCTCCCACAACCTCTGGCTCCCGCGGTGATTTTTG  
 GACGCTGAGATACACAACAACCCTAGACAATGGTAGTCTCGTGGTTTGTGAAAGATCCCTATCTGGTA  
 ATGGGCTGGCCCAAATCCTACTGCTGCTCCCAGTTTGTAAAGAGCTCAAATGCTTCCATCTGGATAT  
 CTGATCCGACCGTGTGATGGTGGAGGATCAATCATAATATTGTTGATCACCTGAATCTTGAGGCATG  
 GAGTGCCCTGAGATTTTGCCTCCACTCTATGAATCGTCGAAAGTTGTGGCACAGAAAATGACTATTG  
 CAGCACTGCGATATGCAAGGCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGG  
 CAACCTGCTGTTCCCGAACATTGAGCCAGAGATTATGCAGAGGGTTCAATGATGCCATCAATGGATT  
 CGGTGACGATGGCTGGTCAATGTTAAGTTGAGATGGTGTGAAAGATGTCATAGTTGCTGTCAATTCAA  
 GGAAGAACCTCGCAACCACCTCCATTCCTCTTCCCGCTTGGTGGCGTCTTTGTACCAAAGCATCA  
 ATGCTACTCCAGCAGAATGTCCCCCTGCCGTAAGTGGTTCGGTTTCTGAGGGAGCACCGTTCAGAA

Tomato REV protein (SEQ ID NO:165)

SSLRRQQLIRECHILSNIEPKQIKVWFQNRRCREKQRKESRLQTVNRKLSAMNKLMEENDRLQKQV  
 SQLVCENGYMRQQLQNVSAATTDVSCESGVTPQHSLRDANNPAGLLPIAETLAEFLSKATGTAVDW  
 VMPGKMPGPDVSGIFAIHSCSGVAARACGLVLEPTKLIADILKDRPSWFRDCRNVEVITMFPAGNG  
 GTVELLYTQIYAPTTLAPARDFWTLRYTTTLDNGSLVVCERSLSGNGPGPNPTAASQFVRAQMLPSGY  
 LIRPCDGGGSIHIVDHLNLEAWSAPEILRPLYESSKVVAQKMTIAALRYARQLAQETSGEVVYGLGR  
 QPAVPRTFSQLCRGFNDINGFGDDGWSMLSSDGAEDVIVAVNSRKNLATTSIPLSPLGGVLCTKAS  
 MLLQONVPPAVLVRFLREHRSE

TG-cDNA (SEQ ID NO:165): GTRAGTGCCCCATACTTGCT

R25AS-J (SEQ ID NO:166): GCCGTTACGGCSTCRITRAANCC

R22S (SEQ ID NO:167): CGACGACTCCTGGAGTCCGTCAG

(SEQ ID NO:168): TGTATCATTTGCCAGCGGAG

Rice Genomic REV1 (SEQ ID NO:169):

1 gtaagtgcc cataacttgc aacattgagc ccaagcagat caaggtctgg ttccagaaca  
 61 gaaggaatg ataatagaat tgaatctttc tacgtttggt ctctgtgaca aaaacttgg

121 atggcagttt tccctgattg tttcatctgt cacctgaaat aacatttcgt  
tagtttcctt

181 ctggggaggc tatggttcta atatgctgcg ttgttggtgt gatgatgagc  
ggtattttga

241 tatgagggga gctgcaatgc caattgttta tcatttcatt tgtttgcgca  
ccagcaaaat

301 gtagtataat tacttttagct gacggctctg catgtatatg attttttcgt  
ttttcatgnt

361 cgctgaagtg gataactgtg cttgtgttgc tctaggtgcc gggataagca  
gcggaaggag

421 tcttcacggc ttcaggctgn caacaggaaa ttgacggcaa tgaacaagct  
acttatggaa

481 gagaatgagc gactccagaa gcaggtctcc caattggttc atgagaatgc  
ccacatgcga

541 cagcagctgt agaatgtaag ctcttgatgt gctggtgctg atgttgctcc  
ccatgcataa

601 acaygttctc actgaaatgc tatctattcy ttgygcattt tgttatacgc  
atggcatgtc

661 cggggatgtg ttgttctgta ctgtatattg tagattagta taactttaa  
atattgatga

721 tgtgtagcta taccagctgg ggccatatgc gtcagttcct ttagaattga  
tatatgaatt

781 aatcctcaga atgtccatga gatcgctaga tttcactgat aacaccactt  
gcttgggtgc

841 agactccgct ggcaaatgat acaagctgtg aatcaaatgt gactaccctt  
caaaaccctt

901 taagggatgc aagtnacccc tctgggtaag taaatagttc tgagtgactc  
aggtagaatt

961 attgttggat ggacktgctc tttcgatata atgctatctt aactgccttt  
tatcttgytc

1021 taggtccttt tcaattgcag aggagacctt gacagagttc ctctcaaagg  
ctactggtac

1081 cagctattga ttgggtccag atgcctggga tgaaggttcc atgctagcac  
tgtttgtttt

1141 tttgttctgt gattcgtgct aagaggtttt tacttgaagt gcttactacc  
cttttgtttt

1201 catgatgtaa gcctgggccg gattcgggtg gtattgtggc catttcacat  
ggttgccgtg

1261 gtgttgctgc ccgtgcctgt ggttcgggtga acctagance aacaaaagta  
agtgttgtag

1321 ctatttgggt acatgggttt ggtattttta tgttncctca gtattccctg  
gtctgtatgt

1381 tttctgaagc atctattttg gggatagtagc aagcctatcc accagtcact  
tagttttctt

1441 tgtgtgcaaa tggtagaaa cctactacct ccacccaaa atatagccaa  
aagttgctat

1501 atcaaaaatc ctatcagaag tggctcctga acacattgct gccgagtgtg  
gaattaagac

1561 aactgtaat tcaacttaat aaatactaaa ctttgaagat gtcactttag  
aggtctaag

1621 atttcatgtc tgccaactgt tatcatcaaa tttaatcgtg aagataagca  
gatattctgc

1681 tttttttgtt actttattca ggagattttg tgtctcatag aactttgtta  
cgtaggtggt

1741 agagatattg aaagatcgtc catcttggtt ccgtgattgt cgaacctgg  
aagtctttac

1801 aatgattcca gcaggaaatg gagggacggt tgaacttgtc tacacacagg  
tgaacctgt

1861 ttcattttac attgtataat ggtatatacct cagtcttctc tatcaatgca  
tgtgcttcat

1921 gccatgaaca ttattacttg tttttgctta cagttgtatg ctccaacaac  
tttagttcct

1981 gcacgagatt tttggacgtt acggtacaca accacaatgg aagatggcag  
tcttgtggta

2041 tgtatgaaca tgaacctgt tttcacccca caatgagtct caatgtgatg  
ttacccttgc

2101 taatattcct ccactccea ggtctgtgag agatctttaa gtggttcagg  
gggcggtcca

2161 agtgctgcct ctgctcagca atatgtgaga gcggaatgc ttccaagtgg  
atacctgggtt  
2221 cgcccatgtg aagggtggggg atcaattgtg cacatagtgg accatctgga  
tcttgaggta  
2281 tttttcacac tttgtacag ttgaacctg tttttgtcc ctttgatgta  
ggaccatfff  
2341 tgtatcctgt caaactaata atacaatttg ggtttaatct tttcaggcat  
ggagtgttcc  
2401 tgagggtgctt cggccactct atgaatcttc aagggtagtc gctcagaaaa  
tgactactgc  
2461 ggtaagctgt cgtgaaatga tattcagctc aaatttcatt gatgtgatta  
caagttcatc  
2521 atttcaagtg aaacttgfff ttaatgaact cttcaagfff cataacattg  
gattttffff  
2581 tagaaaaaat aaaataaaaa tccaatatta tgaaacttga agagttcaag  
ctaatgataa  
2641 agtttgtgtt ttggataaag cttataatat tggatatgtg gtgaaaatga  
tttatatggg  
2701 ttggtacaac taatgataaa atttgcfff ttgatattgtt gaacagttca  
ttttctgcaa  
2761 tctactttat actaacfff tattgtctat cctatatatc aaggcactcc  
ggcacatcag  
2821 acaaattgct caagaaacaa gtggggaagt ggtgtatgcc ttggggaggc  
aaccagcagt  
2881 gctacggact tttagtcaaa ggctgagcag gtgattffff tataaattat  
tactcagcaa  
2941 ttaatatttt tttcacctgt ttaatctaac accaatatta tgcttttctt  
agaggtttca  
3001 acgacgccgt gaacggc

RiceREV1 cDNA (SEQ ID NO:170):

1 cggggccgtg gctgtggggg ggctgtgagg gtgccccgg cggcgcctcc  
ctccgcct

61 gccggcgagg gggctcggac tgaagggatc taggcgagct gaaaattgaa  
gygcaggcaa  
121 ggagataaga gcagcgtcca aattgtgagt acttcattag caggaggtag  
tggttgtgct  
181 tgcttggtc ctttgcaatt tggctttggc gaggtagcaa tggctgcggc  
agtggcaatg  
241 ctaggagta gcagtgatgg aggtggetat gataaggttt ccgggatgga  
ctccggtaaa  
301 tatgtgcgct acacgcctga gcaggtggag gcgcttgagc ggggtgacgc  
cgattgcccc  
361 aagccaacct cctcccgcag gcagcaattg ctgcgtgagt gcccatact  
tgctaacatt  
421 gagcccaagc agatcaaggt ctggttccag aacagaaggt gccgggataa  
gcagcgggaag  
481 gagtcttcac ggcttcaggc tgtcaacagg aaattgacgg caatgaacaa  
gctacttatg  
541 gaagagaatg agcgactcca gaagcaggtc tcccaattgg ttcattgagaa  
tgcccacatg  
601 cgacagcagc tgcagaatac tccgctggca aatgatacaa gctgtgaatc  
aatgtgact  
661 acccctcaaa accctttaag ggatgcaagt aaccctctg ggctccttc  
aattgcagag  
721 gagacctga cagagttcct ctcaaaggct actggtacag ctattgattg  
gtccagatg  
781 cctgggatga agcctggtec ggatteggtt ggtattgtgg ccatttcaca  
tggttgcccc  
841 tgggtgtgct gccgtgctg tggtttggtg aacctagaac caacaaaagt  
ggtagagata  
901 ttgaaagatc gtccatcttg gttccgtgat tgtcgaaacc tggaagtctt  
tacaatgatt  
961 ccagcaggaa atggaggaac ggttgaactt gtctacacac agttgtatgc  
tccaacaact  
1021 ttagttcctg cagagatctt ttggacgta cgtacacaa ccacaatgga  
agatggcagt

1081 cttgtggtct gtgagagatc ttaagtggc tcaggggacg gtccaagtgc  
tgcctctgct  
1141 cagcaatatg tgagagcggc aatgcttcca agtggatacc tggttcgccc  
atgtgaaggt  
1201 ggggatcaa ttgtgcacat agtggaccat ctggatcttg aggcattggag  
tgctcctgag  
1261 gtgcttcggc cactctatga atcttcaagg gtagtcgctc agaaaatgac  
tactgaggca  
1321 ctccggcaca tcagacaaat tgctcaagaa acaagtgggg aagtgggtga  
tgccttgggg  
1381 aggcaaccag cagtgtacg gacttttagt caaaggctga gcagaggctt  
taacgatgcc  
1441 attagtgggt tcaatgatga tgggtggtct ataatgggtg gagacggtg  
tgaagatga  
1501 gttattgctt gcaactcaac taagaaagtt aggagtagca gcaatgcngn  
catcgcttt  
1561 ggagcccccg gaggtattat a

RiceREV1 Protein (SEQ ID NO:171):

maaavamlgsssdggydkvsgmdsgkyvrytpeqvealervyadcpkptssrrqllrecpilanie  
pkqikvwfqnrccrdkqrkessrlqavnrlktamnkllmeenerlqkqvsqlvhenahrqqllqntpl  
andtscesnvttpnplrdasnpsgllsiaetlteflskatgtaidwvqmpgmkpdpdsvgivaish  
gcpwcccracglvnleptkvveilkdrpswfrdcnlevftmipagnggtvelvytglyapttlvpar  
dfwtlrytttmedgslvvcerslsgsgggpsaasaqyvraemlpsgylvrpcegggsivhivdhldl  
eawsvpevlrplyessrvvaqkmttaalrhirqiaqetsgevvyalgrqpavlrtfsqrllsrgfndai  
sgfnddgwsimggdgvedvviacnstkkvrsssnaxiaf

RiceREV2 cDNA (SEQ ID NO:172):

gactgcccc atcctcgcca acatcgagcc caagcagatc aaggtctggt tccagaacag  
aaggtgccga gataagcagc ggaaggaggc atcaaggctt caggccgnga accgaaaatt  
gacggcgatg aataagcttn tcatggagga gaatgagcgt cttcagaagc aggnctccca  
gctggtccat gagaacgcgt acatgaagca gcaacttcag aatccgncat tgggcaatga

tacaagctgn gaatcaaatg tgaccactcc tcagaaccct ctgagagatg caagtaacce  
gtctggactc cttacaattg cggaggagac cctgacagag ttcctctcca aggctacagg  
gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggtat  
tgtggccggt tcacatgggt gccgtgggtg tgctgcccgt gcctgtgggt tggatgaatc  
agaaccaaca aagatcgtgg agatcttaaa agaccgcca tcttggttcc gtgattgtcg  
aagtcttgaa gtcttcacaa tgtttccagc tggaaatggt ggcacgatcg aacttgttta  
catgcagatg tatgctccta ctactttggt tcctgcacga gatttttgga cacttagata  
cacaactaca atggaggatg gcagccttgt ggtctgtgag agatcattga gtggttctgg  
agggtggTcca agtacagcct ccgcacagca atttgtaaga gctgagatgc ttcctagcgg  
ctatctagtg cgcccatgag aggggtgggg ctccatcgtg catattgtgg accatctgga  
tcttgaggct tggagtgttc cagaagtgtc tcggccactc tacgagtcac ctagggtagt  
tgctcagaaa atgactactg cagcngtgcg gcacatcaga caaattgctc aagagacaag  
cggggagggt gtatacgctt tggggaggca acctgctgtt ttgctggacat ttagtcagag  
gttgagtaga ggcttcaatg atgctataag tggtttcaaT gatgatggtt ggtctgtcat  
gggtggggat ggcattgaag atgtgatcat tgcttgcaat gcaaagaa

RiceREV2 protein (SEQ ID NO:173):

DCPILANIEPKQIKVWFQNRRCRDQQRKEASRLQAXNRKLTAMNKLXMEENERLQKQXSQ  
LVHENAYMKQLQNPXLGNDTSXESNVTTQPONPLRDASNPSGLLTIAEETLTFELSKATG  
TAVDWVPMGMPGPGPDSFGIVAVSHGCRGVAARACGLVNLEPTKIVEILKDRPSWFRDCR  
SLEVFMTFPAGNGGTIELVYMQMYAPTTLVPARDFWTLRYTTTMDGSLVVCERSLSGSG  
GGPSTASAQQFVRAEMLPSGYLVRPCEGGGSIVHIVDHLDEAWSVPEVLRPLYESSRVV  
AQKMTTAXVRHIRQIAQETSGEVVYALGRQPAVLRTFSQRLSRGFNDASISGFNDGWSVM  
GGDGIEDVIIACNAK

CHIMERIC PROTEIN (SEQ ID NO:174):

(RICEREV1/AT REV)

maaavamlgsssdgggydkvsgmdsgkyvrytpeqvealervyadcpkptssrrqllrecepilanie  
pkqikvwfqnrccrdkqrkessrlqavnrltamnkllmeenerlqkqvslvhenahmrqqlqntpl  
andtscesnvttqpnlrdasnpsgllsiaeetlteflskatgtaidwvmpgmkgpdsvgivaish  
gcpwcccracglvnleptkvveilkdrpswfrdcnlevftmipagnggtvelvytqlyapttlvpar  
dfwtlrytttmedgslvvcerslsrgsgggpsaasaqqyvraemlpsgylvrpcegggsivhivdhl

eawsvpevlrplyessrvvaqkmttaalrhirqiaqetsgevvyalgrqpavlrtfsqrslsrgfndai  
 sgfnddgwsimggdgvedv/VAINSTKHLNNISNSLSFLGGVLCAKASMLLQNVPPAVLIRFLREHRS  
 EWADFNVDAYSAAATLKAGSFAYPGMRPTRFTGSQIIMPLGHTIEHEEMLEVVRLEGHSLAQEDAFMSR  
 DVHLLQICTGIDENAVGACSELIFAPINEMFPDDAPLVP SGFRVIPVDAKTGDVQDLLTANHRTLDTL  
 SSLEVGPSPENASGNSFSSSSSRCILTIAFQFPFENNLOENVAGMACQYVRSVISSVQRVAMAI SPG  
 ISPSLGSKLSPGSPEAVTLAQWISQSYSHHLGSELLTIDSLGSDSVLKLLWDHQDAILCCSLKPQPV  
 FMFANQAGLDMLETTLVALQDITLEKIFDESGRKAICSDFAKLMQGGFACLPSGICVSTMGRHVSSEQ  
 AVAWKVFAASEENNNLHCLAFS FVNWSFV

REVcentral-1 (SEQ ID NO:175): GGAGCCTGAAGTTTTCACTATG

REVcentral-2 (SEQ ID NO:176): AGGCTGCCTTCCTAATCCAT

REV3'-1 (SEQ ID NO:177): TGAGGAGCGTGATCTCATCAG

REV 3'-2 (SEQ ID NO:178): CAAAATTATCACATCATTCCCTTT

FIL-1 (SEQ ID NO:179): CGTCTATGTCCTCCCCTTCC

FIL-2 (SEQ ID NO:180): AACGTTAGCAGCTGCAGGA

H4-1 (SEQ ID NO:181): TGGAAAGGGAGGAAAAGGTT

H4-2 (SEQ ID NO:182): GCCGAATCCGTAAAGAGTCC

(SEQ ID NO:183): AACGAGAGCATTGGTTCAAG

(SEQ ID NO:184): CAACGAAAGATATGAGAGAG

Tomato REV Exon 1 (SEQ ID NO:185):

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCAATTTCTGTGCGAATATCGAGCCTAAGCAGAT  
 CAAAGTTTGGTTTCAGAACAGAAG

Tomato REV Exon 2 (SEQ ID NO:186):

GTGTCGAGAGAAGCAAAGGAAAGAGTCTTCTCGATTGCAGACTGTGAACAGAAAGTTGTCTGCGATGA  
ATAAACTGTTGATGGAGGAGAATGACCGCTTGCAGAAACAAGTCTCGCAGCTTGTATGTGAAAATGGC  
TATATGCGGCAACAATTGCAAAAT

Tomato REV Exon 3 (SEQ ID NO:187):

GTATCGGCGGCCACTACTGATGTAAGTTGTGAATCAGGGTAACCACTCCTCAGCATTCCCTTAGAGA  
TGCTAACAACCCTGCTGG

Tomato REV Exon 4 (SEQ ID NO:188):

ACTACTACCAATTGCAGAAGAAACCTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTGCGATT  
GGGTCCCAGATGCCTGGGATGAAG

Tomato REV Exon 5 (SEQ ID NO:189):

CCTGGTCCGGATTGAGTTGGGATTTTTGCCATCTCACACAGTTGCAGTGGAGTGGCAGCCCCGAGCATG  
TGGTCTTGTTAGTTTAGAGCCAACAAAG

Tomato REV Exon 6 (SEQ ID NO:190):

ATTGCTGATATCTCAAAGATCGACCTTCTGGTTCCGCGACTGCCGGAATGTTGAAAGTTATCACAAT  
GTTTCTGCTGGAAATGGTGGTACAGTTGAGCTTTTGTATACCCAG

Tomato REV Exon 7 (SEQ ID NO:191):

ATATATGCTCCCAACTCTGGCTCCCGCGGTGATTTTTGGACGCTGAGATACACAACAACCCTAGA  
CAATGGTAGTCTCGTG

Tomato REV Exon 8 (SEQ ID NO:192):

GTTTGTGAAAGATCCCTATCTGGTAATGGGCCTGGCCCAAATCCTACTGCTGCTTCCCAGTTTGTAAAG  
AGCTCAAATGCTTCCATCTGGATATCTGATCCGACCGTGTGATGGTGGAGGATCAATCATACATATTG  
TTGATCACCTGAATCTTGAG

Tomato REV Exon 9 (SEQ ID NO:193):

GCATGGAGTGCCCCTGAGATTTTGGCTCCACTCTATGAATCGTCGAAAGTTGTGGCACAGAAAATGAC  
TATTGCA

Tomato REV Exon 10 (SEQ ID NO:194):

GCACTGCCGATATGCAAGGCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGGCA  
ACCTGCTGTTCCCTCGAACATTCAGCCAGAGATTATGCAG

Tomato REV Exon 11 (SEQ ID NO:195):

AGGGTTCAATGATGCCATCAATGGATTCCGGTGACGATGGCTGGTCAATGTTAAGTTCAGATGGTGCTG  
AAGATGTCATAGTTGCTGTCAATTCAGGAAGAACCCTCGCAACCACCTCCATTCCTCTTTCCCCGCTT  
GGTGGCGTCCTTTGTACCAAAGCATCAATGCTACTCCAG

Tomato REV Exon 12 (SEQ ID NO:196):

CAGAATGTCCCCCTGCCGTA CTGGTTCGGTTTCGAGGGAGCACCGTTCAGAA

**The claims defining the invention are as follows:**

1. An isolated DNA molecule consisting of a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 70% identical to SEQ ID NO: 2, wherein said protein has a *REV* biological activity, and wherein said DNA molecule can optionally contain intervening sequences that do not encode said protein.

2. The DNA molecule according to claim 1 wherein the encoded protein confers a *REV* phenotype.

3. The DNA sequence according to claim 1 wherein the encoded protein comprises an amino acid sequence is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO: 2.

4. The DNA sequence according to claim 1 wherein the encoded protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 171 and SEQ ID NO: 173.

5. An isolated DNA sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 169, SEQ ID NO: 170 and SEQ ID NO: 172.

6. An isolated DNA molecule consisting of a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 80% identical to SEQ ID NO: 2, wherein said protein has a *REV* biological activity, and wherein said DNA molecule can optionally contain intervening sequences that do not encode said protein.

7. An isolated DNA molecule comprising a polynucleotide sequence at least about 80% identical to a polynucleotide selected from the group consisting of nucleotides 3670-3743, 3822-3912, 4004-4099, 4187-4300, 4383-4466, 4542-4697, 4786-4860, 4942-5048, 5132-5306, 5394-5582, 5668-5748, 5834-5968, 6051-6388, 6477-6585, 6663-6812, and 6890-7045 of SEQ ID NO: 1.

8. An isolated DNA molecule comprising a polynucleotide sequence at least about 80% identical to a polynucleotide selected from the group consisting of SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, and SEQ ID NO: 196.

9. An isolated DNA molecule comprising a polynucleotide sequence which encodes a protein comprised of an amino acid sequence at least about 95% identical to a

member selected from the group consisting of SEQ ID NO: 130; SEQ ID NO: 131; SEQ ID NO: 132; SEQ ID NO: 133; SEQ ID NO: 134; SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137.

10 5 10. An isolated protein comprising an amino sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 and SEQ ID NO: 12.

11. An isolated protein comprising an amino sequence that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has a biological activity that is the same as SEQ ID NO: 2.

10 12. A vector comprising a replicon and a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11.

15 13. A vector comprising a replicon and a polynucleotide comprising a sequence that encodes a protein that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has the biological activity of SEQ ID NO: 2.

14. A vector according to claim 12 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO: 2, and wherein the said protein has the biological activity of SEQ ID NO: 2.

20 15. A vector according to claim 13 further comprising a promoter region that is heterologous to the polynucleotide and is operable in a plant cell.

16. A vector according to claim 13 wherein the promoter region is operably linked to the polynucleotide.

17. A vector according to claim 15 wherein the promoter region is transcriptionally active in a tissue and/or organ specific fashion.

25 18. A transformed plant cell comprising a transgene encoding a *REVOLUTA* protein.

19. The transformed plant cell of claim 18 wherein said transgene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11.

30 20. The transformed plant cell of claim 18 wherein said transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO: 2, wherein said protein has a biological activity of SEQ ID NO: 2.

21. The transformed plant cell of claim 20 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO: 2.

22. A transgenic plant comprising a *REVOLUTA* transgene, said transgenic plant comprising cells having modulated cell division as compared to an untransformed plant, wherein said *REVOLUTA* transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO: 2, and wherein said protein has the biological activity of SEQ ID NO: 2.

23. The transgenic plant of claim 22 wherein said transgene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11.

24. The transgenic plant of claim 22 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO: 2, and wherein said protein has the biological activity of SEQ ID NO: 2.

25. A method for modulating plant cell division comprising introducing into a plant cell a *REVOLUTA* transgene, wherein said transgene comprises:

(1) a polynucleotide from a *REVOLUTA* gene, wherein said *REVOLUTA* gene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO: 2 and has the biological activity of SEQ ID NO: 2;

(2) an inverted repeat of a polynucleotide sequence from said *REVOLUTA* gene;

(3) an anti-sense orientation of a polynucleotide from said *REVOLUTA* gene; or

(4) a ribozyme comprising a polynucleotide sequence from said *REVOLUTA* gene.

26. The method of claim 25 wherein said transgene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11.

27. The method of claim 25 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID No. 2, and wherein said protein has the biological activity of SEQ ID NO.: 2.

28. The method of claim 25, wherein the transgene comprises an inverted repeat of a polynucleotide sequence from a *REVOLUTA* gene.

29. The method of claim 25, wherein the transgene comprises an anti-sense orientation of a polynucleotide sequence from a *REVOLUTA* gene.

30. The method of claim 25, wherein the transgene comprises a sense orientation of a polynucleotide sequence from a *REVOLUTA* gene.

31. The method of claim 25, wherein the transgene is a ribozyme comprising a polynucleotide sequence from a *REVOLUTA* gene.

5 32. The method of claim 25 wherein the *REVOLUTA* transgene is expressed in the plant cell and said plant cell exhibits modulated cell division as compared to an untransformed plant cell.

33. A method of isolating a *REVOLUTA* gene comprising:

10 (a) amplifying a plant polynucleotide sequence using a forward and a reverse oligonucleotide primer, said primers selected from a polynucleotide sequence, or its complementary sequence, that encodes a protein that is at least about 70% identical to SEQ ID NO: 2 and which protein has the biological activity of SEQ ID NO: 2;

(b) hybridizing said amplified plant polynucleotide to a library of recombinant plant DNA clones;

15 (c) isolating a DNA molecule from a recombinant DNA clone that hybridizes to said amplified plant polynucleotide;

(d) transforming with a vector comprising said amplified plant polynucleotide or said DNA molecule into a plant cell; and

20 (e) determining that cell division in the transformed plant is modulated by comparing the transformed plant cell with an untransformed plant cell.

34. The method of claim 33, wherein the isolated DNA encodes an amino acid sequence that is at least about 70% identical to an amino acid sequence in SEQ ID NO: 2, and wherein said amino acid sequence has the biological activity of SEQ ID NO: 2.

25 35. The method of claim 33, wherein the isolated DNA is expressed in said transformed plant cell as a transgene selected from the group consisting of a sense gene, an antisense gene, an inverted repeat gene and a ribozyme gene.

36. The method of claim 33, wherein the isolated DNA is expressed by operably linking said isolated DNA adjacent to a heterologous promoter sequence.

30 37. The method of claim 33, wherein the transformed plant has at least one plant organ with a greater number of cells than a corresponding untransformed plant organ.

38. The method of claim 33, wherein the transformed plant has at least one plant organ with a smaller number of cells than a corresponding untransformed plant organ.

39. A plant comprising a chimeric plant gene having:

(i) a promoter sequence which functions in plant cells;  
(ii) a coding sequence which causes the production of RNA encoding a fusion polypeptide which modulates plant growth; and wherein said fusion polypeptide comprises the protein of claim 10 or claim 11.

5 (iii) a 3' non-translated region which encodes a polyadenylation signal which functions in plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, where the promoter is heterologous with respect to the coding sequence and adapted to cause sufficient expression of the fusion polypeptide to modulate plant growth of a plant transformed with said gene.

10 40. An isolated polynucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 103 SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, and SEQ ID NO: 125.

41. A vector comprising the polynucleotide of claim 40.

15 42. A plant transformed with the vector of claim 41.

43. A transgenic seed of, or obtained from, the transgenic plant of any one of claims 22, 23, 24, 39 or 42, wherein the seed comprises:

(a) the DNA molecule according to any one of claims 1 to 9 and 40;

(b) the protein according to either one of claims 10 or 11;

20 (c) the vector according to any one of claims 12 to 17 and 41;

(d) the transformed plant cell according to any one of claims 18 to 21; or

(e) the transgene according to any one of claims 25 to 32.

25 44. The DNA molecule, protein, vector, transformed plant cell, transgenic plant and methods substantially as hereinbefore described with reference to any one of the examples.

45. An isolated nucleotide sequence comprising a heterologous promoter and a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 70% identical to SEQ ID NO:2, wherein said protein has a REV biological activity.

30 **Dated 19 July, 2005**

**University of Washington**

**Patent Attorneys for the Applicant/Nominated Person**

**SPRUSON & FERGUSON**

FIG. 1

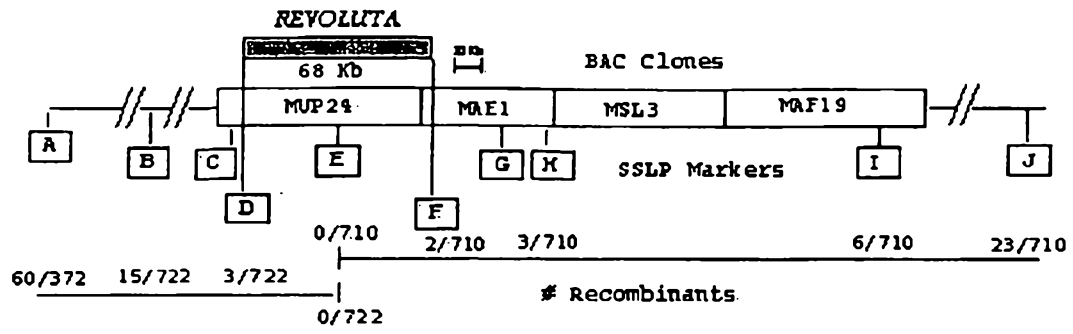
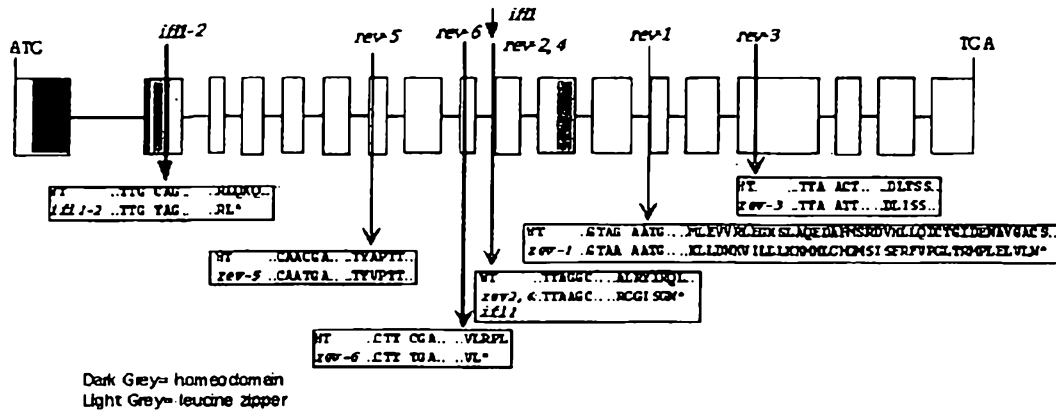


FIG. 2



# FIG. 3

## REVOLUTA Protein and rev mutants

MEMAVANHRBSSDSMNRHL DSSGKYVRYT AEQVEALERV **Homeodomain** YAECPKPSSL PPOQLRECS

**Homeodomain** ELANLEPKQI **L-zipper** KWFONRRQR DKQRKEASR QSVNRKLSAM NKLMEENDR LDKQVSQLV

ENGYMKQQLT TVVNDPSCES VVTTPOHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW

VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF

*rev-5*  
TMFPAGNGGT IELVYMOTYA PTTLAPARDF WTLRYTTS LD NGSEFVCERS LSGSGAGPNA

*rev-6*  
ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT

*rev-2,4*  
ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA  
RCGISGN\*

EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRELREH RSEWADFNDV

*rev-1*  
AYSAATLKAG SFAYPGMRPT RFTGSQIIMP LGHTIEHEEM LEVVRLEGH'S LAQEDAFMSR

DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA

*rev-3*  
NHRTLDLSS LEVGPSPENA SGNSFSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS

VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSYSHHLGSE LLTIDSLGSD

DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI

CSDFAKLMQQ GFACLPSGIC VSTMGRHVS Y EQAVAWKVFA ASEENNNNLH CLAFSEVNWSFV

FIG. 4

FIG. 4 displays a series of 16 groups of data, each consisting of a reference sequence (PVP 19.21) and four variants (Babb-1, Babb-2, Babb-14, and sup 21.4). Each group is represented by a grid of characters, likely nucleotide bases (A, C, G, T) and dashes, indicating sequence alignment or mutations. The groups are numbered 1 through 16 on the left side of the page.

1 PVP 19.21 1 -----ND-----CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 1 -----CCBMM BMM-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 1 -----BMM BMM-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 1 -----BMM BMM-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 1 -----BMM BMM-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

2 PVP 19.21 2P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 2P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 2P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 2P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 2P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

3 PVP 19.21 3P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 3P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 3P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 3P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 3P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

4 PVP 19.21 4P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 4P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 4P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 4P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 4P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

5 PVP 19.21 5P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 5P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 5P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 5P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 5P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

6 PVP 19.21 6P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 6P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 6P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 6P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 6P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

7 PVP 19.21 7P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 7P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 7P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 7P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 7P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

8 PVP 19.21 8P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 8P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 8P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 8P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 8P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

9 PVP 19.21 9P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 9P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 9P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 9P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 9P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

10 PVP 19.21 10P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 10P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 10P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 10P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 10P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

11 PVP 19.21 11P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 11P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 11P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 11P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 11P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

12 PVP 19.21 12P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 12P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 12P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 12P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 12P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

13 PVP 19.21 13P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 13P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 13P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 13P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 13P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

14 PVP 19.21 14P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 14P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 14P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 14P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 14P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

15 PVP 19.21 15P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 15P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 15P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 15P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 15P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

16 PVP 19.21 16P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-1 16P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-2 16P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
Babb-14 16P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV  
sup 21.4 16P CCTA LCC-----CAGVYV EAVGCLVYV-----CAGVYV EAVGCLVYV

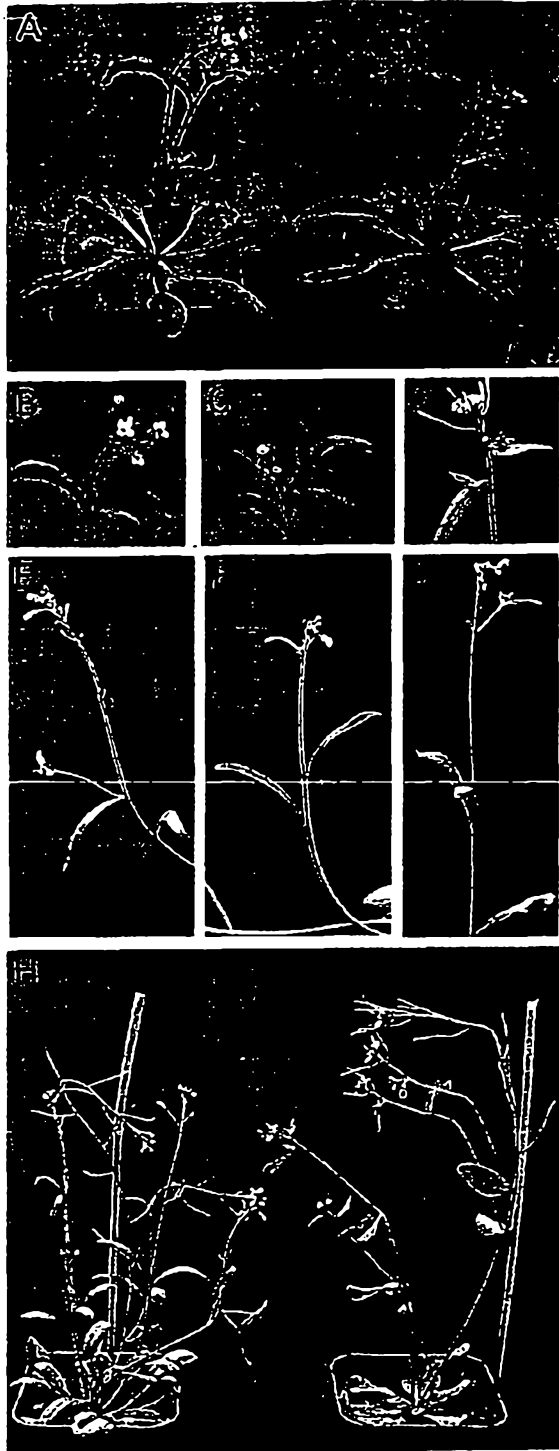


FIGURE 5

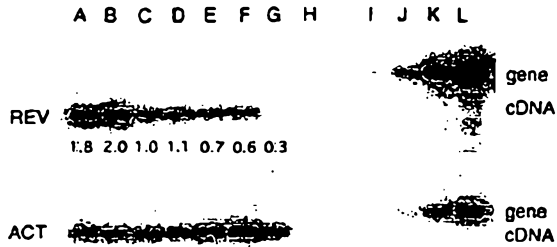


FIGURE 6

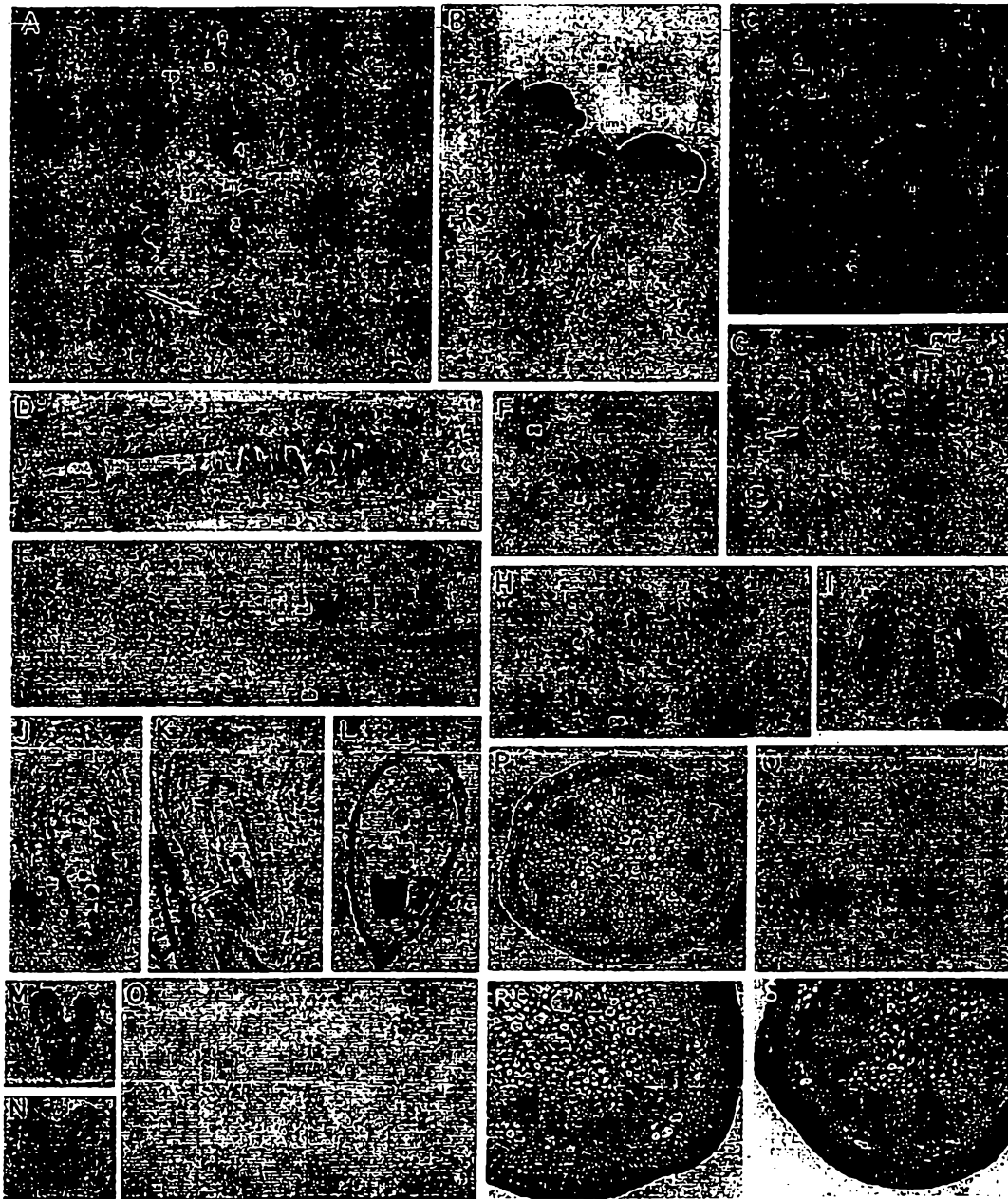


FIGURE 7

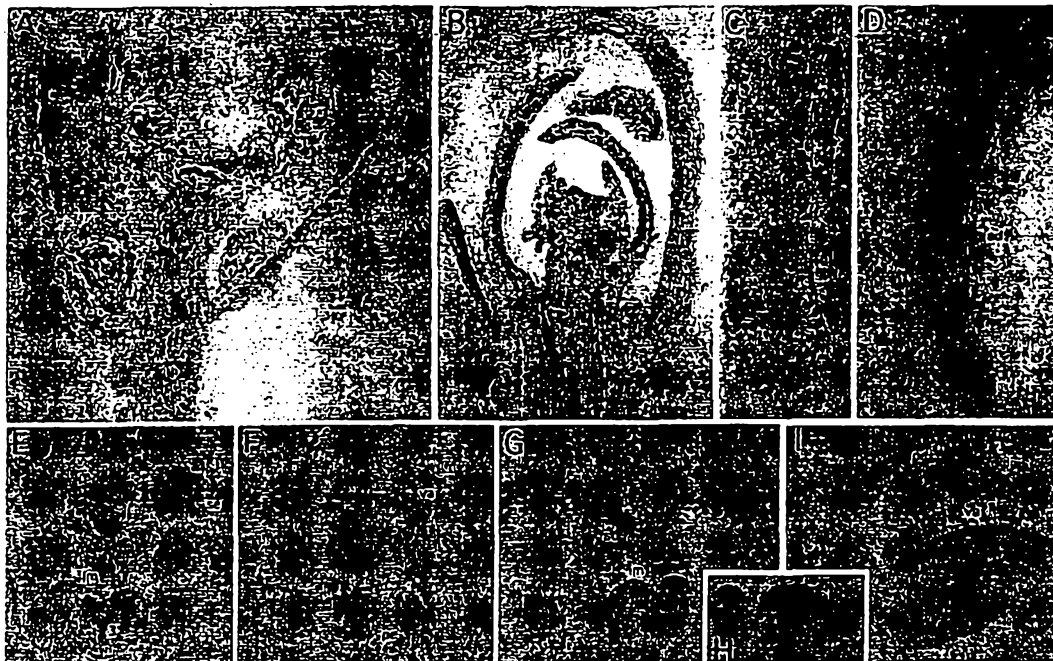


FIGURE 8

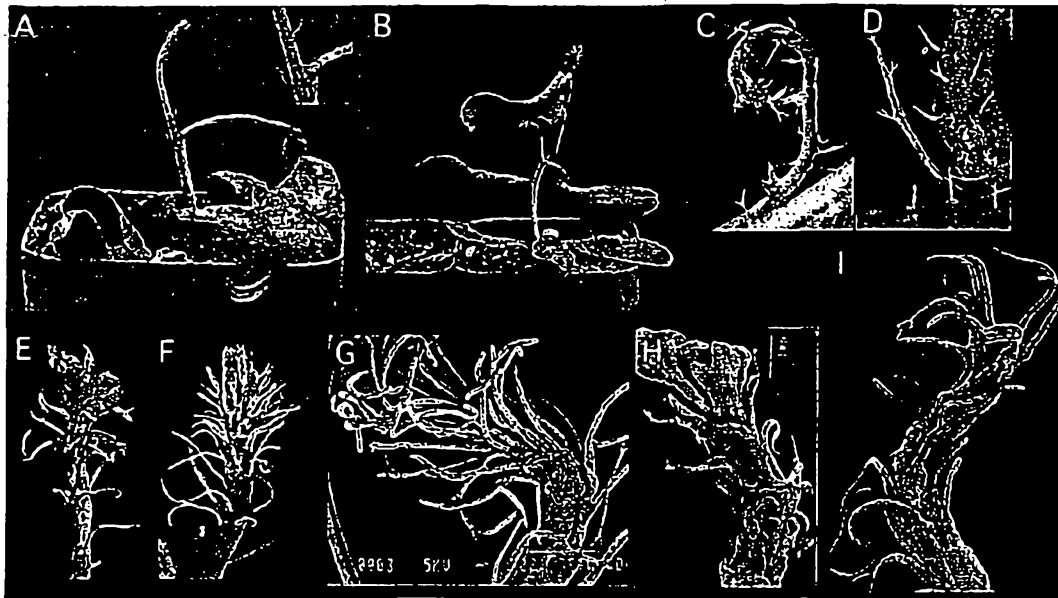


FIGURE 9



FIGURE 10

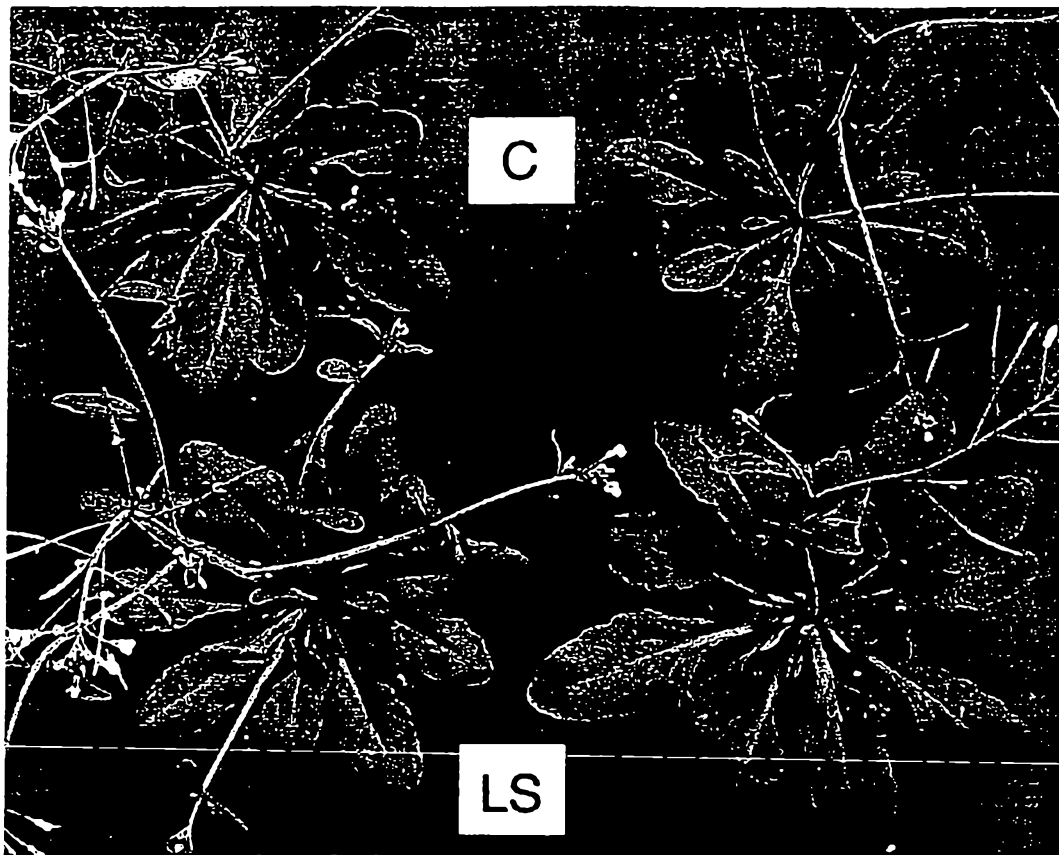


FIGURE 11

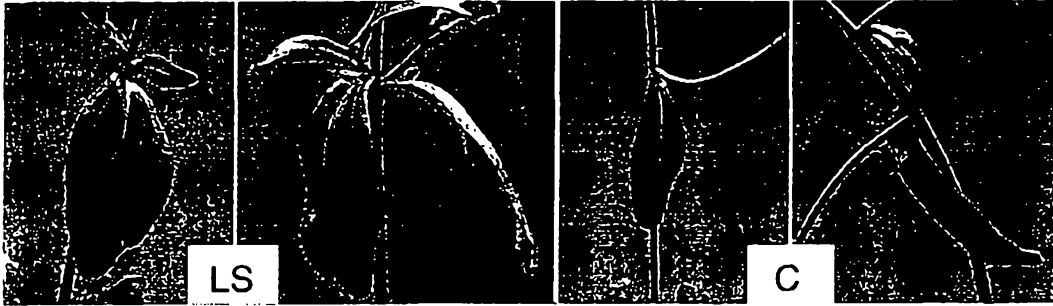


FIGURE 12