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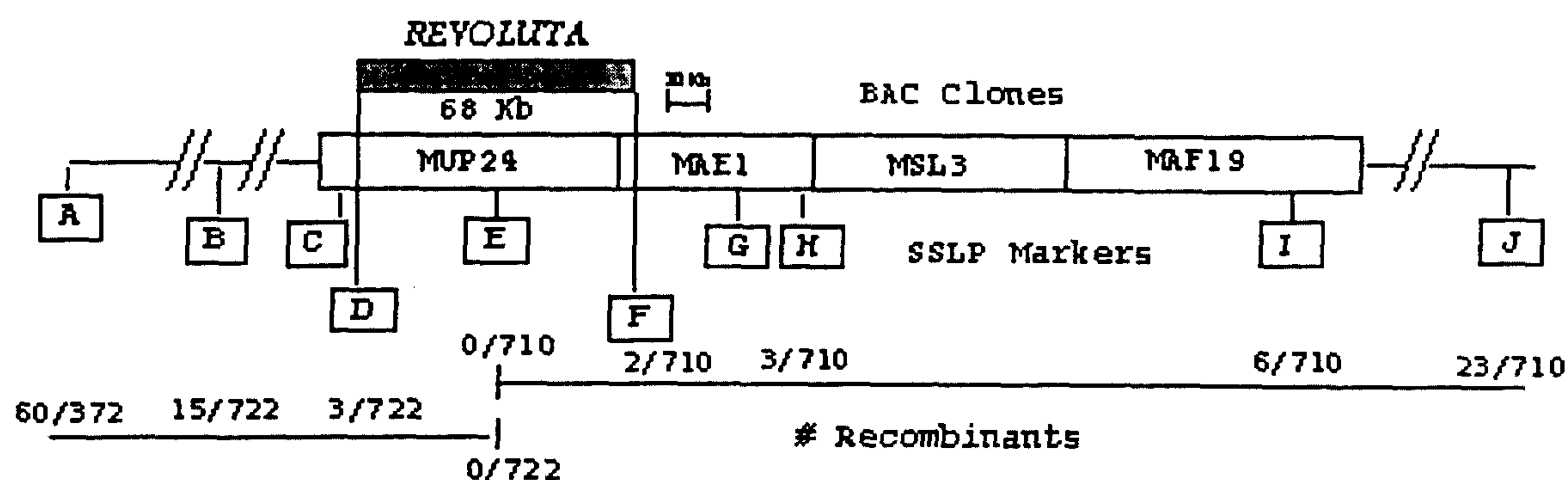
(71) Demandeur/Applicant:
THE UNIVERSITY OF WASHINGTON, US

(72) Inventeurs/Inventors:
SLADE, ANN, US;
MADISEN, LINDA, US;
COMAI, LUCA, US

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : COMPOSITIONS ET PROCEDES DE MODULATION DE LA DIVISION CELLULAIRE D'UNE PLANTE

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



(57) Abrégé/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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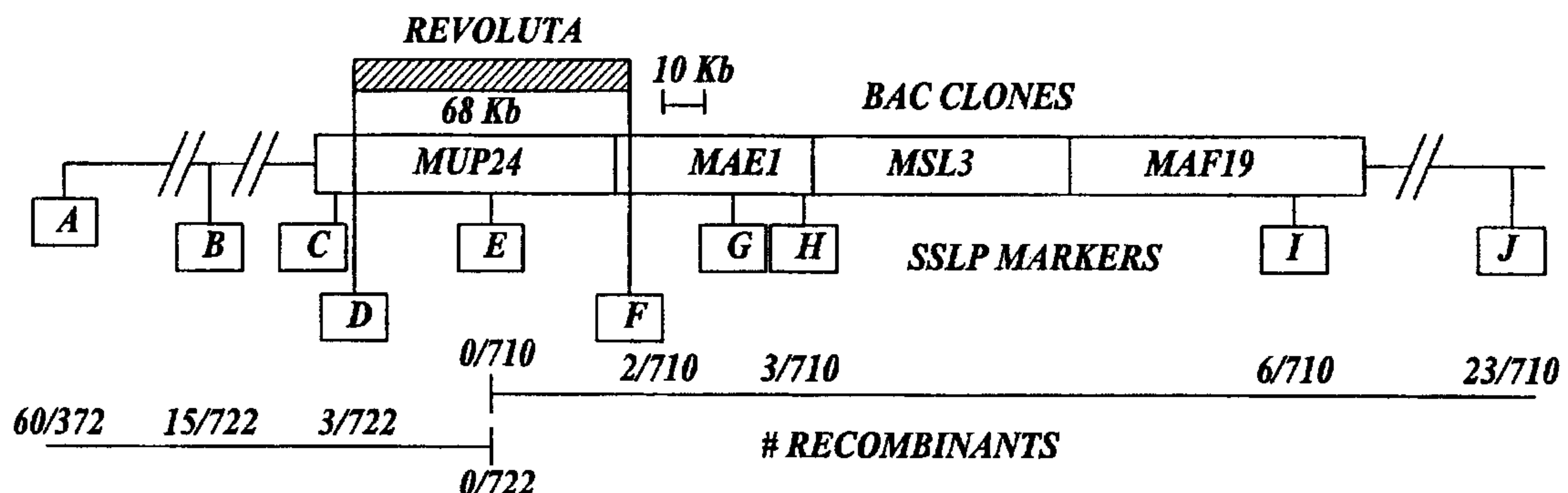
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(71) Applicants and

(72) Inventors: **SLADE, Ann** [US/US]; B104, 9527 Interlake Avenue North, Seattle, WA 98103 (US). **MADISEN, Linda** [US/US]; 6515 Cotton Wood Circle, #H, Dublin, CA 94568 (US). **COMAI, Luca** [US/US]; 2650 NE 184th Place, Seattle, WA 98155 (US).(48) Date of publication of this corrected version:
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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

The present invention relates generally to compositions and methods for
5 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

Elaboration of the plant body pattern depends primarily on the proper
10 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

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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.

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 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 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 putative key controller genes that determine whether a plant cell will grow and divide verses 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.

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 paraclade meristems, floral meristems and the primary shoot apical meristem. Simultaneously, the *REV* gene has an opposing effect on the

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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).

A large number of plant HD-containing genes have been isolated using
35 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

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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

HD-Zip Subfamily and Gene	Expression Pattern	Proposed Function	Reference
<u>Subfamily I</u>			
Athb-1	late plant development	activation of genes related to 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, induced by abscisic acid and water deficit	signal transduction pathway in response to water deficit	Soderman et al., 1994, 1996
CHB1	early embryogenesis	maintenance of indeterminant 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 tissue	?	Kawahara et al., 1995
Hahb-1	stem	?	Chan et al., 1994
VAHOX-1	phloem of adult plants	differentiation of cambium cells to phloem tissue	Tornero et al., 1996

Subfamily II

Athb-2 (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; Carabelli et al, 1993; 1996; Steindler et al., 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

SubfamilyIII

Athb-8	procambial cells of the embryo, induced by auxins	regulation of vascular development	Baima et al., 1995; Altamura et al., 1998; 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 (Gl-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.

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.

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

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.

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

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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.

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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*, *rev-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

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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.

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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 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 chimeric plant gene to modulate plant growth of a plant transformed with the chimeric gene.

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

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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 (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 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 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 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 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 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 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 rev-1 mutant plant with small filamentous appendage. Panel (E) shows the

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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 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- α -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

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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.

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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.; Atlschul 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

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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.

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 Na₂HPO₄ buffer (pH 7.2) containing 1 mM Na₂EDTA, 20% sodium dodecyl sulfate; washing (three washes of twenty minutes each at 65°C) is conducted in 20 mM Na₂HPO₄ buffer (pH 7.2) containing 1 mM Na₂EDTA, 1% (w/v) sodium dodecyl sulfate.

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 Na₂HPO₄ buffer (pH 7.2) containing 1 mM Na₂EDTA, 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 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 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 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) 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 target endogenous gene. The introduced polynucleotide sequence will typically be at least substantially identical (as determined below) to the target endogenous sequence.

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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.

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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 α -carboxy or α -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

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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.

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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.

10 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
15 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
20 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.
25 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
30 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
35 the subsequent linearization of unmutated plasmids results in high mutation

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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 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 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 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 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 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, 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 determine if activity is greatly impaired as a consequence. The properties of the

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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

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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 tumefaciens* 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

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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.

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.

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 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 (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; 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

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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 Number 5,712,135, issued January 27, 1998 "Process for transforming monocotyledonous plants." These preferred marker DNAs encode second proteins or 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 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 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 DNA, for production of single-stranded DNA templates used for site-directed

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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.

The promoters most commonly used in prokaryotic vectors include the
25 β -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).

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

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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

5 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

10 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), α -factor, alkaline phosphatase and invertase may be used to direct secretion from yeast host cells.

15 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

20 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*).

25 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.

30 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.

35 Provided below are examples of a number of different ways in which a *REVOLUTA*

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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

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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
5 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
10 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
15 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
20 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
25 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
30 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
35 virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum

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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

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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 I, 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

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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 .

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

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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.

Examples also include promoters of seed storage proteins which express these
5 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 β -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 β -conglycinin (Beachy et al., 1985 *EMBO J.* **4**:3047-3053; pea vicilin (Higgins et al., 1988), pea convicilin (Newbigin 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 β -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 β -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 β -conglycinin (Harada et al., 1989 *Plant Cell* **1**:415-425); promoters of genes for α - and β -subunits of soybean β -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), β -ketoacyl-ACP synthetase

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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

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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
5 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
10 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
15 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
20 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

25 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,
30 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

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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 *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 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). 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 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 µl 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 µl PEB was added and the material ground until no leaf chunks remain (15 seconds.) After heating at 65°C for 1 hour, 260 µl saturated NaCl was added. The tubes were microfuged for 20 minutes at top speed. The supernatant was transferred to a new tube containing 850 µl 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 µl TE buffer, then 133 µl LiCl 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 µl 10 mM Tris (pH 8.0). For PCR, either 1 µl or 1 µl of a 1:10 dilution of this DNA was used.

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Genomic DNA was amplified in a PCR reaction using either the nga 129 primers or the MBK5 primers (Table 3) in 1X Buffer, 2mM MgCl₂, 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 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 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 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.fhcrc.org/~jorja/blastnew.html>. The *Arabidopsis* database was searched 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). 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 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 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 (SEQ ID NO.)	Primer Sequence
nga129F (SEQ ID NO.:13)	5' TCAGGAGGAACTAAAGTGAGGG 3'
nga129R (SEQ ID NO.:14)	5' CACACTGAAGATGGTCTTGAGG 3'
MBK5-1 (SEQ ID NO.:15)	5' ATCACTGTTGTTTACCATTA 3'
MBK5-2 (SEQ ID NO.:16)	5' GAGCATTTACAGAGACG 3'
K21L19L (SEQ ID NO.:17)	5' CTCCTCCTTTCCAGACACA 3'
K21L19R (SEQ ID NO.:18)	5' TTCCACCAATTCCTCACCA 3'
MUP24-1 (SEQ ID NO.:19)	5' CGTAAAACGTCGTCGTTTCATT 3'
MUP24-2 (SEQ ID NO.:20)	5' ATCGCTGGATTGTTTTGGAC 3'
MAF19L (SEQ ID NO.:21)	5' TTCTAAGAATGTTTTTACCACCAAAA 3'
MAF19R (SEQ ID NO.:22)	5' CCAACTGCGACTGCCAGATA 3'
MUP24-3 (SEQ ID NO.:23)	5' TCCGATTGGTCTAAAGTACGA 3'
MUP24-4 (SEQ ID NO.:24)	5' TGACCAAGGCCAAACATACT 3'
MUP24-13 (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' CACTCGTAGCATCCATGTCG 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 nga129 F/R/rev-1 recombinant chromosomes was screened using K21L19L and K21L19R primers (K21L19 L/R)(Table 3), 6 of the original 60 nga129 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

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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 (SEQ ID NO.)	Primer Sequence
HDAL (SEQ ID NO:33)	5' AAAATGGAGATGGCGGTGGCTAAC 3'
HDAR (SEQ ID NO:34)	5' TGTCAATCGAATCACACAAAAGACCA 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 (SEQ ID NO.)	Primer Sequence
Rev-1 (SEQ ID NO:35)	5' CAG ACT TTG ATC TGC TTA GGA TC 3'
Rev-2 (SEQ ID NO:36)	5' TGA GCC TAA GCA GAT CAA AGT C 3'
Rev-3 (SEQ ID NO:37)	5' ACC GGA AGC TCT CTG CGA TG 3'
Rev-4 (SEQ ID NO:38)	5' TCG CAG AGG AGA CTT TGG CAG 3'
Rev-5 (SEQ ID NO:39)	5' GGA GCC TTG AAG TTT TCA CTA TG 3'
Rev-6 (SEQ ID NO:40)	5' GGT ATT TAA TAA GGC CTT GTG ATG 3'
Rev-7 (SEQ ID NO:41)	5' AGA ACC TTT AGC CAA AGA TTA AGC 3'
Rev-8 (SEQ ID NO:42)	5' AGC ATC GAT CTG AGT GGG CTG 3'
Rev-9 (SEQ ID NO:43)	5' GTA CCG GGA TTG ACG AGA ATG 3'
Rev-10 (SEQ ID NO:44)	5' TGA GGA GCG TGA TCT CAT CAG 3'
Rev-11 (SEQ ID NO:45)	5' GCC AGT GTT CAT GTT TGC GAA C 3'
Rev-12 (SEQ ID NO:46)	5' ATG GCG GTG GCT AAC CAC CGT GAG 3'

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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> 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

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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].

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

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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' TTGCGGCCGCTTCGATTGACAGAAAAAGACTAATTT 3' [SEQ ID NO:51]; 3' primer includes ApaI and KpnI sites:
20 5' TTGGGCCCCGGTACCCTCAACCAACCACATGGAC 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 mosaic viral promoter (35S CaMV) was amplified from the pHomer 102 plasmid by
30 PCR using primers 5' AAGGTACCAAGTTCGACGGAGAAGGTGA 3' [SEQ ID No.:53] and 5' AAGGATCCTGTAGAGAGAGACTGGTGATTTCAG 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

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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 *Revoluta* 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 *Revoluta* 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

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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 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. 10 Alternatively, *Revoluta* gene expression is measured by performing reverse transcriptase reactions on isolated mRNA samples and then 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. 15

Partial Complementation of the rev-1 mutant

We confirmed that the HD-Zip protein encoded by *MUP24.4* was the *REV* 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 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 results supported the conclusion that the HD-Zip coding region was the *REV* gene, 25 30 35

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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/. 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 *REV*central-1
GGAGCCTTGAAGTTTTCACTATG [SEQ ID NO:175] and *REV*central-2
AGGCTGCCTTCCTAATCCAT [SEQ ID NO:176]; or the primers *REV*3'-1
30 TGAGGAGCGTGATCTCATCAG [SEQ ID NO:177] and *REV* 3'-2
CAAAATTATCACATCATTCCTTT [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

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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 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 (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 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 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) 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 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 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).

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Example 5REV double mutantsFIL is properly expressed in rev mutantsREV 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 *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).

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 *AP1* during vegetative development can result in precocious flower formation (Weigel and Nilsson (1995) Nature 377, 495; Mandel and Yanofsky (1995) Nature 377: 522). *AP1* 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

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(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.

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>	¹ 24 GK YVRYTAEQVEALERVYAEC PKPSSLRRQQLIRECSILANIE
SEQ ID No.:2	
Athb-14	24 GK YVRYTPEQVEALERVYTECPKPSSLRRQQLIRECPILSNIE
SEQ ID No.:55	
Athb-8	14 GK YVRYTPEQVEALERLYNDCKPSSMRQQLIRECPILSNIE
SEQ ID No.:56	
Athb-9	20 GK YVRYTPEQVEALERVYAEC PKPSSLRRQQLIRECPILCNIE
SEQ ID No.:57	
CRHB1	19 GK YVRYTSEQVQALEKLYCECPKPTLLQRQQLIRECSILRNVD
SEQ ID No.:58	

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F5F19.21 16 GKYVRYTPEQVEALERLYHDCPKPSSIRRQQLIRECPILSNIE
SEQ ID No.:59

HD-ZipIII: Block B

(block width = 42)

REVOLUTA 70 IKVWFQNRRCRDKQRKEASRLQSVNRKLSAMNKLLMEENDRL
SEQ ID No.:2

Athb-14 70 IKVWFQNRRCREKQRKEAARLQTVNRKLNAMNKLLMEENDRL
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 VLCAKASMLLQNVPPAVLIRFLREHRSEWAD
SEQ ID No.:2	
<i>Athb-14</i>	464 VLCAKASMLLQNVPPAVLVRFLREHRSEWAD
SEQ ID No.:55	
<i>Athb-8</i>	452 VLCAKASMLLQNVPPSILLRFLREHRQEWAD
SEQ ID No.:56	
<i>Athb-9</i>	460 VLCAKASMLLQNVPPPLVLIRFLREHRAEWAD
SEQ ID No.:57	
<i>CRHB1</i>	138 LMNIYAIVRLQHVPPIPECRS ² XXXXXXXXXXXX
SEQ ID No.:58	
<i>F5F19.21</i>	453 VLCAKASMLLQNVPPAILLRFLREHRSEWAD
SEQ ID No.:59	

¹The 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.

²X 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 ¹
<u>HD-ZipIII: Block A</u>	
Forward	
Rice A1F	5'-GGCGGCAGCAGCTGathmgngartg-3'
SEQ ID No.:60	R Q Q L I R E C
	Degen ² =48, temp ³ =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'-TGCGGTACACCCCCgarcargtnsa-3'
SEQ ID No.:62	R Y T P E Q V E
	Degen=32, temp=63.3
Rice A4F	5'-TGCGGTACACCCCCGarcargtnsarg-3'
SEQ ID No.:63	R Y T P E Q V E A
	Degen=64, temp=63.3
Rice A5F	5'-CGGTACACCCCCGAGcargtnsargc-3'
SEQ ID No.:64	R Y T P E Q V E A
	Degen=32, temp=64.2
Rice A6F	5'-TGGAGAGGGTGTACTGCGantgyccnaa-3'
SEQ ID No.:65	E R V Y C E C P K

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Degen=32, temp=60.4

Rice A7F

SEQ ID No.:66

5' -TGGAGAGGGTGTACTGCGAntgyccnaarc-3'

E R V Y C E C P K P

Degen=64, temp=60.4

Rice A8F

SEQ ID No.:67

5' -CCGACCTCCATGCGGmgncarcaryt-3'

P S S L R R Q Q L

Degen=64 temp=60.8

Rice A9F

SEQ ID No.:68

5' -CCATGCGGCGGcarcarytnat-3'

L R R Q Q L I

Degen=32, temp=62.4

HD-ZipIII: Block A**Reverse**

Rice A1R

SEQ ID No.:69

5' -CGGGGGTGTACCGCacrtayttnc-3'

Degen=16, temp=62.1

⁴Complementary to:

G K Y V R Y T P E

ccnttyatrcaCGCCATGTGGGGGC

Rice A2R

SEQ ID No.:70

5' -CTGCTCGGGGGTGTACcknacrtaytt-3'

Degen=32, temp=60.1

Complementary to:

K Y V R Y T P E Q

ttyatrcankcCATGTGGGGGCTCGTC

Rice A3R

SEQ ID No.:71

5' -ACCTGCTCGGGGGTGTancknacrt-3'

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Degen=64, temp=60.1

Complementary to:

Y V R Y T P E Q V

atrcankcnatGTGGGGGCTCGTCCA

Rice A4R

SEQ ID No.:72

5'-CCACCTGCTCGGGGgtrtancknac-3'

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

ctygtycanstCCGGGACCTCTCCCAC

Rice A6R

SEQ ID No.:74

5'-CAGTACACCCTCTCCAGGGcytsnacytgyt-3'

Degen=64 temp=62.0

Complementary to:

Q V E A L E R V Y C

tygtycanstycGGGACCTCTCCCACATGAC

Rice A7R

SEQ ID No.:75

5'-CAGTACACCCTCTCCAGGgcytsnacytg-3'

Degen=32, temp=62.0

Complementary to:

Q V E A L E R V Y C

gtycanstycgGGACCTCTCCCACATGAC

HD-ZipIII: Block B**Forward**

Rice B1F

SEQ ID No.:76

5'-CCATGAACAAGATGCTGatggargaraa-3'

M N K M L M E E N

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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' -CCGCCATGAACAAGATGCTnatggargara-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 F Q N R R C R

accaargtyttGGCCGCCACGGC

Rice B2R

SEQ ID No.:82

5' -ATCTGGTTCATGGCGGTCaryttncbrtt-3'

Degen=96, temp=60.1

Complementary to:

N R K L T A M N K M

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ttrbcnttyraCTGGCGGTACTTGTCTA

Rice B3R

5'-CGCCGGTTCTGGaaccanacytt-3'

SEQ ID No.:83

degen=8, temp=64.3

Complementary to:

K V W F Q N R R

ttycanaccaaGGTCTTGGCCGC

Rice B4R

5'-GCACCGCCGGTTctgraaccanac-3'

SEQ ID No.:84

degen=8, temp=61.0

Complementary to:

V W F Q N R R C

canaccaargtCTTGGCCGCCACG

HD-ZipIII: Block D**Forward**

Rice D1F

5'-CCAAGGCCACCATGCTGytncarmaygt-3'

SEQ ID No.:85

K A S M L L Q N V

Degen=64, temp=62.3

Rice D2F

5'-AAGGCCACCATGCTGCTncarmaygtnc-3'

SEQ ID No.:86

K A S M L L Q N V P

Degen=128, temp=60.4

Rice D3F

5'-CCACCATGCTGCTGcarmaygtnc-3'

SEQ ID No.:87

S M L L Q N V P

Degen=32, temp=60.1

Rice D4F

5'-CCCGTCTGCATCCGGttytnmgnga-3'

SEQ ID No.:88

A V C I R F L R E

Degen=128, temp=63.1

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Rice D5F

SEQ ID No.:89

5'-CCGTCTGCATCCGGTTCytnmgngarca-3'

V C I R F L R E H

Degen=128, temp=61.2

Rice D6F

SEQ ID No.:90

5'-GTCTGCATCCGGTTCCTGmgngarcaymg-3'

V C I R F L R E H R

Degen=64, temp=60.2

Rice D7F

SEQ ID No.:91

5'-TGCGGGAGCACCGGnvnngartgggc-3'

R E H R S E W A

Degen=96, temp=62.9

Rice D8F

SEQ ID No.:92

5'-GCGGGAGCACCGGTCngartgggcng-3'

R E H R S E W A D

Degen=32, temp=62.6

Rice D9F

SEQ ID No.:93

5'-GGAGCACCGGTCGgartgggcnga-3'

E H R S E W A D

Degen=8, temp=60.3

HD-ZipIII: Block D**Reverse**

Rice D1R

SEQ ID No.:94

5'-GACGGGCGGCggnacrtskytg-3'

Degen=32, temp=60.4

Complementary to:

Q N V P P A V

gtyktrcanggCGGCGGGCAG

Rice D2R

5'-GACGGGCGGCggnacrtskytgna-3'

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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

¹First 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.

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

³"Temp." means the mean melting temperature of the degenerate oligonucleotide primer pool.

⁴"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 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 PCR reaction using 2 Units AmpliTaq Gold (Perkin Elmer), the supplied buffer, 2 mM MgCl₂, 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. *indica* var. IR36) cDNA library

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(Stratagene FL1041b); 1.5 μ L of purified genomic rice (*Oryza sativa*) DNA (about 400 ng); 1.5 μ L of purified genomic barley (*Hordeum vulgare*) DNA (about 400 ng); 1.5 μ 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

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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 *Lycopersicum 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).

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Table 10

TomatoREV

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

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; GCCGTTACGGCSTCRTTRAANCC (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,

20 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). 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.

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Example 7Modulation 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 CaCl₂, 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
20 protein-3 polynucleotide sequence as set forth in SEQ ID No.:107. Transcription of 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 Mah1 VII substrate (macro- and micro-elements and vitamins of N6 medium supplemented with 100 mg/l casein
35 hydrolysate, 6 mM proline, 0.5 g/l MES, 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-

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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). pHYGI1 is a plasmid that contains the hygromycin coding sequence (Gritz

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

pHD-Zip-invp-1 plasmid DNA and pHYGI1 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.

All of the tubes contain 25 µl of 50 mg/ml M-10 tungsten in water, 25 µl of 2.5M CaCl₂, 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.

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.

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.

Immediately after all samples are bombarded, callus from all of the plates treated with the pHYGI1 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.

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After about 14 days of selection, tissue appear essentially identical on both selective and nonselective media. All callus from plates of the pHYGI1/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 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 sectors of callus. Viable sectors of callus are dissected from a background of necrotic tissue on the plantes treated with pHYGI1/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 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 pHYGI1/pHD-Zip-invp-1 treated callus has acquired the hygromycin resistance gene, genomic DNA is isolated from the callus that has 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 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 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 at -20°C. for 15 min. and the DNA pelleted 5 min. in an Eppendorf microcentrifuge

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(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 μ 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 pHYGII 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

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taken from plant tissues and frozen in liquid nitrogen. Samples are then ground with 120 grit carborundum in 200 µl 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 µl volumes in 50 mM KCl, 10 mM Tris-HCl pH 8.4, 3 mM MgCl₂, 100 µg/ml gelatin, 0.25 µM 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 µl 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 µl 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.

R₁ 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

30 Modulation of Cell Division in Rice Seed

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 Phytagel, pH 5.8), and
35 cultured at 27°C. in the dark. Callus develops from the scutella of the embryos

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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 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 washed twice in EPM (5 mM CaCl₂, 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 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 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 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 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 regeneration medium N6M25 (N6 medium (Chu et al. 1975), supplemented with 0.5

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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 R₁ 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-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.

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).

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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 3×10^9 - 5×10^9 cells/ml and the suspensions are used for inoculation of rice callus.

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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 (R₀ 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 hygromycin. Twenty seeds of each plant of the progeny of the transformants are

30 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.

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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: TGTCGAATCGAATCACACAAAAGACCA [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: TTGCGGCCGCTTCGATTGACAGAAAAAGACTAATTT [SEQ ID NO:51]; 3' primer includes ApaI and KpnI sites: TTGGGCCCCGGTACCCTCAACCAACCACATGGAC [SEQ ID NO:52]). Clones

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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.

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

GGGAACACTTAAAGTATAGTGCAATTGTATTCAAACCTGACAAATTAATCATTAAATTGTATTAGAAAAT
 GATATATTGTCATCGTGACTAATTGTCTTTTGAATTCAATTGTAACCTACTACTGGTTTTTCATT
 TTTCAGATTATTTCTCGGTTCTTAAAAAGAAGAAAACACATACCTAATATCGTGTGTATCAAAAAGCC
 ATGTGCGAGCAACCATCTTCCTTTGTAAATTTGACCCTTTTGTGTATCATTTATATCGAGTGTTTGTA
 ATTGTTGGTTGATTTTGTGTTATTTGAGAGGCTAGTCATTTACGCAATTTCTAAATTTATCTTTTAGT
 ACGTATCAAGATGTTGGAGCAACTGTGTCCAACATACATGACATGTGAAATTATAATTGTTAAAACAA
 AAGGACATGTGAAATTACTATCTACTTAAAGAAAAAAACCAGAAAGAAAAAAAGGTATTAAATTTGGA
 ACTAAAAGAAGGTTAAAAAGTTTTTTTACAGAAAGTAATTACACTTGCAGATGAAAGAAAAAAGGCAGC
 ATCATGATATGTAAAAAATTGTCGAAGAGGTTTAGTCGTATCACTTTGTTTGACTGATCACTGTCTTC
 TGATTCATTTTTTCAGTTTTTCTTTTTCAAATTGTAGCTCACAACATTAAAGTTATTCAGTGCTTTAAT
 CAGATAGTTTAATACTAGTAACTAGCTCATTTAGGCTTTAAACACCTCTTTCTGATTACTAGCCCACT

CTTTGGTGGTTCTTACATATCACACCTAACTATACTGTGTATCCTTGAAGTGAAAATCAAATTTACCA
TTCGTATCTTACTTACATACACTATTATTTTTCCTTTTTTTTTTCACTCAAGGTCCTACTCTTTGATA
CCATAGCTATAATTTGGAAATAACTATTTACAGTGTATTAATTATACCTAAACAGTTTAATCTGGACT
AAATATTTAGATAGATGTTACAAATTTGGTTCGTCTAATAAATGAAGACAAGACATGCTAACAAATAA
AACACTACCACAAAGGGATAGTGAGAGAATGTGTTTTGCAACAAGACATAACTTTGATTGCTTGATGT
GTTAAAATGATTATGTCAGAGACAGAGAGCGATCATAGGCTTTCTTTATTTCTAATAACGTCGATTTT
CTTCTTCTTTTCTGGCGTTCAATAATGTCGAATTTTAATTCTTGATTTTTGCAACTCTAAATATCCTT
AACGACATTGAAGCATGGTGCATGTCGATCGTTAATAATAAAGTTGAACAAAAATCTTGTTGAATTAA
TTACAAGCACAGCTTCAATAGCATAACTTTACGAGACGACCAGATCTTATAGACGAGTTTCGCTTTTA
CTTTTTTAATGATTAAAACTTTCATCGGAGAACATAAGTCTTCCTCTTAATTAAAATTACTACCCGTG
CATAACTTCATTTTTTAAACATCAATAATTAATATACGATTACAACCCTAAAAATTAGTCACCCTAT
AGTACATAACAATGATGATAGTTTTTTCTTTTTGGTGTAATGTTAAAATAAAATGTTAGCCATATTAA
ACGATAGTTCTTTTAACTTAGCCATTGTAAGATATTTCTTACTTTAGTTTTTCCGTAGAAGATATTCA
TTATGGTATGGATAGTATATACCTTAACTGAGTTTAAATATTGGATCAATACCATCTAATAACACATA
TCTGATGTTCAATACTTAATAATTTTGCATAAATGTTAAGCGTGACAACTTAAAAAAAAAACACATCAA
CAGAGTAAAAACATATCTGTAAATAAGAAAAATGTCATTTTTATAACACTTAAAAAAAAAATGCATGA
AGCGTTTCATAGTTTTTTTTTTTTTCAAAGTAATGTAGGCGTTAGATATTTCTTACAATTTTTTGAAA
AATATTTTTTATGTTGTGATTGGCTGATATCAGGTAACATAAACTTCTTTAAAGAATTGAAGAAAATT
TGAAAAGTAAATAGATGGATTCTTATATTGTCATTTTCAAGAAAACAGTAGGGACAACCTTCGTAAATGA
TAGCCGTATTATTAAACAAATAAATTTAAATTAGAAAAAAGGAAAAAACGCACCACTTTTCTTTTTTC
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TCTCTTCTCTTTTTATTCTCTGTTCCAAAGTTTCAAGCAGAAAGCAAACACACACATCACTTACTATCTC
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CTTTAAAACTTTTTTTAACTTTATTTTTTGTACTCGCTTCTTTGCCTTCATTTTTCTCGTATCCACAT
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GTTTCTCTTACGATACGACGGACTTTCCGAAGAAATTAATTTAAAGAGAAAAGAAGAAGAAGCCAAAG
AAGAAGAAGAAGCTAGAAGAAACAGTAAAGTTTGAGACTTTTTTTTGAGGGTCGAGCTAAAATGGAGAT
GGCGGTGGCTAACCACCGTGAGAGAAGCAGTGACAGTATGAATAGACATTTAGATAGTAGCGGTAAGT
ACGTTAGGTACACAGCTGAGCAAGTCGAGGCTCTTGAGCGTGTCTACGCTGAGTGTCTAAGCCTAGC
TCTCTCCGTCGACAACAATTGATCCGTGAATGTTCCATTTTGGCCAATATTGAGCCTAAGCAGATCAA
AGTCTGGTTTCAGAACCGCAGGTATTGCTTCTCTTTAATATGGCCAGGATTAATTTTTTAATTAAGGAT

TTTGAATTTGATTCTATTGGATTTAGTGTGTTATATTCAATGGATATGAAGGACCACTTTTGTGTGTTA
TTTCAAGATTTGATGCTTCAATTCATTTCTCCGACACAATTTCTGTTTTTTACAAAAGGGTTCCTTTG
AATCTGTCTGGTAGATTTGGTTATTCAATAGCTTGGTGTAACGTTCCTGTGACGATATGGTTACTGT
CTGATCTGGTGTCTAATCTTAGGAGTTTTGTGATTCTGTTTTGTGTGTGGTTTCAGGTGTCGAGATA
AGCAGAGGAAAGAGGCGTCGAGGCTCCAGAGCGTAAACCGGAAGCTCTCTGCGATGAATAAACTGTTG
ATGGAGGAGAATGATAGGTTGCAGAAGCAGGTTTCTCAGCTTGTCTGCGAAAATGGATATATGAAACA
GCAGCTAACTACTGTTGTATGTAACCTAACATTTCTTTTTGTCAAATGTGTTCTTAAAGAATCATTTG
TTACTCCTATCAGTTCAACATGTAGCTTGAGTTATAAAGTTACTGACTTGTGTGTTTTAACTTCAGGTT
AACGATCCAAGCTGTGAATCTGTGGTCACAACTCCTCAGCATTCGCTTAGAGATGCGAATAGTCCTGC
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ACTGCTGTTGATTGGGTTGAGATGCCTGGGATGAAGGTTATACGCATCTCGTATCATTAAGTGT
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TAGCTTAGAACCTATGAAGGTAAGAAAGGGACACTCTTTTCGTTGCTAAAGATAACAAGTCATAATGTT
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GATTCATATAACATATCTAACGGTTGGAGGTGCTTCATGTTTTAGGCGTTGCGGTATATCAGGCAATT
AGCCCAAGAGTCTAATGGTGAAGTAGTGTATGGATTAGGAAGGCAGCCTGCTGTTCTTAGAACCTTTA
GCCAAAGATTAAGCAGGTACTTCGATCTTGAGCTAAAACCTAATTGTTCTTTGCTCTGTTTGCTCATT
GTCATTTTTTCTGTTCTTGGTTTTCTTGAAGGGGCTTCAATGATGCGGTTAATGGGTTTGGTGACGAC
GGGTGGTCTACGATGCATTGTGATGGAGCGGAAGATATTATCGTTGCTATTAACCTTACAAAGCATTT
GAATAATATTTCTAATTCTCTTTCTGTTCCCTTGGAGGCGTGCTCTGTGCCAAGGCTTCAATGCTTCTCC
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TGAGTGGGCTGATTTCAATGTTGATGCATATTCCGCTGCTACACTTAAAGCTGGTAGCTTTGCTTATC

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GACGAGAATGCCGTTGGAGCTTGTTCTGAACTGATATTTGCTCCGATTAATGAGATGTTCCCGGATGA
TGCTCCACTTGTTCCCTCTGGATTCCGAGTCATACCCGTTGATGCTAAAACGGTACTCTTCTTTGCTG
TACCACTGATTTTTCTTTTACTTAGAGATGGTTTGTTTCAAGGCTCATTTTTTCTTACTCATACAGGG
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TTTCAATTCCTTTTGAAAACAACCTGCAAGAAAATGTTGCTGGTATGGCTTGTCAGTATGTGAGGAG
CGTGATCTCATCAGTTCAACGTGTTGCAATGGCGATCTCACCGTCTGGGATAAGCCCGAGTCTGGGCT
CCAAATTGTCCCCAGGATCTCCTGAAGCTGTTACTCTTGCTCAGTGGATCTCTCAAAGTTACAGGTGG
GGGTGTAAATGTTTACTCTCGTCTCTTTCTTATAATCCTCGAACTTATCGATGATGCCTTATGCTGAT
ATGTTTGTTTTTCCAGTCATCACTTAGGCTCGGAGTTGCTGACGATTGATTCACCTTGAAGCGACGAC
TCGGTACTAAAACCTTCTATGGGATCACCAAGATGCCATCCTGTGTTGCTCATTAAGGTATGTGTCTCT
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ACAGCCAGTGTTTCATGTTTGCGAACCAAGCTGGTCTAGACATGCTAGAGACAACACTTGTAGCCTTAC
AAGATATAACACTCGAAAAGATATTCGATGAATCGGGTCGTAAGGCTATCTGTTCCGACTTCGCCAAG
CTAATGCAACAGGTAAAGAACCAAAACAAAACATCTGCAGATAAATGGTTTTGATTCATTTGTCTGA
GAACTATCTTTGCGTCTACAGGGATTTGCTTGCTTGCTTCAGGAATCTGTGTGTCAACGATGGGAAG
ACATGTGAGTTATGAACAAGCTGTTGCTTGGAAGTGTTTGCTGCATCTGAAGAAAACAACAACATC
TGCATTGTCTTGCTTCTCCTTTGTAAACTGGTCTTTTGTTGTGATTGATTGACAGAAAAGACTAAT
TTAAATTTACGTTAGAGAACTCAAATTTTTGGTTGTTGTTTAGGTGTCTCTGTTTTGTTTTTTAAAT
TATTTTGATCAAATGTTACTCACTTTCTTCTTTCACAACGTATTTGGTTTTAATGTTTTGGGGAAAAA
AGCAGAGTTGATCAATCTCTATATATAAAGGGAATGATGTGATAATTTTGTTAAACTAAGCTTACAA
CATTTTTTCTATCGCATTTGACAGTTTCATTTTCACATCTCTCGCTATATATTAGTAATATAAACTAT
TTCAAAAAACAAAGAATCAACAAGAATCCACAGATGTAAGAAAGAAAAATCACAGCCAAATAACTTTT
TTATTTATTTGGCCGTTAGATAAAACTACCTTCAGAATTTTCATGCATCTAGCCGGTAAACCTGTCTGA
TGATTGACGGCGACAATCTCAGAGACATTGTTGCAACGAAGAACATCTTGACCAAGCTTAGCTCCTGC
AGCTTTAAGACCTTTAAGCGAAACCGGCATGTTGAAGAGATTGAGTGATGGAAGCTTAGAAAGCGGAG
GGAGTTTCTGGTTCGGTAAGAAGTTTCTGAAATCATCCATAAGCAATGGAACCTTCGAAATCGGTTTTG
TTGTGTTTCACCACATTGTCTTTAGCTGCGATGTGAGCTCCTGGTTCCATGTGGTTGGTTGAG

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

WO 01/33944

PCT/US00/30794

841

871

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

901

931

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

961

991

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

1021

1051

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

WO 01/33944

PCT/US00/30794

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

WO 01/33944

PCT/US00/30794

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

WO 01/33944

PCT/US00/30794

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

2491

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

2521

2551

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

2581

2611

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

2641

2671

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

rev-5

C

2701

2731

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

2761

2791

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

rev-1

A

2821

2851

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

2881

2911

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

2941

2971

TTT TAC CAT TTC AAA GTT AAA ATT GAC CTT AAT GAT TTA TGG TAG ATT TGT
ACC GGG ATT

3001

3031

GAC GAG AAT GCC GTT GGA GCT TGT TCT GAA CTG ATA TTT GCT CCG ATT AAT
GAG ATG TTC

3061

3091

CCG GAT GAT GCT CCA CTT GTT CCC TCT GGA TTC CGA GTC ATA CCC GTT GAT
GCT AAA ACG

3121

3151

GTA CTC TTC TTT GCT GTA CCA CTG ATT TTT CTT TTA CTT AGA GAT GGT TTG
TTT CAA GGC

3181

3211

TCA TTT TTT CTT ACT CAT ACA GGG AGA TGT ACA AGA TCT GTT AAC CGC TAA
TCA CCG TAC

3241

3271

ACT AGA CTT AAC TTC TAG CCT TGA AGT CGG TCC ATC ACC TGA GAA TGC TTC
TGG AAA CTC

rev-3

T

3301

3331

TTT TTC TAG CTC AAG CTC GAG ATG TAT TCT CAC TAT CGC GTT TCA ATT CCC
TTT TGA AAA

3361

3391

CAA CTT GCA AGA AAA TGT TGC TGG TAT GGC TTG TCA GTA TGT GAG GAG CGT
GAT CTC ATC

3421

3451

WO 01/33944

PCT/US00/30794

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 KWWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQ LVC
ENGYMKQQLT TVVNDPSCES VVTTTPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF
TMFPAGNGGT IELVYMQTYA PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA
ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT
ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA
EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNVD
AYSAATLKAG SFAYPGMRPT RFTGSQIIMP LGHTIEHEEM LEVVRLEGHS LAQEDAFMSR
DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA
NHRTLDTLSS LEVGPSPEN A SGNSFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS
VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSYSHHLGSE LLTIDSLGSD
DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI
CSDFAKLMQQ GFACLP SGIC VSTMGRHVS Y EQAVAWKVFA ASEENNNNLH CLAFS FVNWS
FV

Arabidopsis rev-3 Protein SEQ ID NO:6

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQQLIRECS
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQ LVC
 ENGYMKQQLT TVVNDPSCES VVTTTPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF
 TMFPAGNGGT IELVYMPTYA PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA
 ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT
 ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA
 EDIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNVD
 AYS AATLKAG SFAYPGMRPT RFTGSQIIMP LGHTIEHEEM LEVVRLEGHS LAQEDAFMSR
 DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA
 NHRTL DLISS LEVGPS PENA SGNSFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS
 VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSYSHHLGSE LLTIDSLGSD
 DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI
 CSDFAKLMQQ GFACLP SGIÇ VSTMGRHVS Y EQAVAWKVFA ASEENNNNLH CLAFS FVNWS
 FV

Arabidopsis rev-4 Protein SEQ ID NO:8

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQQLIRECS
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQ LVC
 ENGYMKQQLT TVVNDPSCES VVTTTPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF
 TMFPAGNGGT IELVYMPTYA PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA
 ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT
 ISRCGISGN

Arabidopsis REV-5 Protein SEQ ID NO:10

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQQLIRECS
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQ LVC
 ENGYMKQQLT TVVNDPSCES VVTTTPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF
 TMFPAGNGGT IELVYMPTYV PTTLAPARDF WTLRYTTSLD NGSFVVCERS LSGSGAGPNA
 ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVLRPLYE SSKVVAQKMT
 ISALRYIRQL AQESNGEVVY GLGRQPAVLR TFSQRLSRGF NDAVNGFGDD GWSTMHCDGA

EDIIIVAINST KHLNNISNSL SFLGGVLC AK ASMLLQNVPP AVLIRFLREH RSEWADFNVD
 AYSAA TLKAG SFAYPGMRPT RFTGSQIIMP LGHTIEHEEM LEVVRLEGHS LAQEDAFMSR
 DVHLLQICTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA
 NHRTL DLTSS LEVGPS PENA SGNSFSSSSS RCILTIAFQF PFENNLQENV AGMACQYVRS
 VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSYSHHLGSE LLTIDSLGSD
 DSVLKLLWDH QDAILCCSLK PQPVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI
 CSDFAKLMQQ GFACLPSGIC VSTMGRHVS Y EQAVAWKVFA ASEENNNNLH CLAFS FVNWS
 FV

Arabidopsis REV-6 Protein SEQ ID NO:12

MEMAVANHRE RSSDSMNRHL DSSGKYVRYT AEQVEALERV YAECPKPSSL RRQQLIRECS
 ILANIEPKQI KVWFQNRRCR DKQRKEASRL QSVNRKLSAM NKLLMEENDR LQKQVSQ LVC
 ENGYMKQQLT TVVNDPSCS VVTTPOHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
 VQMPGMKPGP DSVGIFAISQ RCNGVAARAC GLVSLEPMKI AEILKDRPSW FRDCRSLEVF
 TMFPAGNGGT IELVYMQTYA PTT LAPARDF WTLRYTTS LD NGSFVVCERS LSGSGAGPNA
 ASASQFVRAE MLSSGYLIRP CDGGGSIIHI VDHLNLEAWS VPDVL

Barley Genomic Clones:

>Barley 2 SEQ ID NO:97

AGCTCTATCCACCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
 CAAAGTATGGTTTCAGAACCGAAGGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT
 CTTTGATCAAAATCTTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT
 CAAGCGGTG

>Barley 9 SEQ ID NO:99

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
 CAAAGTATGGTTTCAGAACCGAAGGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT
 CTTTGATCAAAATCTTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT
 CGAGCGGTG

>Barley 10 SEQ ID NO:101

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
 CAAAGTATGGTTTCAGAACCGAAGGTAATGATGATGCTAACACTCCTTATAATGCAGTTTTAGTGATT

CTTTGATCAAAATCTTTTATAAAAATTCAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTT
CAAGCGGTG

>Barley 2 intron spliced out SEQ ID NO:127

AGCTCTATCCACCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTTCAAGCGG
TG

>Barley 9 intron spliced out SEQ ID NO:128

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGAGGCTTCACGGCTTCGAGCGG
TG

>Barley 10 intron spliced out SEQ ID NO:129

AGCTCTATCCGCCGTCAGCAGTTGATCAGAGAGTGTCTTATTCTCTCCAACATTGAGCCTAAACAGAT
CAAAGTATGGTTTCAGAACCGAAGATGCAGAGAGAAGCAAAGGAAAGGGCTTCACGGCTTCAAGCGGT
G

>Barley 2 protein SEQ ID NO:98

SSIHRQQLIRECPILSNIEPKQIKVWFQNRRCREKQORKEASRLQAV

>Barley 9 protein SEQ ID NO:100

SSIRRQQLIRECPILSNIEPKQIKVWFQNRRCREKQORKEASRLRAV

>Barley 10 protein SEQ ID NO:102

SSIRRQQLIRECPILSNIEPKQIKVWFQNRRCREKQORKEASRLQAV

Corn Genomic Clones:

>Corn 1 SEQ ID NO:103

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT
TAAAGTC

>Corn 2 SEQ ID NO:105

AGCTCCGCGCGCAGGCAGCAGCTGCTACGCGAGTGCCCCATCCTCTCAAACATCGAGGCCAAGCAGAT
TAAAGTC

>Corn 3 SEQ ID NO:107

ACCTCCTCCCGCAGGCAGCAATTGCTGCGTGAGTGCCCCACACTTGCTAACATTGAGGCCAAGCAGAT
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

SSARRQQLLRECPILSNIEAKQIKV

>Corn Protein 2 SEQ ID NO:106

SSARRQQLLRECPILSNIEAKQIKV

>Corn Protein 3 SEQ ID NO:108

TSSRRQQLLRECPTLANIEPKQIKV

>Corn Protein 7 SEQ ID NO:110

SSARRQQLLRECPILSNIEAKQIKV

Corn Protein 8 SEQ ID NO:112

SSARRQQLLRECPILSNIEAKQIKV

Corn Protein 9 SEQ ID NO:114

SSARRQQLRECPILSNIEAKQIKV

Rice Genomic Clones

Rice Genomic 1 SEQ ID NO:115

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT
CAAGGTGTGGTTCCAGAACCGCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTCGCGGCTGCAGGCGC
TC

Rice Genomic 4 SEQ ID NO:117

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT
CAAGGTGTGGTTCCAGAACCGCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTCGCGGCTGCAGGCGC
TC

Rice Genomic 10 SEQ ID NO:119

AGCTCGCTGCGGCGGCAGCAGCTGGTGCGGGAGTGCCCGGCGCTGGCGAACGTGGACCCGAAGCAGAT
CAAGGTGTGGTTCCAGAACCGCCGGTGCCGGGAGAAGCAGCGCAAGGAGTCGTCGCGGCTGCAGGCGC
TC

Rice Genomic protein 1 SEQ ID NO:116

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQQRKESSRLQAL

Rice Genomic protein 4 SEQ ID NO:118

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQQRKESSRLQAL

Rice Genomic protein 10 SEQ ID NO:120

SSLRRQQLVRECPALANVDPKQIKVWFQNRRCREKQQRKESSRLQAL

Rice cDNA Clones:

rice cDNA 1 SEQ ID NO:121

TCCTCCCGCAGGCAGCAATTGCTGCGTGAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA
GGTCTGGTTCCAGAACAGAAAGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

rice cDNA 4 SEQ ID NO:123

TCCTCCCGCAGGCAGCAATTGCTGCGTAAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA
GGTCTGGTTCCAGAACAGAAGGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

rice cDNA 8 SEQ ID NO:125

TCCTCCCGCAGGCAGCAATTGCTGCGTAAGTGCCCCATACTTGCTAACATTGAGCCCAAGCAGATCAA
GGTCTGGTTCCAGAACAGAAGGTGCCGGGATAAGCAGCGGAAGGAGTCTTCACGGCTTCAGGCTGTC

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

///

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

GYMRQQLQSVTTDVSCESGVTTTPQ (SEQ ID NO:131)

Corresponding rice REV1 sequence

AHMRQQLQNTPLANDTSCESNVTTTPQ (SEQ ID NO:132)

Corresponding rice REV2 sequence

AYMKQQLQNPXLGNDTSXESNVTTPO (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

ATGCCACTAGGACATACAATTGAACACGAAGAAATGCTAGAAGTTGTTAGACTGGAAGGT

CATTCTCTTGCTCAAGAAGATGCATTTATGTCACGGGATGTCCATCTCCTTCAGATTTGT

ACCGGGATTGACGAGAATGCCGTTGGAGCTTGTTCTGAACTGATATTTGCTCCGATTAAT

GAGATGTTCCCGGATGATGCTCCACTTGTTCCCTCTGGATTCCGAGTCATACCCGTTGAT
GCTAAAACGGGAGATGTACAAGATCTGTTAACCGCTAATCACCGTACACTAGACTTAACT
TCTAGCCTTGAAGTCGGTCCATCACCTGAGAATGCTTCTGGAAACTCTTTTTCTAGCTCA
AGCTCGAGATGTATTCTCACTATCGCGTTTCAATTCCCTTTTGAAAACAACTTGCAAGAA
AATGTTGCTGGTATGGCTTGCGCGGCCGAGTTCAACGTGTTGCAATGGCGATCTCACCG
TCTGGGATAAGCCCGAGTCTGGGCTCCAAATTGTCCCCAGGATCTCCTGAAGCTGTTACT
CTTGCTCAGTGGATCTCTCAAAGTTACAGTCATCACTTAGGCTCGGAGTTGCTGACGATT
GATTCACTTGGAAGCGACGACGCTAGCGCATGCCAAGCCATACCAGCAACATTTTCTTGC
AAGTTGTTTTCAAAGGGAATTGAAACGCGATAGTGAGAATACATCTCGAGCTTGAGCTA
GAAAAGAGTTTCCAGAAGCATTCTCAGGTGATGGACCGACTTCAAGGCTAGAAGTTAAG
TCTAGTGTACGGTGATTAGCGGTTAACAGATCTTGTACATCTCCCGTTTTAGCATCAACG
GGTATGACTCGGAATCCAGAGGGAACAAGTGGAGCATCATCCGGGAACATCTCATTAATC
GGAGCAAATATCAGTTCAGAACAAGCTCCAACGGCATTCTCGTCAATCCCGGTACAAATC
TGAAGGAGATGGACATCCCGTGACATAAATGCATCTTCTTGAGCAAGAGAATGACCTTCC
AGTCTAACAACCTTCTAGCATTTCTTCGTGTTCAATTGTATGTCCTAGTGGCATTATGATC
TGACTCCCAGTGAATCTTGTTGGTCTCATTCCCGGATAAGCAAAGCTCCGCGG

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):

ATCGATGTTAACGATCCAAGCTGTGAATCTGTGGTCACAACTCCTCAGCATTGCTTAGA
GATGCGAATAGTCCTGCTGGATTGCTCTCAATCGCAGAGGAGACTTTGGCAGAGTTCCTA
TCCAAGGCTACAGGAACTGCTGTTGATTGGGTTCAGATGCCTGGGATGAAGCCTGGTCCG
GATTCGGTTGGCATCTTTGCCATTTTCGCAAAGATGCAATGGAGTGGCAGCTCGAGCCTGT
GGTCTTGTTAGCTTAGAACCTATGAAGATTGCAGAGATCCTCAAAGATCGGCCATCTTGG
TTCCGTGACTGTAGGAGCCTTGAAGTTTTCACTATGTTCCCGGCTGGTAATGGTGGCACA
ATCGAGCTTGTTTATATGCAGACGTATGCACCAACGACTCTGGCTCCTGCCCCGCGATTTC
TGGACCCTGAGATACACAACGAGCCTCGACAATGGGAGTTTTGTGCGCGGCCGC

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

GGAGATGTACAAGATCTGTTAACCGCTAATCACCGTACACTAGACTTAACTTCTAGCCTT
GAAGTCGGTCCATCACCTGAGAATGCTTCTGGAACTCTTTTTCTAGCTCAAGCTCGAGA
TGTATTCTCACTATCGCGTTTCAATTCCCTTTTGAAAACAACTTGCAAGAAAATGTTGCT
GGTATGGCTTGTCAGTATGTGAGGAGCGTGATCTCATCAGTTCAACGTGTTGCAATGGCG

ATCTCACCGTCTGGGATAAGCCCGAGTCTGGGCTCCAAATTGTCCCAGGATCTCCTGAA

GCTGTTACTCTTGCTCAGTGGATCTCTCAAAGTTACAGGCTAGCGCATGCCTGCCCCGCGA

TTTCTGGACCCTGAGATACACAACGAGCCTCGACAATGGGAGTTTTGTGCGCGGCCGC

(SEQ ID NO:147):

CACAAAACCTCCCATTTGTCGAGGCTCGTTGTGTATCTCAGGGTCCAGAAATCGCGGGCAGG

AGCCAGAGTCGTTGGTGCATACGTCTGCATATAACAAGCTCGATTGTGCCACCATTACC

AGCCGGGAACATAGTGAAAACCTCAAGGCTCCTACAGTCACGGAACCAAGATGGCCGATC

TTTGAGGATCTCTGCAATCTTCATAGGTTCTAAGCTAACAAGACACAGGCTCGAGCTGC

CACTCCATTGCATCTTTGCGAAATGGCAAAGATGCCAACCGAATCCGGACCAGGCTTCAT

CCCAGGCATCTGAACCCAATCAACAGCAGTTCCTGTAGCCTTGGATAGGAACTCTGCCAA

AGTCTCCTCTGCGATTGAGAGCAATCCAGCAGGACTATTCGCATCTCTAAGCGAATGCTG

AGGAGTTGTGACCACAGATTCACAGCTTGGATCGTTAACCCGCGG

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

ATCGATATTGCTGATATCCTCAAAGATCGACCTTCTTGGTTCCGCGACTGCCGGAATGTTGAAGTTAT
CACAATGTTTCCTGCTGG

AAATGGTGGTACAGTTGAGCTTTTGTATACCCAGATATATGCTCCCACAACTCTGGCTCCCG
CGCGTGATTTTGGACGC

TGAGATACACAACAACCCTAGACAATGGTAGTCTCGTGGTTTGTGAAAGATCCCTATCTGGT
AATGGGCCTGGCCCAAAT

CCTACTGCTGCTTCCCAGTTTGTAAAGAGCTCAAATGCTTCCATCTGGATATCTGATCCGACC
GTGTGATGGTGGAGGATC

AATCATACATATTGTTGATCACCTGAATCTTGAGGCATGGAGTGCCCCTGAGATTTTGCGTC
CACTCTATGAATCGTCGA

AAGTTGTGGCACAGAAAATGACTATTGCAGCACTGCGATATGCAAGGCAACTAGCTCAAGAG
ACTAGCGGCGAGCGCGGCCGC

Linker (SEQ ID NO:149):

GTAGTATATGGTCTAGGAAGGCAACCTGCTGTTCTCGAACATTCAGCCAGAGATTATGCAGAGGGTT
CAATGATGCCATCAATGGATTCCGGTGACGATGGCTGGTCAATGTTAAGTTCAGATGGTGCTGAAGATG
TCATAGTTGCTGTCAATTCAAGGAAGAACCTCGCAACCACCTCCATTCTTTCCCCGCTTGGTGCG
GTCCTTTGTACCAAAGCATCAATGCTACTCCAGAATGTCCCCCTGCCGTACTGGTTCGGTTTCTGAG
GGAGCACCGTTCAGAATGGGCCGATTATGCTAGCGCATGC

(SEQ ID NO:150):

CTCGCCGCTAGTCTCTTGAGCTAGTTGCCTTGCATATCGCAGTGCTGCAATAGTCATTTTCT
GTGCCACAACCTTCGACGATTCATAGAGTGGACGCAAATCTCAGGGGCACTCCATGCCTCAAGATTC
AGGTGATCAACAATATGTATGATTGATCCTCCACCATCACACGGTCGGATCAGATATCCAGATGGAAG
CATTTGAGCTCTTACAACTGGGAAGCAGCAGTAGGATTTGGGCCAGGCCCATACCAGATAGGGATC
TTTCACAAACCACGAGACTACCATTGTCTAGGGTTGTTGTGTATCTCAGCGTCCAAAAATCACGCGCG
GGAGCCAGAGTTGTGGGAGCATATATCTGGGTATACAAAAGCTCAACTGTACCACCATTTCAGCAGG
AAACATTGTGATAACTTCAACATTCCGGCAGTCGCGGAACCAAGAAGGTCGATCTTTGAGGATATCAG
CAATCCGCGG

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

ATCGATtgggttcattgagaatgccacatgacagcagctgcagaataactccgctggcaa
gatacaagctgtgaatcaaatgtgactacccctcaaaaccctttaagggtgcaagtaaccctctgg
gctcctttcaattgcagaggagaccttgacagagttcctctcaaaggctactggtacagctattgatt
gggtccagatgcctgggatgaagcctgggtccggttcggttggtattgtggccatttcacatggttgc
ccgtgggtggtgctgccgtgcctgtggtttggtgaacctagaaccaacaaaagtggtagagatattgaa
agatcgatccatcttggttccgtgattgtcgaaacctggaagtctttacaatgattccagcaggaaatg
gaggaacgggtgaacttgtctacacacagttgtatgctccaacaactttagttcctgcaCGCGGCCGC

linker(SEQ ID NO:152):

atgctagggagtagcagtgatggaggtggctatgataagggtttccgggatggactccggtaaatatgt
 gcgctacacgcctgagcaggtggaggcgcttgagcgggtgtacgcgattgccccaaagccaacctct
 cccgcaggcagcaattgctgcgtgagtccccatacttgctaacattgagcccaagcagatcaaGCTA
GCGCATGC

(SEQ ID NO:153):

tgcaggaactaaagttggttgagcatacaactgtgtgtagacaagttcaaccgttcctccat
 ttcttgctggaatcattgtaaagacttccaggtttcgacaatcacggaaccaagatggacgatctttc
 aatatctctaccacttttggttggttctaggttcaccaaaccacaggcacggcagcaacaccacgggca
 accatgtgaaatggccacaataccaaccgaatccggaccaggcttcacccaggcatctggaccaat
 caatagctgtaccagtagcctttgagaggaactctgtcaaggtctcctctgcaattgaaaggagccca
 gaggggttacttgcattcccttaaagggttttgaggggtagtcacatttgattcacagcttgatcatt
 tgccagcggagtatctgcagctgctgtcgcatgtgggcattctcatgaaccaCCGCGG

Example for RiceREV2 (SEQ ID NO:154):

ATCGAT gaatcaaattg tgaccactcc tcagaaccct ctgagagatg caagtaaccg
 gtctggactc cttacaattg cggaggagac cctgacagag ttctctcca aggctacagg
 gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggtat
 tgtggccggt tcacatgggt gccgtgggtg tgctgcccgt gcctgtgggt tggatgaatct
 agaaccaaca aagatcgtgg agatcttaaa agaccgcca tcttggttcc gtgattgtcg
 aagtcttgaa gtcttcacaa tgtttccagc tggaaatggt ggcacgatcg aacttggtta
 catgcagatg tatgctccta ctactttggt tctgcacga gatttttgga cacttagata
 cacaactaca atggatgatg gcagccttgt ggtctgtgagCGCGGCCGC

Linker (SEQ ID NO:155):

ccgcacagca atttgtaaga gctgagatgc ttcttagcgg
 ctatctagtgc cggccatgcg aggggtgggtg ctccgtcgtg catattgtgg accatctgga
 tcttgaggct tggagtgttc cagaagtgtc tcggccactc tacgagtcac ctagggtagt
 tgctcagaaa atgactgctg cagcGCTAGCGCATGC

(SEQ ID NO:156):

ctcacagaccacaaggctgccatcatccattgtagttgtgtatctaagtgtccaaaaatctcgtgcag
 gaaccaaagtagtaggagcatacatctgcatgtaaacaagttcgatcgtgccaccatttccagctgga
 aacattgtgaagacttcaagacttcgacaatcacggaaccaagatgggcgggtcttttaagatctccac
 gatctttggttggttctagattcaccaaaccacaggcacgggcagcaacaccacggcaaccatgtgaaa

cggccacaataccaaacgaatccggaccaggcttcatcccaggcattggcacccaatcaacagcagtc
cctgtagccttggagaggaactctgtcaggggtctcctccgcaattgtaaggagtcagacgggttact
tgcattctctcagaggggttctgaggagtggtcacatttgattcCCGCGG

Rice REV1 (SEQ ID NO:157):

atg ctagggagta gcagtgatgg aggtggctat gataaggttt ccgggatgga
ctccggtaaa
tatgtgcgct acacgcctga gcaggtggag gcgcttgagc ggggtgtacgc cgattgcccc
aagccaacct cctcccgag gcagcaattg ctgcgtgagt gcccataact tgctaacatt
gagcccaagc agatcaaggt ctggttccag aacagaaggt gccgggataa gcagcgggaag
gagtcttcac ggcttcaggc tgtcaacagg aaattgacgg caatgaacaa gctacttatg
gaagagaatg agcgactcca gaagcaggtc tcccaattgg ttcattgagaa tgcccacatg
cgacagcagc tgcagaatac tccgctggca aatgatacaa gctgtgaatc aaatgtgact
accctcaaa accctttaag ggatgcaagt aaccctctg ggctccttcc aattgcagag
gagaccttga cagagttcct ctcaaaggct actggtacag ctattgattg ggtccagatg
cctgggatga agcctgggtcc ggattcgggtt ggtattgtgg ccatttcaca tgggtgcccg
tgggtgttgc gccgtgcctg tgggtttggtg aacctagaac caacaaaagt ggtagagata
ttgaaagatc gtccatcttg gttccgtgat tgcgaaacc tgggaagtctt tacaatgatt
ccagcaggaa atggaggaac ggttgaactt gtctacacac agttgtatgc tccaacaact
ttagttcctg cacgagattt ttggacgtta cggtagacaa ccacaatgga agatggcagt
cttgtgggtc gtgagagatc tttaagtggg tcagggggcg gtccaagtgc tgcctctgct
cagcaatatg tgagagcggg aatgcttcca agtggatacc tggttcgccc atgtgaaggt
gggggatcaa ttgtgcacat agtggaccat ctggatcttg aggcattggag tgttcctgag
gtgcttcggc cactctatga atcttcaagg gtagtcgctc agaaaatgac tactgcggca
ctccggcaca tcagacaaat tgctcaagaa acaagtgggg aagtgggtgta tgccttgggg
aggcaaccag cagtgtctacg gacttttagt caaaggctga gcagaggctt taacgatgcc
attagtgggt tcaatgatga tgggtgggtc ataatgggtg gagacgggtg tgaagatgta
gttattgctt gcaactcaac taagaaagtt aggagtagca gcaatgcngn catcgcttt

RICE REV2 (SEQ ID NO:158)

cccaaaacc cagctcctcc cgccgccagc agntgctccg
 ngactgcccc atcctcgcca acatcgagcc caagcagatc aaggtctggt tccagaacag
 aaggtgccga gataagcagc ggaaggaggc atcaaggctt caggccgnga accgaaaatt
 gacggcgatg aataagcttn tcatggagga gaatgagcgt cttcagaagc aggnctccca
 gctgggtccat gagaacgcgt acatgaagca gcaacttcag aatccgncat tgggcaatga
 tacaagctgn gaatcaaatg tgaccactcc tcagaaccct ctgagagatg caagtaaccc
 gtctggactc cttacaattg cggaggagac cctgacagag ttcctctcca aggctacagg
 gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggtat
 tgtggccggt tcacatggtt gccgtggtgt tgctgcccggt gcctgtggtt tgggtgaatct
 agaaccaaca aagatcgtgg agatcttaaa agaccgcca tcttggttcc gtgattgtcg
 aagtcttgaa gtcttcacaa tgtttccagc tggaaatggt ggcacgatcg aacttgttta
 catgcagatg tatgctccta ctactttggt tcctgcacga gatTTTTTgga cacttagata
 cacaactaca atggatgatg gcagccttgt ggtctgtgag agatcattga gtggttctgg
 aggtggtnc aagtcagcct ccgcacagca atttgtaaga gctgagatgc ttcctagcgg
 ctatctagtg cggccatgcg aggggtggtg ctccgtcgtg catattgtgg accatctgga
 tcttgaggct tggagtgttc cagaagtgtc tcggccactc tacgagtcac ctagggtagt
 tgctcagaaa atgactgctg cagcngtgcg gcacatcaga caaattgctc aagagacaag
 cggggagggt gtatacgctt tggggaggca acctgctggt ttgcggacat ttagtcagag
 gttgagtaga ggcttcaatg atgctattag tggtttcaac gatgatggtt ggtctgtcat
 ggggtggggat ggcacgaag atgtgatcat tgcttgcaat gcaaagaggg ttaggaatac
 tagcncttcg gccaatgctt tt

Rice REV1 protein (SEQ ID NO:159):

maaavamlgsssdgggydkvsgmdsgkyvrytpeqvealervyadc pkptssrrqqlrecp
 ilaniepkqikvwfqnrrcrdkqrkessrlqavnrlktamnkllmeenerlqkqvsqlvhenamrqg
 lqntplandtscesnvttpqnplrdasnp sgllsiaetlteflskatgtaidwvqmpgmkpgpds v
 ivaishgcpwcccracglvnleptkvveilkdrpswfrdcnlevftmipagnggtvelvytqlyapt
 tlvpardfwtlrytttmedgslvvcerslsgsgggpsaasaqgyvraemlpsgylvrpcegggsivhi
 vdhlidleawsvpevlrplyessrvvaqkmttaalrhirqiaqetsgevvaylgrqpavlrtfsqrlsr
 gfndaisgfnddgwsimggdgvedvviacnstkkvrsssnaxiafgapggii

Rice REV2 protein (SEQ ID NO:160):

ervycecpkpssrrqqlrdcpilaniepkqikvwfqnrrcrdkqrkeasrlqaxnrklta
 mnklxmeenerlqkqxsqlvhenaymkqqlqnp xlgndtsxesnvttpqnplrdasnp sglltiaet
 lteflskatgtavdwvmpgmkpgpdsfgivavshgcr gvaaracglvnleptkiveilkdrpswfrd

crslevftmfpagnnggtielvymqmyapttlvpardfwtlrytttmdgslvvcerslsrgsgggxsxa
 saqqfvraemlpsgylvrpcegggsvvhivdhldleawsvpevlrplyessrvvaqkmtaaavrhirq
 iagetsgevvyalgrqpavlrftfsqrllsrgfndaisgfnddgwsvmggdgiedviiacnakrvrntsx
 sanafvtpggvi

tomato J1R (SEQ ID NO:162):

CAGCAGAATAAGCATCAACATTATAATCNGCCCAYT

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

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCATATTCTGTCGAATATCGAGCCTAAGCAGAT
CAAAGTTTGGTTTCAGAACAGAAGGTATACTTCCATTGTTCAATTTTGCCCAAATTTTGGTTTATGTT
 TTGTTGTTAATTGCATACATTTTATATGTCTATTGTGTACGATTGATCTGCACTTTACTTTGTTTAG
 TACTGCTCGAATCTTGTATTAGTTAGATCAGTGATGATAAACTGAATGTATCACTGTAGTTCTCCTTG
 CCTAGGCTTGTTGGTTGAGTGGTAGGGTATGTGTTAACCTTAGGTGATTGGGAAATTGAGCTTAGAGT
 TTGGTATGGAGGGTAAACGTTGTATCATTTCAGGTGTCGAGAGAAGCAAAGGAAAGAGTCTTCTCGAT
TGCAGACTGTGAACAGAAAGTTGTCTGCGATGAATAAACTGTTGATGGAGGAGAATGACCGCTTGCAG
AAACAAGTCTCGCAGCTTGTATGTGAAAATGGCTATATGCGGCAACAATTGCAAAATGTAAGCTAACT
 TAACTCTTCGTTTATTTTTTATGTCCAAAAGCTCCATGTGTTGCTTACTATATAGTAGATTAATGTCA
 AACATATCTTGTCTTTTTTGTTCACCTTGATCTATGCTGCTGAAATGGCTACTCACTGTGTAGTCTAGA
 TTATACAATATTCCACCGCTATTGAGTCCATGATTTTAATCAGTCAGTCTTATAATTCTGGAATGCGT
 TACTTTATATATGGGACTAAATTGGCATGGCATTATTTTTGTGTAGTAGTACAAGAAACATTTAAGGT
 CCTGTGACTTCAAAATTGTAAGATGACAGATATCACCAGTCATTTGTGGATCAAGAGGACTTAATTTA
AGCTTACTTAAGACTCTAATTGTGTTTGCTGCAGGTATCGGCGGCCACTACTGATGTAAGTTGTGAAT
CAGGGGTAACCACTCCTCAGCATTCCTTAGAGATGCTAACAACCTGCTGGGTAATAATTTAAAACA
 GCTATTTCTTTCACCTCCTTACTTATATGATGTTAATTCTAAAACGTGTTCACTACTGTATCTTTGGAGG
 AAGTAAATAGCAAATTTCACAATTTAAGGGACTGATTATTTATCTCTAAGTCATGTTTATTCTCTATG
CAGACTACTACCAATTGCAGAAGAAACCTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTCTG
ATTGGGTCCCGATGCCTGGGATGAAGGTGAACTCTAGTCAATCACCTTTTATTTTAAATTCAGT
 ATTTCCATCTGTATCATTGACCAGACGGCTAAAAGGCAATATTATCATTCAATTGTCAGCCTGGTCCG
GATTCAGTTGGGATTTTGGCCATCTCACACAGTTGCAGTGGAGTGGCAGCCCGAGCATGTGGTCTTGT
TAGTTTAGAGCCAACAAAGGTAAACAATTGGAAGTCTATTCAGAAATATTACTGCTGCTCCATTGCTA
GTTTTAGTCCATTAATGATTGTAGATGTTGTCAGCTTTTCTTACTAAAACATTTTACAGATTGCTGA
TATCCTCAAAGATCGACCTTCTTGGTTCCGCGACTGCCGGAATGTTGAAGTTATCACAATGTTTCCTG
CTGGAAATGGTGGTACAGTTGAGCTTTTGTATACCCAGGTGAATACCTTCTCCTCAATCTCTATGTAC

ACTTCTGATTTGATTAGATACAGCATTGAGGGGATCAATGAATCATTTCTTTCAGATATATGCTCCCA
CAACTCTGGCTCCCGCGCGTGATTTTTGGACGCTGAGATACACAACAACCCTAGACAATGGTAGTCTC
GTGGTAAGCAATCCTTCACATTTAAGTGAGCTTGTGTTGGCGACCTGGCCACTTTTATACTTAGTTCT
 GGCATTCCCTGGTTTAACTAGTCTTTTAAACATCTCAACCTTTCAATCCTTGGATTGAACAGAAGTCCT
 GAAATGTAATATTTTTGGGTCATATTTAACCAAATGCTGCATTATAATCCCCGTCTAGACCTTTGAGT
 ATCTTGCTACTTCAGTATAATACTTGGCTCCATTATTTGTGATTCTTAATAGTGAATTCTATTAGCTG
 CGTCATTTGGTAGATGTTGCTCACAGTTTCTTTTTGTGTGGCATCAATTTATCCTCCTCACCAAGGTT
TGTGAAAGATCCCTATCTGGTAATGGGCCTGGCCCAAATCCTACTGCTGCTTCCAGTTTGTAAGAGC
TCAAATGCTTCCATCTGGATATCTGATCCGACCGTGTGATGGTGGAGGATCAATCATAATATTGTTG
ATCACCTGAATCTTGAGGTAAGATTTTGTAAGTACTGCTTACCTTTGTCATGAACCTGTTTTGCATG
 GTAGCTGCAATTCACCTCATATATTTTTTCAGGCATGGAGTGCCCCCTGAGATTTTGCGTCCACTCTATG
AATCGTCGAAAGTTGTGGCACAGAAAATGACTATTGCAGTGAGTTCAACCCTTCGTTATCATTTAATA
 CGGCATATAGATTTATATGTTTGTGAGGTTTAAAGTACTTGTGCAGTATCACACTTCCCATAGCTTAC
 TGCCACAGAAGAAGAACCATGATTTTATGCTTTACTTTCTTTTCTGTGAAGGCACTGCGATATGCAAG
GCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGGCAACCTGCTGTTCTCGAA
CATTCAGCCAGAGATTATGCAGGTGATGCTTATTTCTGATTTTTGTTATGTGGCTTTGAGATGATGAA
 AATTTATGCACTTCTGAGATGCCAATTCTGAAGTACATATACAAGTACCTTATTAGGCCATTTCTATA
TTGCAGAGGGTTCAATGATGCCATCAATGGATTCGGTGACGATGGCTGGTCAATGTTAAGTTCAGATG
GTGCTGAAGATGTCATAGTTGCTGTCAATTCAGGAAGAACCTCGCAACCACCTCCATTCCTCTTTCC
CCGCTTGGTGGCGTCCTTTGTACCAAAGCATCAATGCTACTCCAGGTGAATAGTGGATCTTTCTTGAA
 CTGAATAGAATTTTTTCAATTCGACAACTACCTTGCTCTTGTTAATACACAACAAACAGAAGTTCACAAG
 TTCATATTTGCATCCTCTTTTACGATACCAACTGAGAGACTGGTCCATATCAGCAATAGATGGAGTTA
 ATTGTTAAGACAAGTGTAAGTGGATAAATGAGAATAATTTGACTCTTTTGTTCCTGGCAGAAATGTCC
CCCCTGCCGTACTGGTTCGGTTTCTGAGGGAGCACCGTTCAGAA

Tomato REV coding (SEQ ID NO:164):

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCATATTTCTGTCGAATATCGAGCCTAAGCAGAT
 CAAAGTTTGGTTTCAGAACAGAAGGTGTCGAGAGAAGCAAAGGAAAGAGTCTTCTCGATTGCAGACTG
 TGAACAGAAAGTTGTCTGCGATGAATAAACTGTTGATGGAGGAGAATGACCGCTTGCAGAAACAAGTC
 TCGCAGCTTGTATGTGAAAATGGCTATATGCGGCAACAATTGCAAAATGTATCGGCGGCCACTACTGA
 TGTAAGTTGTGAATCAGGGGTAACCACTCCTCAGCATTCCTTTAGAGATGCTAACAACCCTGCTGGAC
 TACTACCAATTGCAGAAGAAACCTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTCGATTGG
 GTCCCGATGCCTGGGATGAAGCCTGGTCCGGATTCAGTTGGGATTTTTGCCATCTCACACAGTTGCAG
 TGGAGTGGCAGCCCGAGCATGTGGTCTTGTTAGTTTAGAGCCAACAAAGATTGCTGATATCCTCAAAG

ATCGACCTTCTTGGTTCCGCGACTGCCGGAATGTTGAAGTTATCACAATGTTTCCTGCTGGAAATGGT
 GGTACAGTTGAGCTTTTGTATACCCAGATATATGCTCCCACAACTCTGGCTCCCGCGCGTGATTTTTG
 GACGCTGAGATACACAACAACCCTAGACAATGGTAGTCTCGTGGTTTGTGAAAGATCCCTATCTGGTA
 ATGGGCCTGGCCCAAATCCTACTGCTGCTTCCCAGTTTGTAAAGAGCTCAAATGCTTCCATCTGGATAT
 CTGATCCGACCGTGTGATGGTGGAGGATCAATCATAATATTGTTGATCACCTGAATCTTGAGGCATG
 GAGTGCCCCTGAGATTTTTCGCTCCACTCTATGAATCGTTCGAAAGTTGTGGCACAGAAAATGACTATTG
 CAGCACTGCGATATGCAAGGCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGG
 CAACCTGCTGTTTCTCGAACATTCAGCCAGAGATTATGCAGAGGGTTCAATGATGCCATCAATGGATT
 CGGTGACGATGGCTGGTCAATGTTAAGTTCAGATGGTGGTGAAGATGTCATAGTTGCTGTCAATTCAA
 GGAAGAACCTCGCAACCACCTCCATTCTTCTTCCCGCTTGGTGGCGTCCTTTGTACCAAAGCATCA
 ATGCTACTCCAGCAGAATGTCCCCCTGCCGTACTGGTTCGGTTTCTGAGGGAGCACCGTTCAGAA

Tomato REV protein (SEQ ID NO:165)

SSLRRQQLIRECHILSNIEPKQIKVWFQNRRCREKQKRESSRLQTVNRKLSAMNKLLMEENDRLQKQV
 SQLVCENGYMRQQLQNVSAATTDVSCESGVTTTPQHSLRDANNPAGLLPIAEETLAEFLSKATGTAVDW
 VPMPGMKPGPDSVGIFAISHSCSGVAARACGLVSLEPTKIADILKDRPSWFRDCRNVEVITMFPAGNG
 GTVELLYTQIYAPTTLAPARDFWTLRYTTTLDNGSLVVCERSLSGNGPGPNPTAASQFVRAQMLPSGY
 LIRPCDGGGSIIHIVDHLNLEAWSAPEILRPLYESSKVVAQKMTIAALRYARQLAQETSGEVVYGLGR
 QPAVPRTFSQRLCRGFNDAINGFGDDGWSMLSSDGAEDVIVAVNSRKNLATTSIPLSPLGGVLCTKAS
 MLLQQNVPPAVLVRFLREHRSE

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

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

R22S (SEQ ID NO:167): CGACGACTCCTGGAGTCCGTCAG

(SEQ ID NO:168): TGTATCATTTGCCAGCGGAG

Rice Genomic REV1 (SEQ ID NO:169):

1 gtaagtgcc cataacttgct aacattgagc ccaagcagat caaggtctgg ttccagaaca
 61 gaaggtaatg ataatagaat tgaatctttc tacgtttggt ctctgtgaca aaaacttggt

121 atggcagttt tccctgattg tttcatctgt cacctgaaat aacatttcgt
tagtttcctt
181 ctggggaggg tatggttcta atatgctgcg ttgttggtgt gatgatgagc
ggtattttga
241 tatgagggga gctgcaatgc caattgttta tcatttcatt tgtttgcgca
ccagcaaaat
301 gtagtataat tacttttagct gacggctctg catgtatatg attttttcgt
ttttcatgnt
361 cgctgaagtg gatacttggtg cttgtgttgc tctaggtgcc gggataagca
gcggaaggag
421 tcttcacggc ttcaggctgn caacaggaaa ttgacggcaa tgaacaagct
acttatggaa
481 gagaatgagc gactccagaa gcaggctctc caattggttc atgagaatgc
ccacatgcga
541 cagcagctgt agaatgtaag ctcttgatgt gctgggtgctg atgttgctcc
ccatgcataa
601 acaygttctc actgaaatgc tatctattcy ttgygcattt tgttatacgc
atggcatgtc
661 cggggatgtg ttgttctgta ctgtatattg tagattagta taactttaaa
atttgatgta
721 tgtgtagcta taccagctgg ggccatatgc gtcagttcct ttagaattga
tatatgaatt
781 aatcctcaga atgtccatga gatcgctaga tttcactgat aacaccactt
gcttgggtgc
841 agactccgct ggcaaatgat acaagctgtg aatcaaagt gactaccct
caaaaccctt
901 taagggatgc aagtnacccc tctgggtaag taaatagttc tgagtgactc
aggtagaatt
961 attggttgat ggacktgctc tttcgatctc atgctatctt aactgccttt
tatcttgytc
1021 taggctcctt tcaattgcag aggagacctt gacagagttc ctctcaaagg
ctactggtac
1081 cagctattga ttgggtccag atgcctggga tgaaggttcc atgctagcac
tgtttgtttt

1141 tttgttctgt gattcgtgct aagaggtttt tacttgaagt gcttactacc
cttttgtttt
1201 catgatgtaa gcctgggtccg gattcgggtg gtattgtggc catttcacat
ggttgccgtg
1261 gtggtgctgc ccgtgcctgt gggtcgggtga acctagancc aacaaaagta
agtgttgtag
1321 ctatttggtt acatgggttt ggtattttta tgttncctca gtattccctg
gtctgtatgt
1381 tttctgaagc atctattttg gggatgtagc aagcctatcc accagtcact
tagttttctt
1441 tgtgtgcaaa tggtagaaa cctactacct ccatcccaa atatagcaa
aagttgctat
1501 atcaaaaatc ctatcagaag tggctcctga acacattgct gccgagtgtg
gaattaagac
1561 aactgtaat tcactttaat aaatactaaa ctttgaagat gtcactttag
aggtctaatg
1621 atttcatgtc tgccaactgt tatcatcaa tttaatcgtg aagataagca
gatattctgc
1681 tttttttgtt actttattca ggagattttg tgtctcatag aactttgtta
cgtagggtgt
1741 agagatattg aaagatcgtc catcttggtt ccgtgattgt cgaaacctgg
aagtctttac
1801 aatgattcca gcaggaaatg gagggacggt tgaacttgtc tacacacagg
tgaacactgt
1861 ttcattttac attgtataat ggtatatcct cagtcttctc tatcaatgca
tgtgcttcat
1921 gccatgaaca ttattacttg tttttgctta cagttgtatg ctccaacaac
tttagttcct
1981 gcacgagatt tttggacgtt acggtacaca accacaatgg aagatggcag
tcttgtggta
2041 tgtatgaaca tgaacactgt tttcacccca caatgagtct caatgtgatg
ttacccttgc
2101 taatattcct ccatctccaa ggtctgtgag agatctttaa gtggttcagg
gggcggtcca

2161 agtgctgcct ctgctcagca atatgtgaga gcggaaatgc ttccaagtgg
atacctgggt
2221 cgcccatgtg aaggtggggg atcaattgtg cacatagtgg accatctgga
tcttgaggta
2281 tttttcacac tttgtacag ttgaaccatg tttttgtcc ctttgatgta
ggaccatttt
2341 tgtatcctgt caaactaata atacaatttg ggtttaatct tttcaggcat
ggagtgttcc
2401 tgaggtgctt cggccactct atgaatcttc aagggtagtc gctcagaaaa
tgactactgc
2461 ggtaagctgt cgtgaaatga tattcagctc aaatttcatt gatgtgatta
caagttcatc
2521 atttcaagtg aaacttgttt ttaatgaact cttcaagttt cataacattg
gatttttttt
2581 tagaaaaaat aaaataaaaa tccaatatta tgaaacttga agagttcaag
ctaatgataa
2641 agtttgtgtt ttggataaag cttataatat tggatatgtg gtgaaaatga
tttatatggg
2701 ttggtacaac taatgataaa atttgccttt tggatatgtt gaacagttca
ttttctgcaa
2761 tctactttat actaaccttt tattgtctat cctatatatc aaggcactcc
ggcacatcag
2821 acaaattgct caagaaacaa gtggggaagt ggtgtatgcc ttggggaggg
aaccagcagt
2881 gctacggact tttagtcaaa ggctgagcag gtgatttttt tataaattat
tactcagcaa
2941 ttaatatttt tttcacctgt ttaatctaac accaatatta tgcttttctt
agaggtttca
3001 acgacgccgt gaacggc

RiceREV1 cDNA (SEQ ID NO:170):

1 cggggccgtg gctgtggggt ggctgtgagg gtgccccgg cggcgctccc
ctccgcgcct

61 gccggcgagg gggctcggac tgaagggatc taggcgagct gaaaattgaa
gygcaggcaa
121 ggagataaga gcagcgtcca aattgtgagt acttcattag caggaggtag
tggttggtgct
181 tgcttggctc ctttgcaatt tggctttggc gaggtagcaa tggctgcggc
agtggcaatg
241 ctagggagta gcagtgatgg aggtggctat gataaggttt ccgggatgga
ctccggtaaa
301 tatgtgcgct acacgcctga gcagggtggag gcgcttgagc ggggtgtacgc
cgattgcccc
361 aagccaacct cctcccgcag gcagcaattg ctgcgtgagt gcccataact
tgctaacatt
421 gagcccaagc agatcaaggt ctggttccag aacagaaggt gccgggataa
gcagcggaag
481 gagtcttcac ggcttcaggc tgtcaacagg aaattgacgg caatgaacaa
gctacttatg
541 gaagagaatg agcgactcca gaagcaggtc tcccaattgg ttcattgagaa
tgcccacatg
601 cgacagcagc tgcagaatac tccgctggca aatgatacaa gctgtgaatc
aaatgtgact
661 acccctcaaa accctttaag ggatgcaagt aaccctctg ggctcctttc
aattgcagag
721 gagaccttga cagagttcct ctcaaaggct actggtacag ctattgattg
ggtccagatg
781 cctgggatga agcctgggtcc ggattcggtt ggtattgtgg ccatttcaca
tggttgcccg
841 tgggtgttgct gccgtgcctg tggtttggtg aacctagaac caacaaaagt
ggtagagata
901 ttgaaagatc gtccatcttg gttccgtgat tgtcgaaacc tggaagtctt
tacaatgatt
961 ccagcaggaa atggaggaac ggttgaactt gtctacacac agttgtatgc
tccaacaact
1021 ttagttcctg cacgagattt ttggacgtta cggtacacaa ccacaatgga
agatggcagt

1081 cttgtggtct gtgagagatc ttttaagtggc tcagggggcg gtccaagtgc
 tgcctctgct
 1141 cagcaatatg tgagagcgga aatgcttcca agtggatacc tggttcgccc
 atgtgaaggt
 1201 gggggatcaa ttgtgcacat agtggaccat ctggatcttg aggcattggag
 tgttcctgag
 1261 gtgcttcggc cactctatga atcttcaagg gtagtcgctc agaaaatgac
 tactgcggca
 1321 ctccggcaca tcagacaaat tgctcaagaa acaagtgggg aagtgggtga
 tgccttgggg
 1381 aggcaaccag cagtgcctacg gacttttagt caaaggctga gcagaggctt
 taacgatgcc
 1441 attagtgggt tcaatgatga tgggtggtct ataatgggtg gagacgggtg
 tgaagatga
 1501 gttattgctt gcaactcaac taagaaagtt aggagtagca gcaatgcngn
 catcgcttt
 1561 ggagcccccg gaggtattat a

RiceREV1 Protein (SEQ ID NO:171):

maaavamlgsssdgggydkvsgmdsgkyvrytpeqvealervyadcpkptssrrqqlrecpilanie
 pkqikvwfqnrrcrdkqrkessrlqavnrkltamnkllmeenerlqkqvslvhenahmrqqqlqntpl
 andtscesnvttpqnplrdasnpzglslsiaeetlteflskatgtaidwvqmpgmkpgpdsvgivaish
 gcpwcccracglvnleptkvveilkdrpswfrdcnlevftmipagnggtvelvytqlyapttlvpar
 dfwtlrytttmedgslvvcerslsqsgggpsaasaqqyvraemlpsgylvrpcegggsivhivdhldl
 eawsvpevlrplyessrvvaqkmttaalrhirqiagetsgevyalgrqpavlrtsqrslsrgfndai
 sgfnddgwsimggdgvedvviacnstkkvrsssnaxiaf

RiceREV2 cDNA (SEQ ID NO:172):

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

tacaagctgn gaatcaaattg tgaccactcc tcagaaccct ctgagagatg caagtaaccc
gtctggactc cttacaattg cggaggagac cctgacagag ttcctctcca aggctacagg
gactgctggt gattgggtgc caatgcctgg gatgaagcct ggtccggatt cgtttggtat
tgtggccggt tcacatgggt gccgtgggtg tgctgcccgt gcctgtgggt tggatgaatct
agaaccaaca aagatcgtgg agatcttaaa agaccgccc tcttggttcc gtgattgtcg
aagtcttgaa gtcttcacaa tgtttccagc tggaaatggt ggcacgatcg aacttggtta
catgcagatg tatgctccta ctactttggt tcctgcacga gatttttgga cacttagata
cacaactaca atggaggatg gcagccttgt ggtctgtgag agatcattga gtggttctgg
aggtgggtCca agtacagcct ccgcacagca atttgtaaga gctgagatgc ttcctagcgg
ctatctagtg cgcccatgcg aggggtgggtg ctccatcgtg catattgtgg accatctgga
tcttgaggct tggagtgttc cagaagtgtc tcggccactc tacgagtcac ctagggtagt
tgctcagaaa atgactactg cagcngtgcg gcacatcaga caaattgtc aagagacaag
cggggagggt gtatacgctt tggggaggca acctgctgtt ttgcggacat ttagtcagag
gttgagtaga ggcttcaatg atgctataag tggtttcaaT gatgatgggt ggtctgtcat
gggtggggat ggcattgaag atgtgatcat tgcttgcaat gcaaagaa

RiceREV2 protein (SEQ ID NO:173) :

DCPILANIEPKQIKVWFQNRRCRDKQRKEASRLQAXNRKLTAMNKLXMEENERLQKQXSQ
LVHENAYMKQQLQNPXLGNDTSXESNVTTTPQNPLRDASNPSGLLTIAEETLTEFLSKATG
TAVDWVPMMPGMPGDSFGIVAVSHGCRGVAARACGLVNLEPTKIVEILKDRPSWFRDCR
SLEVFTMFPAGNGGTIELVYMQMYAPTTLPARDFWTLRYTTTMDGSLVVCERSLSGSG
GGPSTASAQQFVRAEMLPSGYLVRPCEGGGSIVHIVDHLDEAWSVPEVLRPLYESSRVV
AQKMTTAXVRHIRQIAQETSGEVVYALGRQPAVLRTFSQRLSRGFNDASGFNDGWSVM
GGDGIEDVIIACNAK

CHIMERIC PROTEIN (SEQ ID NO:174) :

(RICEREV1/AT REV)

maaavamlgsssdgggydkvsgmdsgkyvrytpeqvealervyadcpkptssrrqllrecpilanie
pkqikvwfqnrrcrdkqrkessrlqavnrltamnkllmeenerlqkqvsglvhenahmrqqqlqntpl
andtscesnvttppqnplrdasnpssllsiaeetlteflskatgtaidwvqmpgmkpgpdsfgivaish
gcpwcccracglvnleptkvveilkdrpswfrdcnlevftmipagnggtvelvytqlyapttlvpar
dfwtlrytttmdgslvvcerslsrgsgggpsaasaqqyvraemlpsgylvrpcegggsivhivdhldl

eawsvpevlrplyessrvvaqkmttaalrhirqiagetsgevvyalgrqpavlrtsqrslsrgfndai
 sgfnddgwsimggdgvedv/VAINSTKHLNNISNSLSFLGGVLCAKASMLLQNVPPAVLIRFLREHRS
 EWADFNVDAYSAAATLKAGSFAYPGMRPTRFTGSQIIMPLGHTIEHEEMLEVVRLEGHSLAQEDAFMSR
 DVHLLQICTGIDENAVGACSELIFAPINEMFPDDAPLVPSGFRVIPVDAKTGDVQDLLTANHRTLDLT
 SSLEVGPSPENASGNSFSSSSSRCILTIAFQFPFENNLQENVAGMACQYVRSVISSVQRVAMAI SPG
 ISPSLGSKLSPGSPEAVTLAQWISQSYSHHLGSELLTIDSLGSDDSVLKLLWDHQDAILCCSLKPQPV
 FMFANQAGLDMLETTLVALQDITLEKIFDESGRKAICSDFAKLMQQGFACLP SGICVSTMGRHVS YEQ
 AVAWKVFAASEENNNNLHCLAFS FVNWSFV

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

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

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

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

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):

AGCTCGTTGCGTCGACAGCAATTGATCCGTGAATGTCATATTCTGTCGAATATCGAGCCTAAGCAGAT
 CAAAGTTTGGTTTCAGAACAGAAG

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

GTGTCGAGAGAAGCAAAGGAAAGAGTCTTCTCGATTGCAGACTGTGAACAGAAAGTTGTCTGCGATGA
ATAAACTGTTGATGGAGGAGAATGACCGCTTGCAGAAACAAGTCTCGCAGCTTGTATGTGAAAATGGC
TATATGCGGCAACAATTGCAAAAT

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

GTATCGGCGGCCACTACTGATGTAAGTTGTGAATCAGGGGTAACCACTCCTCAGCATTCCCTTAGAGA
TGCTAACAACCCTGCTGG

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

ACTACTACCAATTGCAGAAGAAACCTTGGCAGAGTTCCTTTCTAAGGCTACAGGAACTGCTGTCGATT
GGGTCCCGATGCCTGGGATGAAG

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

CCTGGTCCGGATTCAGTTGGGATTTTTGCCATCTCACACAGTTGCAGTGGAGTGGCAGCCCGAGCATG
TGGTCTTGTTAGTTTAGAGCCAACAAAG

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

ATTGCTGATATCCTCAAAGATCGACCTTCTTGGTTCGCGACTGCCGGAATGTTGAAGTTATCACAAT
GTTTCCTGCTGGAAATGGTGGTACAGTTGAGCTTTTGTATACCCAG

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

ATATATGCTCCCACAACTCTGGCTCCCGCGCGTGATTTTTGGACGCTGAGATACACAACAACCCTAGA
CAATGGTAGTCTCGTG

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

GTTTGTGAAAGATCCCTATCTGGTAATGGGCCTGGCCCAAATCCTACTGCTGCTTCCCAGTTTGTAAG
AGCTCAAATGCTTCCATCTGGATATCTGATCCGACCGTGTGATGGTGGAGGATCAATCATAATATTG
TTGATCACCTGAATCTTGAG

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

GCATGGAGTGCCCCTGAGATTTTGC GTCCACTCTATGAATCGTCGAAAGTTGTGGCACAGAAAATGAC
TATTGCA

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

GCACTGCGATATGCAAGGCAACTAGCTCAAGAGACTAGCGGCGAGGTAGTATATGGTCTAGGAAGGCA
ACCTGCTGTTCTCTCGAACATTCAGCCAGAGATTATGCAG

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

AGGGTTCAATGATGCCATCAATGGATTCGGTGACGATGGCTGGTCAATGTTAAGTTCAGATGGTGCTG
AAGATGTCATAGTTGCTGTCAATTCAAGGAAGAACCTCGCAACCACCTCCATTCCTCTTTCCCCGCTT
GGTGGCGTCCTTTGTACCAAAGCATCAATGCTACTCCAG

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

CAGAATGTCCCCCTGCCGTACTGGTTCGGTTTCTGAGGGAGCACCGTTCAGAA

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An isolated DNA molecule comprising a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 70% identical to SEQ ID NO:2.
2. The DNA molecule according to Claim 1 wherein the encoded protein has a *REV* biological activity.
3. The DNA molecule according to Claim 1 wherein the encoded protein confers a *REV* phenotype.
4. 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.
5. 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.
6. The DNA sequence according to Claim 1 wherein 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, and 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.
7. An isolated DNA molecule comprising a polynucleotide sequence that encodes a protein comprised of an amino acid sequence at least about 80% identical to SEQ ID NO:2.
8. 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.

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9. 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.

10. 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.

11. 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.

12. An isolated protein comprising an amino sequence that is at least about 70% identical to SEQ ID NO:2.

13. The protein of Claim 12 wherein the protein has a biological activity that is the same as SEQ ID NO:2.

14. 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. 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.

16. A vector according to Claim 15 wherein the protein encoded by the polynucleotide sequence has the biological activity of SEQ ID NO:2.

17. A vector according to Claim 15 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO:2.

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

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19. A vector according to Claim 15 wherein the promoter region is operably linked to the polynucleotide.

20. A vector according to Claim 18 wherein the promoter region is transcriptionally active in a tissue and/or organ specific fashion.

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

22. The transformed plant cell of Claim 21 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.

23. The transformed plant cell of Claim 21 wherein said transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO:2.

24. The transformed plant cell of Claim 21 wherein the protein encoded by the polynucleotide sequence has the biological activity of SEQ ID NO:2.

25. The transformed plant cell of Claim 24 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO:2.

26. A transgenic plant comprising a *REVOLUTA* transgene, said transgenic plant comprising cells having modulated cell division as compared to an untransformed plant.

27. The transgenic plant of Claim 26 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.

28. The transgenic plant of Claim 26 wherein said transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID NO:2.

29. The transgenic plant of Claim 26 wherein the protein encoded by the polynucleotide sequence has the biological activity of SEQ ID NO:2.

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30. The transgenic plant of Claim 29 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID NO:2.

31. A method for modulating plant cell division comprising introducing into a plant cell a *REVOLUTA* transgene.

32. The method of Claim 31 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.

33. The method of Claim 31 wherein said transgene comprises a polynucleotide that encodes a protein that is at least about 70% identical to SEQ ID No.:2.

34. The method of Claim 33 wherein the protein encoded by the polynucleotide sequence has the biological activity of SEQ ID No.:2.

35. The method of Claim 33 wherein the protein is at least about 70% identical to amino acids 114 to 842 of SEQ ID No.:2.

36. The method of Claim 31, wherein the transgene comprises an inverted repeat of a polynucleotide sequence from a *REVOLUTA* gene.

37. The method of Claim 31, wherein the transgene comprises an anti-sense orientation of a polynucleotide sequence from a *REVOLUTA* gene.

38. The method of Claim 31, wherein the transgene comprises a sense orientation of a polynucleotide sequence from a *REVOLUTA* gene.

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

40. The method of Claim 33 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.

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

- 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 70% 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;
- 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
- e) determining that cell division in the transformed plant is modulated by comparing the transformed plant cell with an untransformed plant cell.

42. The method of Claim 41, 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.

43. The method of Claim 41, 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.

44. The method of Claim 41, wherein the isolated DNA is expressed by operably linking said isolated DNA adjacent to a heterologous promoter sequence.

45. The method of Claim 41, wherein the transformed plant has at least one plant organ with a greater number of cells than a corresponding untransformed plant organ.

46. The method of Claim 41, wherein the transformed plant has at least one plant organ with a smaller number of cells than a corresponding untransformed plant organ.

47. 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

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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.

48. 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.

49. A vector comprising the polynucleotide of Claim 48.

50. A plant transformed with the vector of Claim 49.

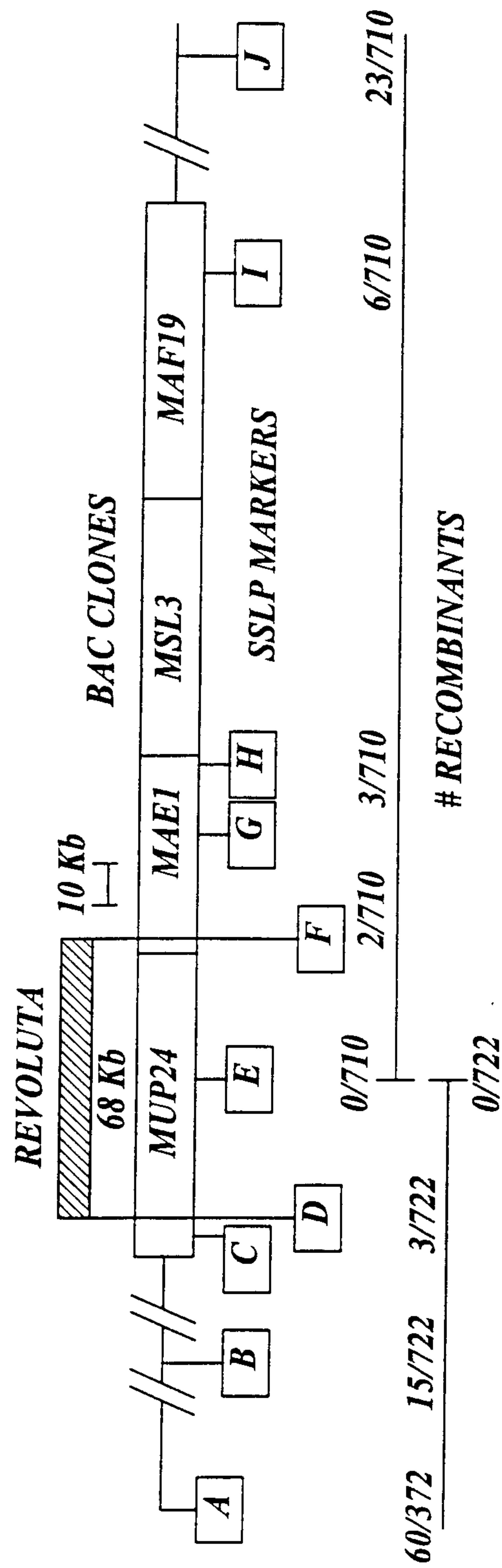
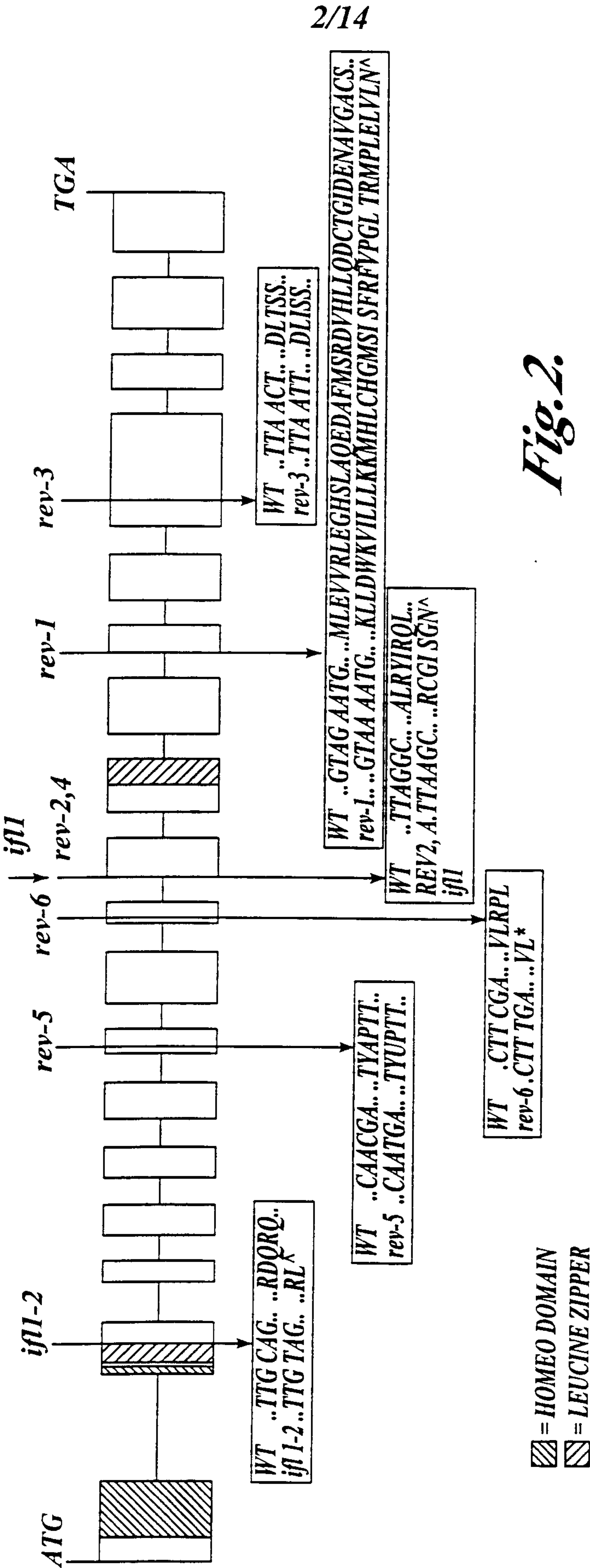


Fig. 1.



REVOLUTA Protein
and rev mutants

MEMAVANHRBSSDSMNRHL DSSGKYVRYT AEQVEALE^{rev-2,4}RVVAEC^{rev-5}PK^{rev-6}PSYRRQQLRECS
ILANIEPKQK^{rev-3}AVV^{rev-1}QVRR^{rev-1}CRDQ^{rev-1}QKEASRLQSYVRA^{rev-1}LS^{rev-1}THNALLME^{rev-1}ENDR^{rev-1}IQKQVSQLV^{rev-1}C
ENGYMKQQLT TV^{rev-1}NDPSCS VV^{rev-1}TPQHSLR DANSPAGLLS IAEETLAEFL SKATGTAVDW
VQMPGMK^{rev-1}PGP DSVGIFAISQ RCNGVAARAC GLVSLEPMK^{rev-1} AEILKDRPSW FRDCRSLEVF
TMFPAGNGGT IELVYM^{rev-1}QTYA P^{rev-1}TLAPARDF WTLRYTTSLD NGSFV^{rev-1}VCERS LSGSGAGPNA
ASASQ^{rev-2,4}FVRAE MLSSGYLIRP CDGGGSHIHI VDHLNLE^{rev-6}AWS VPDV^{rev-6}L^{rev-6}RP^{rev-6}LYE SSKVV^{rev-6}AQKMT
IS^{rev-2,4}ALRYI^{rev-2,4}ROL AQESNGEVVY GLGRQPAVLR TFSQRLSR^{rev-6}GF NDAVNGFGDD GIVSTMHC^{rev-6}DGA
RCGISGN^{rev-2,4}*
EDIIVAINST KHLNNISNSL SFLGGV^{rev-1}LCAK ASML^{rev-1}LQ^{rev-1}NVPP AVLIRFLREH RSEWADFNV^{rev-1}D
AYSAAATLKAG SFAYPGMRPT RFTGSQ^{rev-1}UIMP LGHTIEHEEM^{rev-1}LEVVRLEGHS LAQEDAFMSR
DVHLL^{rev-3}Q^{rev-3}CTG IDENAVGACS ELIFAPINEM FPDDAPLVPS GFRVIPVDAK TGDVQDLLTA
NHRTLDTSS LEVGPSPENA SGNSFS^{rev-1}SSSS RCILTIAFQF PFENNLOENV AGMACQYVRS
VISSVQRVAM AISPSGISPS LGSKLSPGSP EAVTLAQWIS QSY^{rev-1}SHHLGSE LLTIDSLGSD
DSVLKLLWDH QDAILCCSLK^{rev-1}Q^{rev-1}PVFMFANQ AGLDMLETTL VALQDITLEK IFDESGRKAI
CSDFAKLMQ^{rev-1}Q^{rev-1}GFACLP^{rev-1}SGIC VSTMGRHVSYEQAVAWKVFA ASEENNNNLH CLAFSFVNW^{rev-1}SFV

Fig. 3.

1 - - - - - NAM S - - - - - CND GKL GCLDN - GKXVRYTPEQVEALERLYH QCPKPSSTPRQOLI R
1 - - - - - MG - - - - - GGSNNSNNNDN - GKXVRYTPEQVEALERLYN QCPKPSSTPRQOLI R
1 - - - - - MAHHSM D - - - - - DRDSPDKGFDS - GKXVRYTPEQVEALERLYA ECPKPSSTPRQOLI R
1 - - - - - MMVHSM SRDMN RSPDKGLDS - GKXVRYTPEQVEALERLYT ECPKPSSTPRQOLI R
1 - - - - - MAVANHRER - - - - - SSDSVNRHLDS - GKXVRYTAEQVEALERLYA ECPKPSSTPRQOLI R

50 ECPIL SNI EPKCI KVWFQNRRCREKORKEASRLCAVNRKLTAMNKLLVEENDRLOKOV'SH
48 ECPIL SNI EPKCI KVWFQNRRCREKORKEASRLCAVNRKLTAMNKLLVEENDRLOKOV'SH
54 ECPIL CNI EPRCI KVWFQNRRCREKORKEASRLCAVNRKLTAMNKLLVEENDRLOKOV'SH
58 ECPIL SNI EPKCI KVWFQNRRCREKORKEASRLCAVNRKLTAMNKLLVEENDRLOKOV'SH
58 ECSILANI EPKCI KVWFQNRRCREKORKEASRLCAVNRKLTAMNKLLVEENDRLOKOV'SH

110 LVHENSYFRQHPTPNP - SLPAKDTSCESVATSC - QHQLASQNPORDAS - PAGLLSIAE
108 LVYENSYFRQHPQNCQSNLATTDTSCESVATSC - QHHLTPQHQPORDAS - PAGLLSIAE
114 LVYENG - FKKHRLHTASGTTTNDN SCESVATVSGQQRCQCP THQHPORDVKN PAGLLSIAE
118 LVYENG - HMKHQLHTASGTTTNDN SCESVATVSGQQHCQCP NPHQCORDVKN PAGLLSIAE
118 LVCENG - NKKQQLTTVVN - - - - - DPSCESVAT - - - - - TPCHSLRDVNS PAGLLSIAE

164 ETLAEFLSKATGTAVEMVQNPCKPKPGPDSIGINAI8HGLGNAARACGLVCLPTIRVAEI
163 ETLTEFLSKATGTAVEMVQNPCKPKPGPDSIGIVAI8HGLGCLAARACGLVCLPTIRVAEI
173 ETLAEFLCKATGTAVEDWVOMCKPKPGPDSIGIVAN8HGLGCLAARACGLVSLPTIRVAEI
177 ETLAEFLSKATGTAVEDWVOMCKPKPGPDSIGIVAI8HGLGCLAARACGLVSLPTIRVAEI
164 ETLAEFLSKATGTAVEDWVOMCKPKPGPDSIGIFAI8HGLGCLAARACGLVSLPTIRVAEI

Fig. 4A.

224 VKDRPSWEFRECRAVENMVLPTANGGTNELLYVCLYAPTTLAPPRDFWLLRYTSVLEDGS
223 LKDRPCLRDGRSLDIYMWLSTANGGTLELLVMCLYAPTTLAPARDFWMLRYTSVLEDGS
233 LKDRPSWEFROCRCEVETLMMVPTGNGGTLELLYNTQIYAPTTLAARDFWTLRYSTVLEDGS
237 LKDRPSWLRDGRSVDTLSMVPAGNGGTLELLYNTQIYAPTTLAARDFWTLRYSTVLEDGS
224 LKDRPSWEFROGRSLENFTNFPAGNGGTLELLYVMOITYAPTTLAPARDFWTLRYTTSVLEDGS

284 LNVYCERSLKSTQNGPSNPLVQNEFVRAEMLSSGYLIRPCDGGGSSIIHIVDHNDLAEACSVPE
283 LNVYCERSLNNQNGPSNPLVQNEFVRAEMLSSGYLIRPCDGGGSSIIHIVDHNDLAEACSVPE
293 LNVYCERSLTSATGGPNOPLSSSEFVRAEMLSSGYLIRPCDGGGSSIIHIVDHNDLAEACSVPE
297 LNVYCERSLTSATGGPNOPLSSSEFVRAEMLSSGYLIRPCDGGGSSIIHIVDHNDLAEACSVPE
284 LNVYCERSLSGSGAGPNAASAGQNEFVRAEMLSSGYLIRPCDGGGSSIIHIVDHNDLAEACSVPE

344 VLRPLYESPKVLAOKTITNAALRYLQKQIAGEVLTQTNSSVNGMGRPAAALRALSORLSRGEN
343 VLRPLYESSTLLAOKTITNAALRYLQKQIAGEVLTQTNSSVNGMGRPAAALRALSORLSRGEN
353 VLRPLYESSKI LACKNTNAALRYLQKQIAGEVLTQTNSSVNGMGRPAAALRALSORLSRGEN
357 VLRPLYESSKI LACKNTNAALRYLQKQIAGEVLTQTNSSVNGMGRPAAALRALSORLSRGEN
344 VLRPLYESSKI LACKNTNAALRYLQKQIAGEVLTQTNSSVNGMGRPAAALRALSORLSRGEN

404 EAVNGFTDEGWSVIGDSMDDVTITNNSSPDKLMMGLNLTFRANGFAPVSNVVLCAKAS
401 EAVNGFSOEGRWSLESDGIDDDVTLLNNSSPTKNNMTSSLPFRANGTSTSNVVLCAKAS
411 DAVNGFVDDGWSPPMSDGGGEDVTMMN8SSAKFAAGSSQ--XCS8FLPSFGSGVLCAKAS
415 DAVNGFVDDGWSPPMSGDGGGEDVTMMNLS8GKFGSSQ--XCN8FLPSFGSGVLCAKAS
402 DAVNGFGDDGWS TMHCDGNEETI VAIL8TKHLNNI S---NSL8FLG---GVLCAKAS

Fig. 4B.

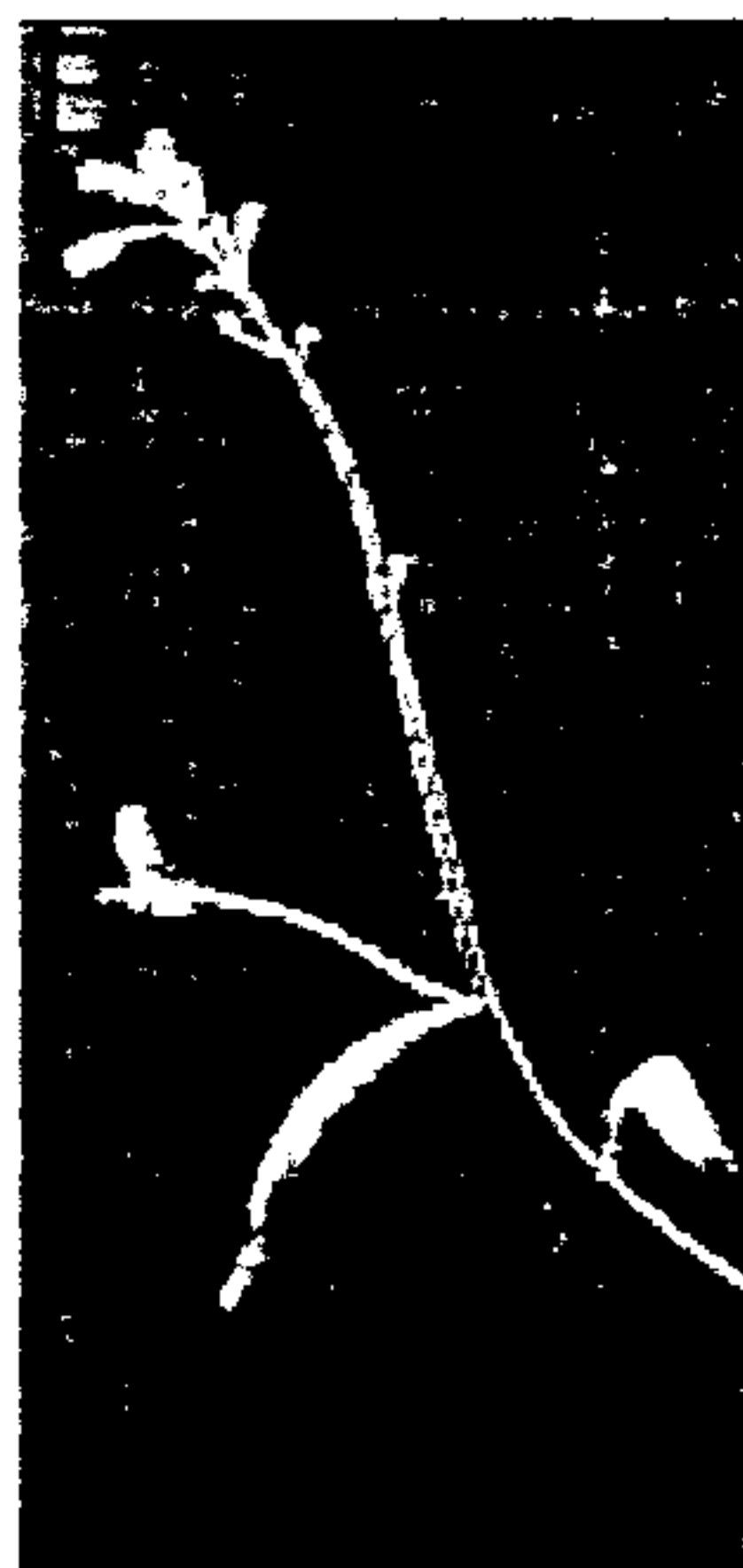
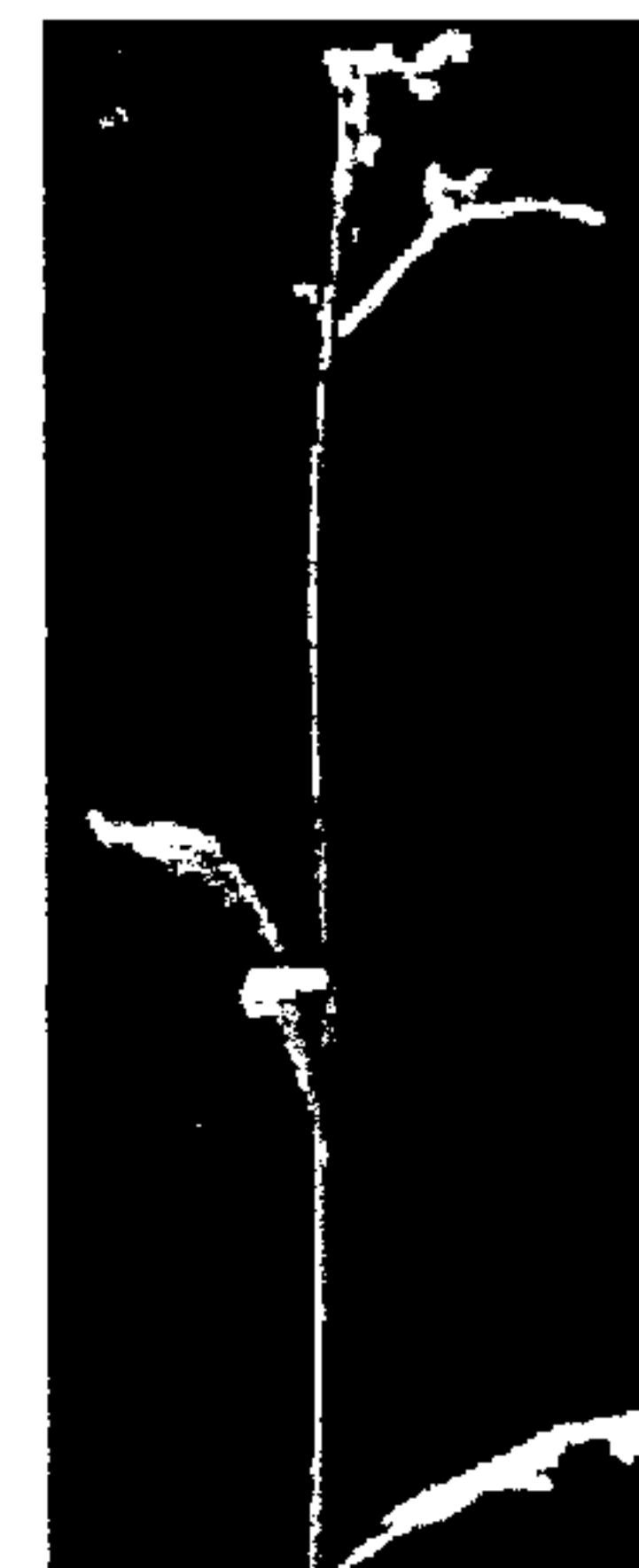
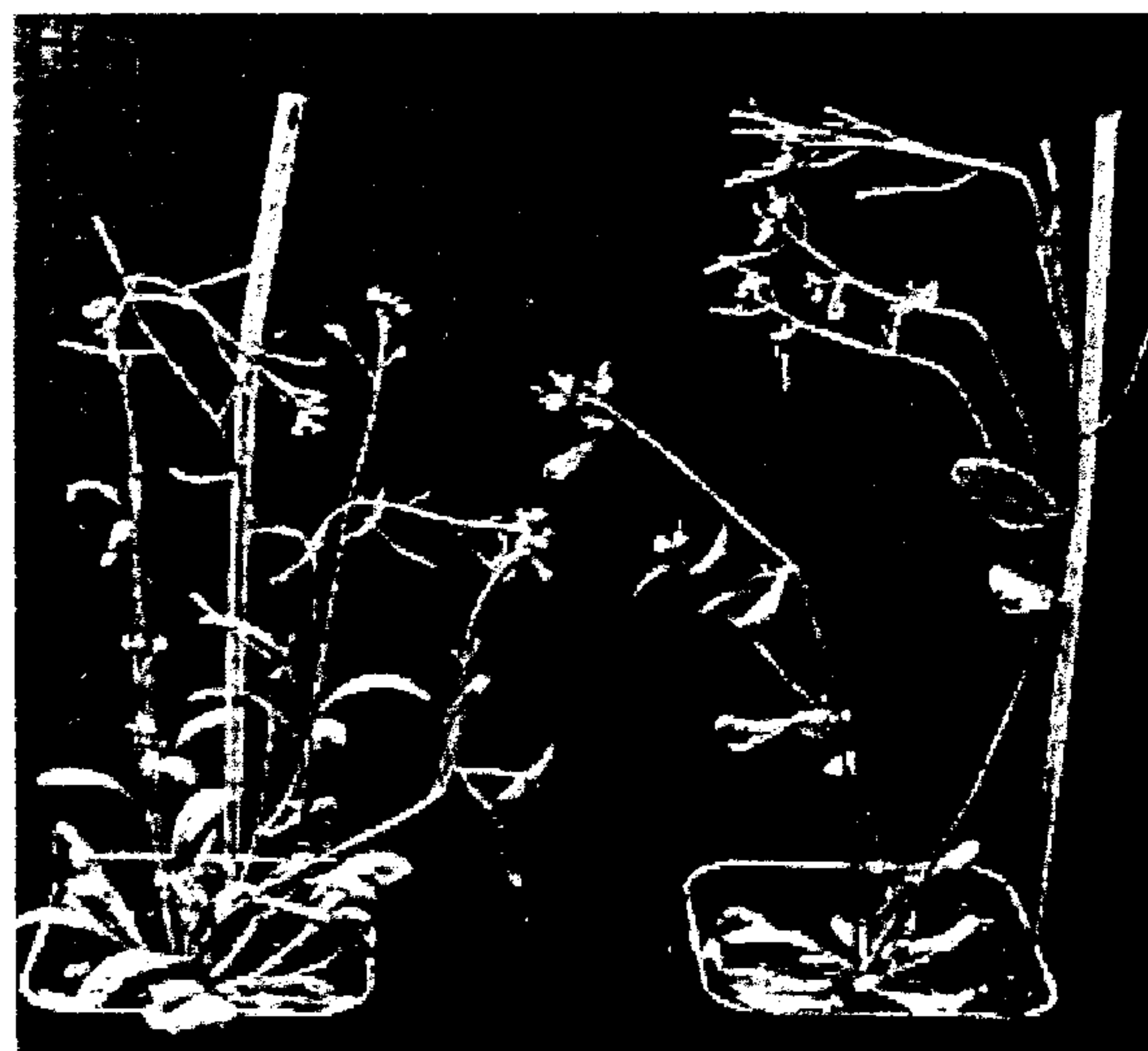
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460	LLQNVPPS	LLRELRCEVADNS	DAYSAAKAGP	CSLP	PRPGSF	GG-	QVILPLAH
468	LLQNVPP	LLRELRSEVADN	CVDAYSAATP	YANP	CVRTGGF	PSN	QVILPLAQ
472	LLQNVPPAVL	LLRELRSEVADN	CVDAYSAATSP	YANP	CAHACGF	PSN	QVILPLAQ
454	LLQNVPPAVL	LLRELRSEVADN	CVDAYSAATL	KAGS	YANP	GMPTRE	TGSONINPLCH
517	TIHEEFFNEV	TIHESPEDAI	PRDI	ELLQLOCSGNDENAV	CTCAELI	PAPI	DA SFAD
519	TIENEEFNEV	TIHESPEDAI	MPADI	ELLQLOCSGVDENAV	ESCAELI	FAPI	DA SFAD
528	TIHEEEFLEVRLE	TIHESPEDAI	SRDNY	ELLQLOCSGVDENAV	VGSCAOL	VF	API DE SFAD
532	TIHEEESLEVRLE	TIHESPEDAI	ARDNY	ELLQLOCSGVDENAV	VGSCAOL	VF	API DE SFAD
514	TIHEEEMLEVRLE	TIHESPEDAI	SRDNY	ELLQLOCSGVDENAV	VGSCAOL	VF	API DE SFAD
577	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI
579	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI
588	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI
592	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI
574	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI	LAPILLPSCFFRI
632	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI
631	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI
638	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI
646	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI
633	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI	VNTI AFECI

Fig. 4C.

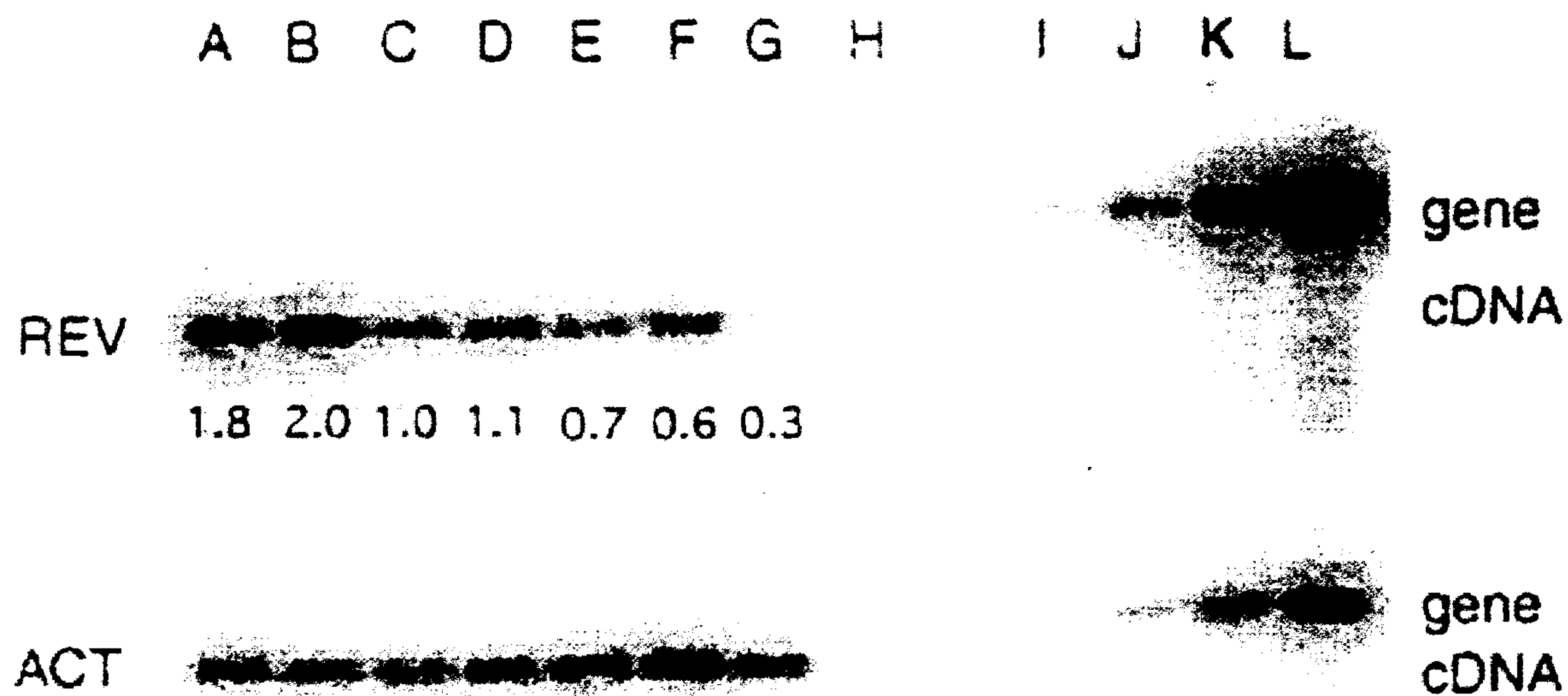
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690	HTLARWI	SHSYRCY	LC	VLLKPHG	----	TDL	LN	SL	LV	HPDANN	CCSLKA	LS--	PVF	TE	A							
693	LTLVRWI	TRSYSL	HTC	ALLFGAD	S	CSCGG	D	LN	LV	HSDAIL	CCSLK	TN-A	SPVF	TE	A							
705	LTLVRWI	SRSYSL	HTC	ALLFGSD	S	C-TSGD	T	LN	LV	HSDAIL	CCSLK	TN-A	SPVF	TE	A							
693	NTLAQWI	SCSYSHN	LC	SELLTI	D	L-GSD	DS	LN	LV	HSQDAIL	CCSLK	P---	CPVF	NE	A							
747	NCAGL	ENLETTL	VAL	CEI	SL	EKI	FD	N-	GRK	TL	CS	EF	P	CI	NCCCF	ACL	CG	CI	CL	SSN	GR	P
743	NCAGL	ENLETTL	VAL	CEI	TL	DK	FD	N	NGN	TL	S	EF	P	CI	NCCCF	ACL	CG	CI	CL	SSN	GR	A
752	NCAGL	ENLETTL	VAL	CEI	N	L	DK	TL	ES	GR	AL	CS	EF	AKI	NCCCF	ACL	CG	CI	CL	SSN	GR	P
763	NCAGL	ENLETTL	VAL	CEI	N	L	DK	TL	ES	GR	AL	CS	EF	PKI	NCCCF	ACL	CG	CI	CL	SSN	GR	M
749	NCAGL	ENLETTL	VAL	CEI	TL	EKI	FD	N	GRK	TL	CS	EF	P	CI	NCCCF	ACL	CG	CI	CL	SSN	GR	H
806	VSYE	RA	AV	WK	VL	NE	EE	NA	----	HCL	CF	EF	LN	SV	FN	836						
803	VTYE	K	AV	WK	VL	N	DE	CP	----	HCL	CF	EF	LN	SV	FN	833						
811	VSYE	QA	TV	WK	VL	N	DE	SN	----	HCL	AF	TL	SV	FN	841							
822	VSYE	QA	TV	WK	VL	E	DE	SN	----	HCL	AF	TL	SV	FN	852							
808	VSYE	QA	AV	WK	VL	FA	AS	EN	NNNL	HCL	AF	SE	VL	SV	FN	842						

Fig. 4D.

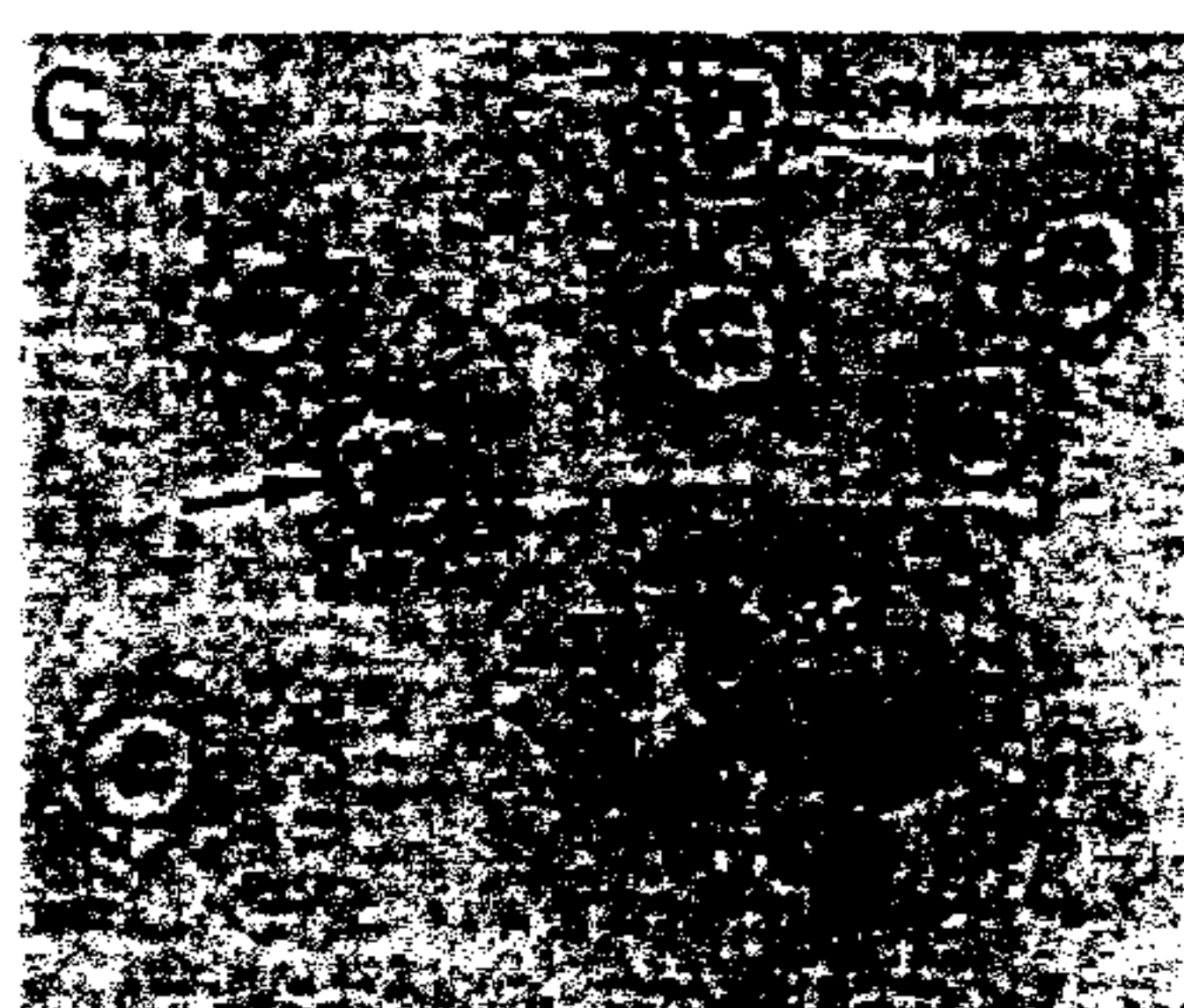
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*Fig. 5A.**Fig. 5B.**Fig. 5C.**Fig. 5D.**Fig. 5E.**Fig. 5F.**Fig. 5G.**Fig. 5H.*

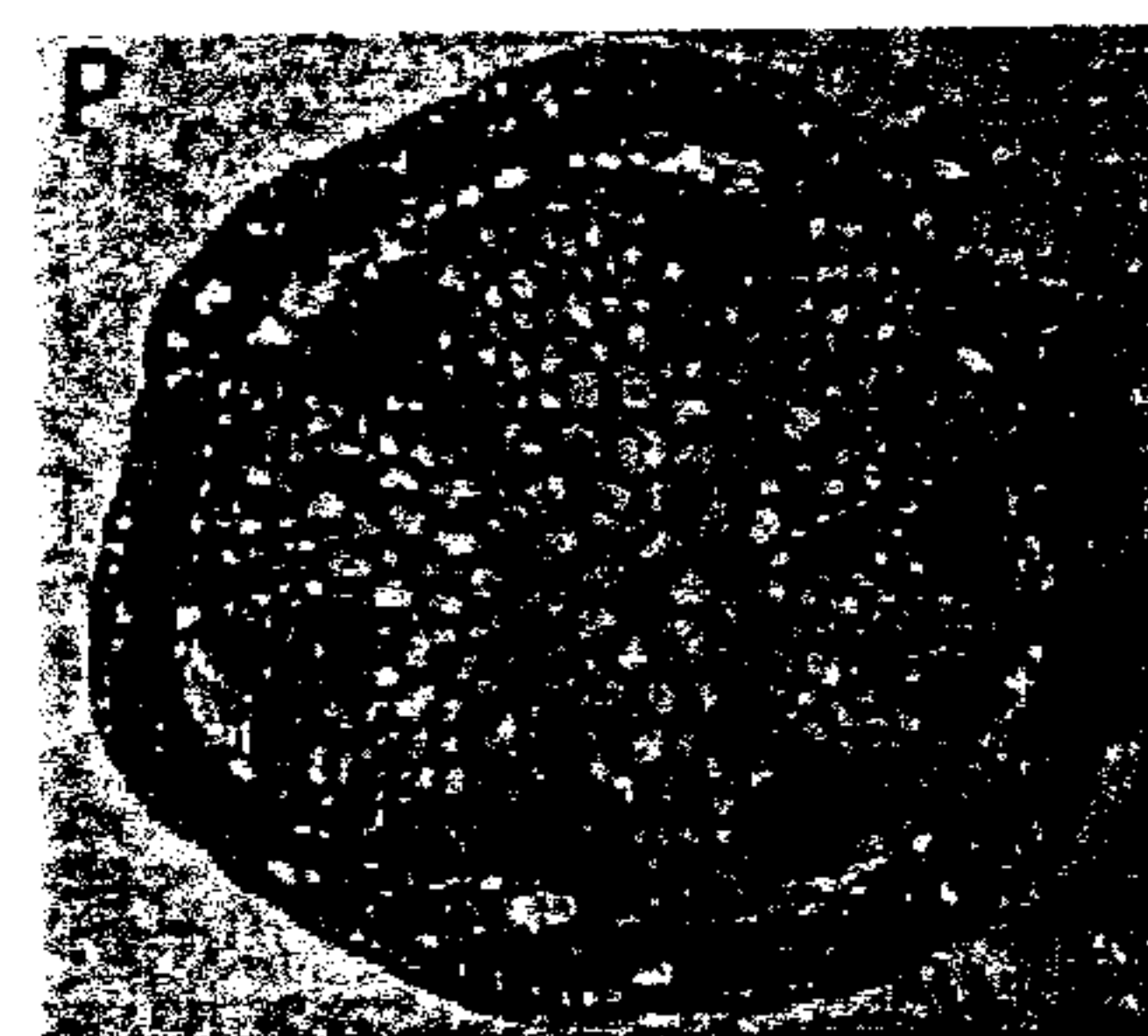
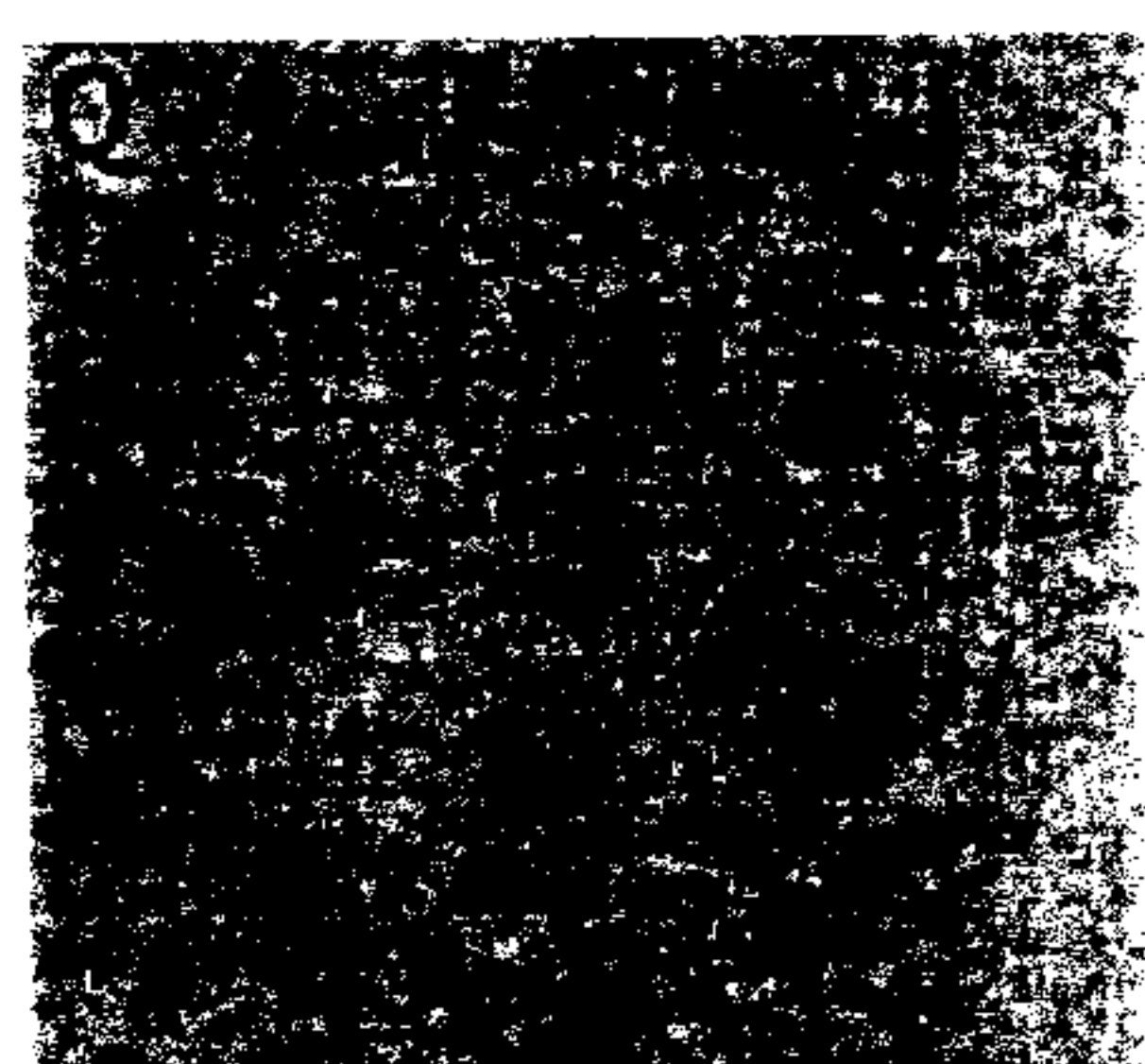
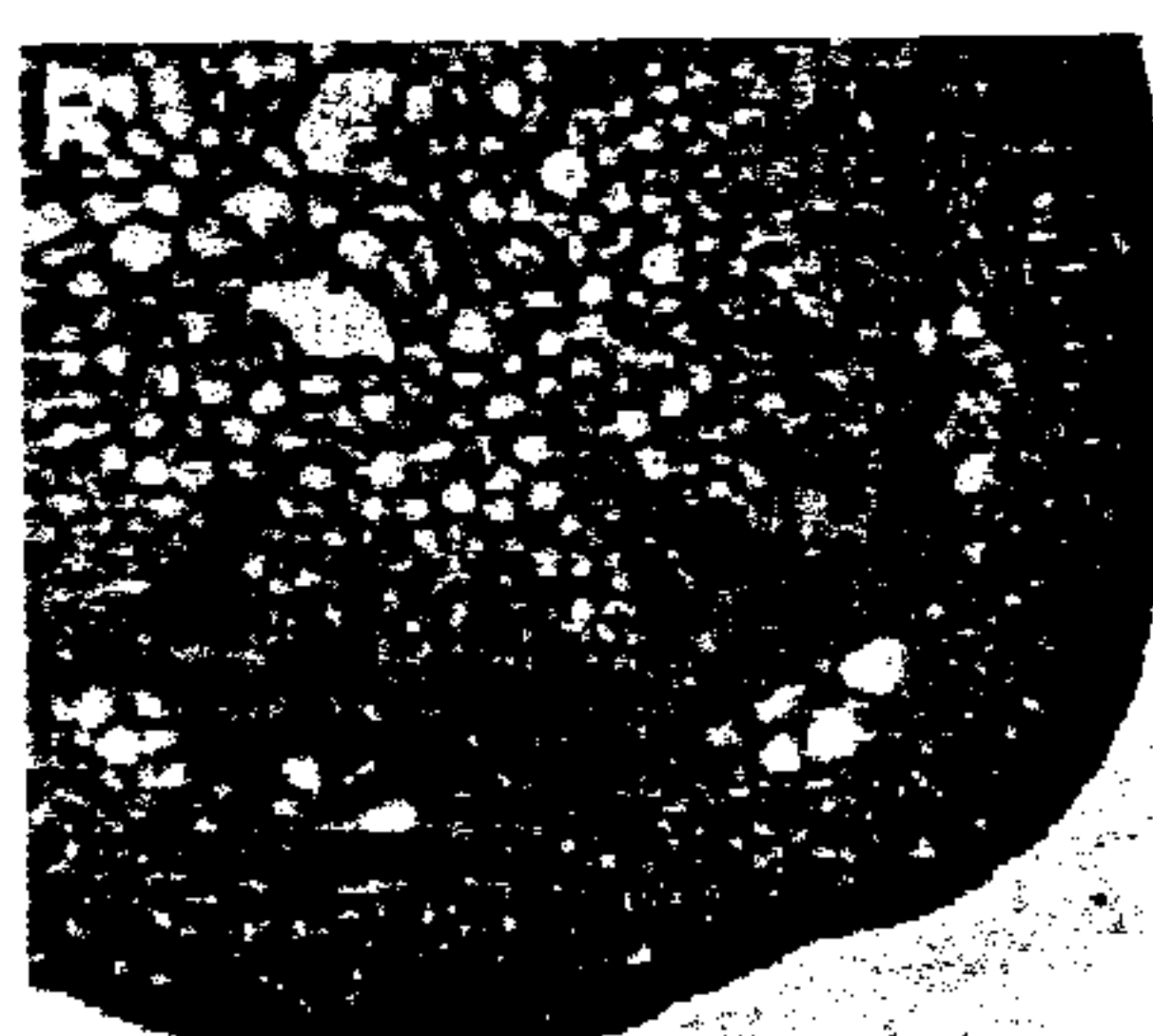
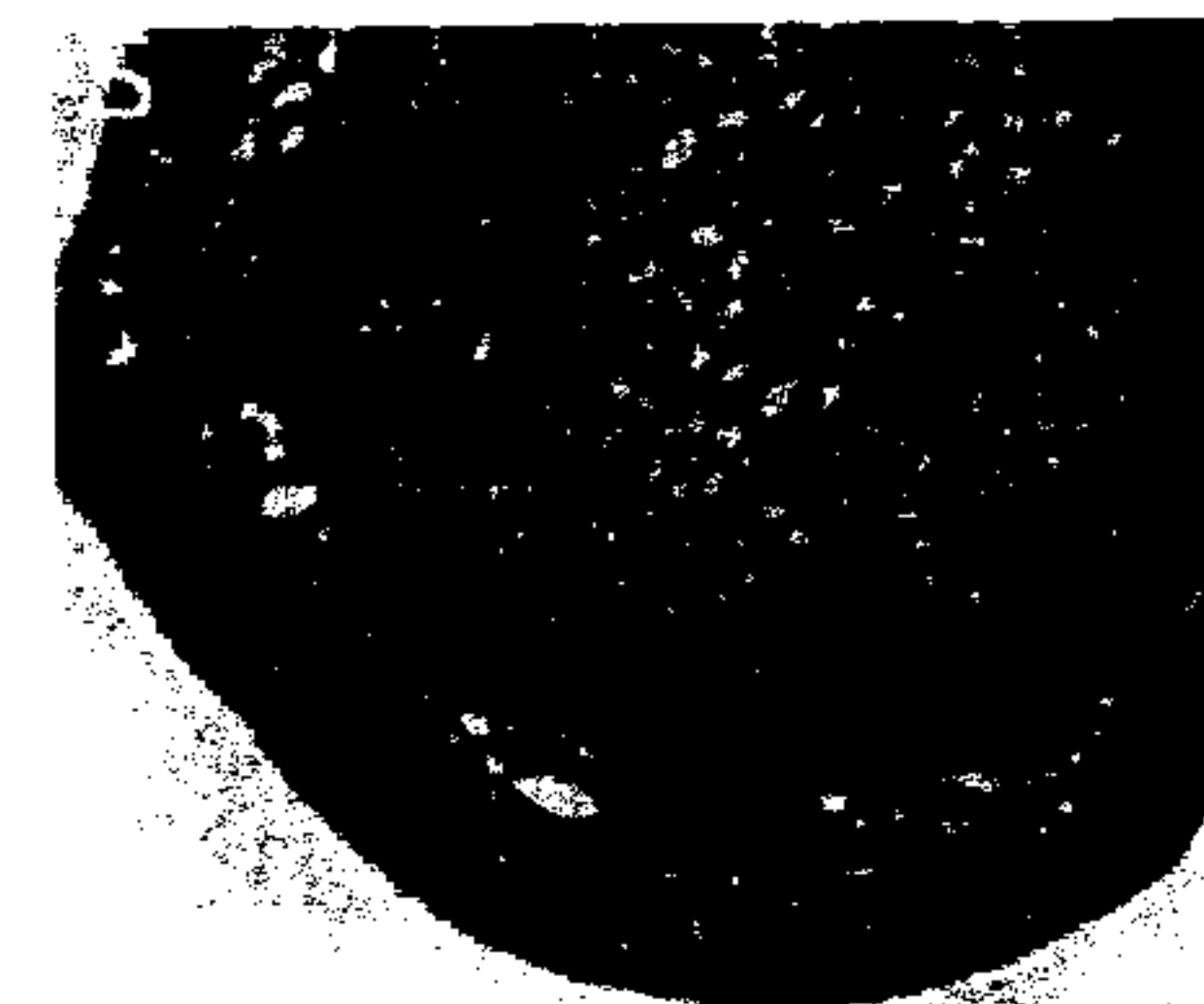
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*Fig. 6.*

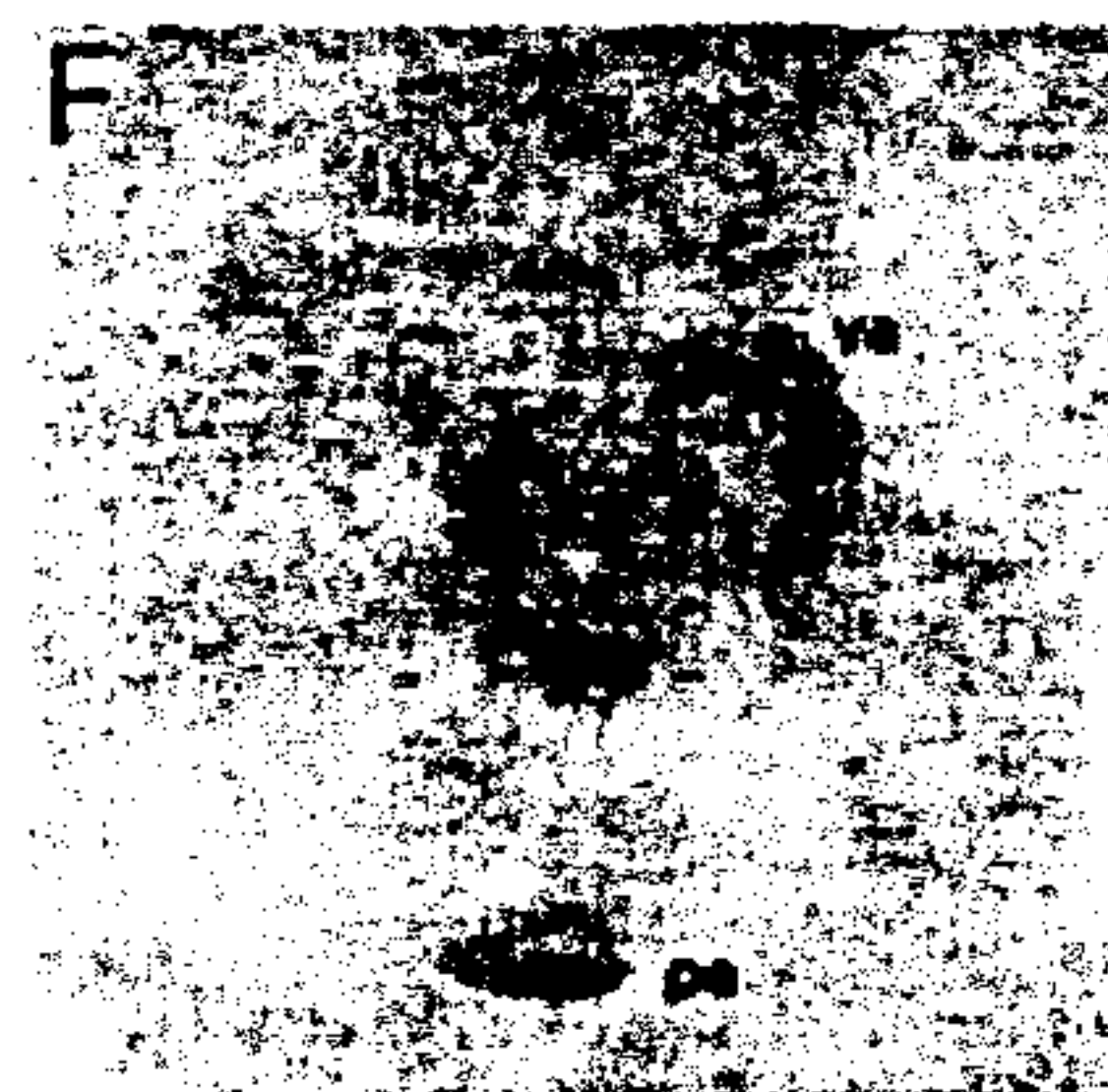
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*Fig. 7A.**Fig. 7B.**Fig. 7C.**Fig. 7D.**Fig. 7E.**Fig. 7F.**Fig. 7G.**Fig. 7H.*

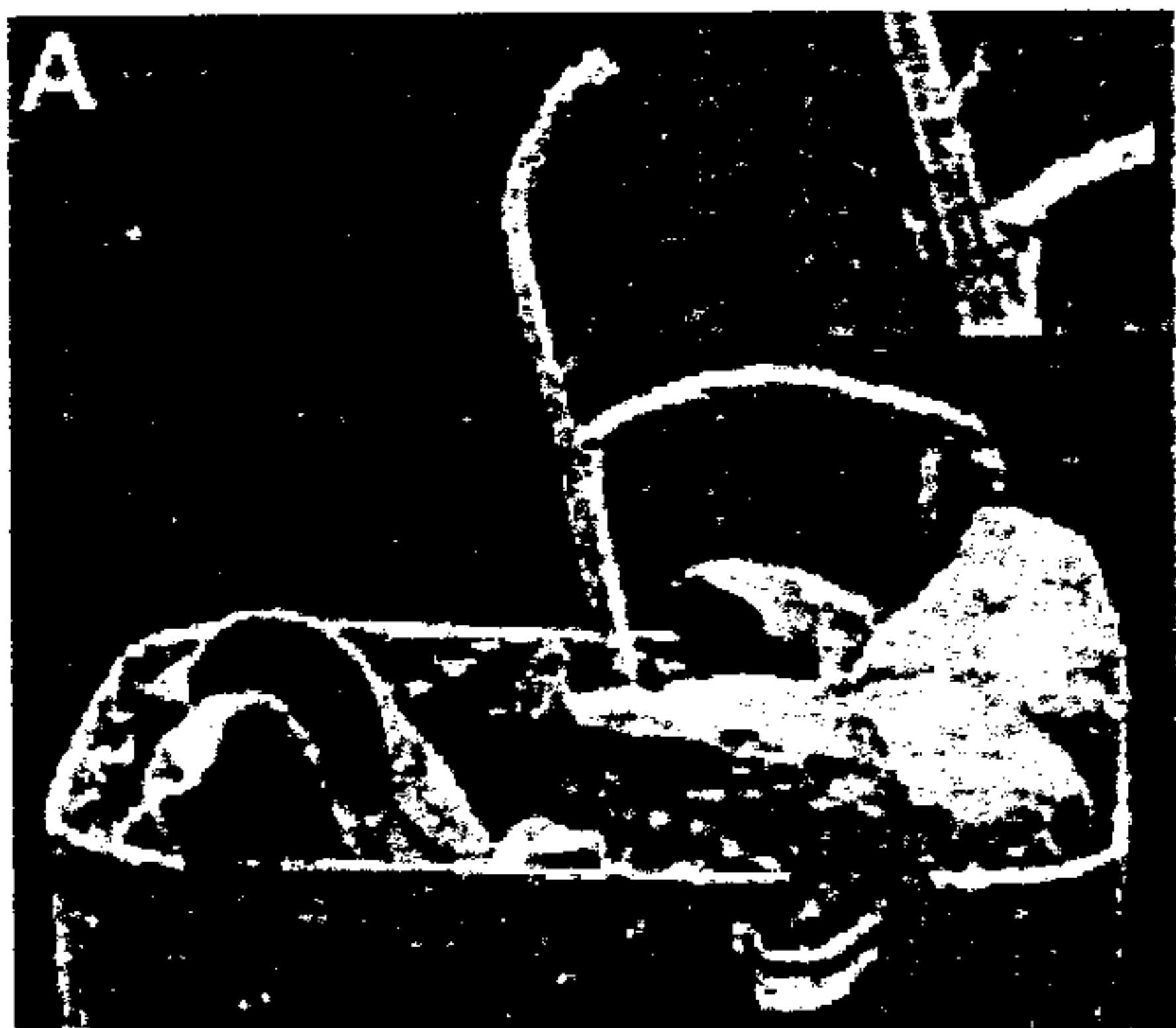
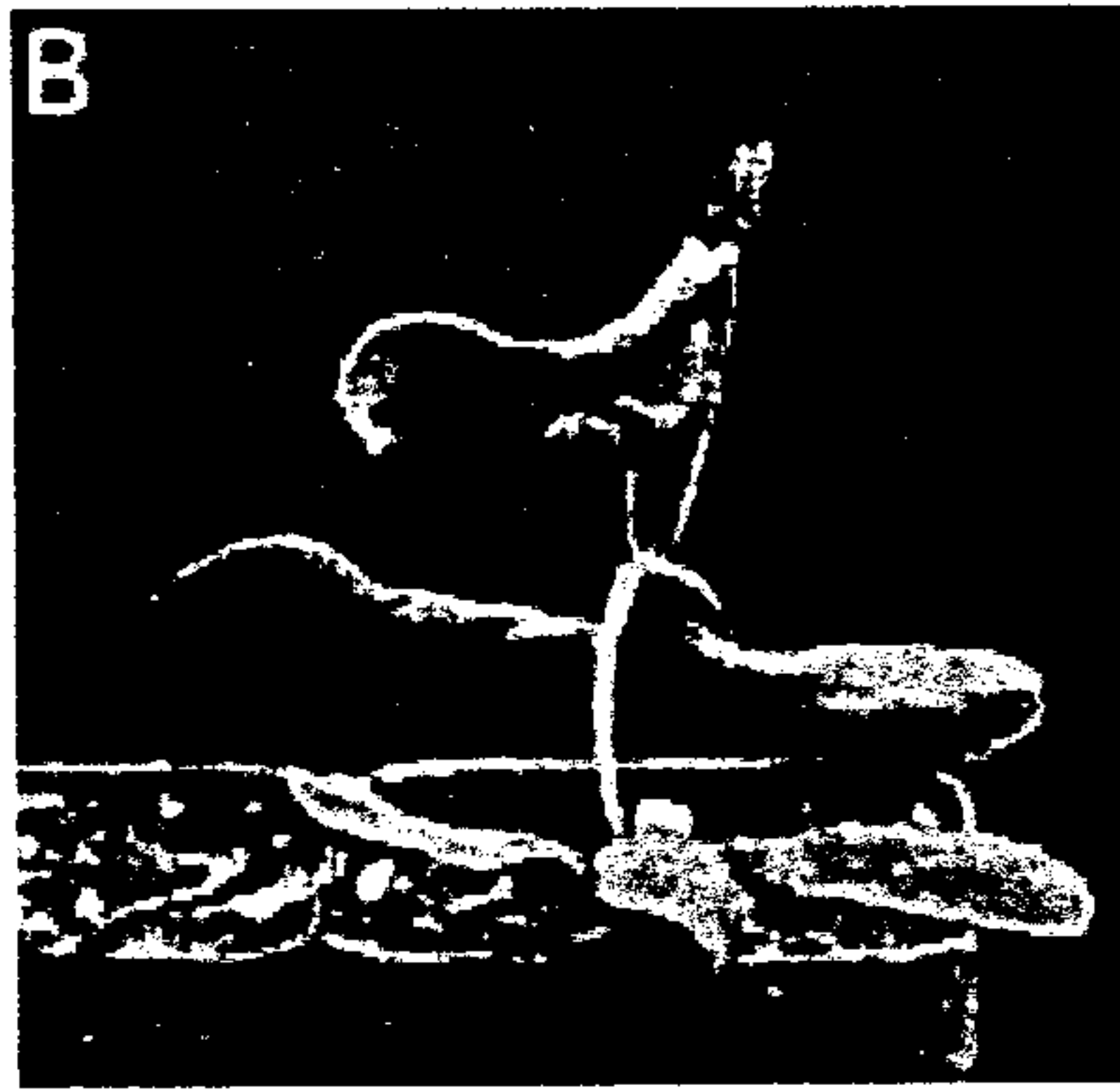
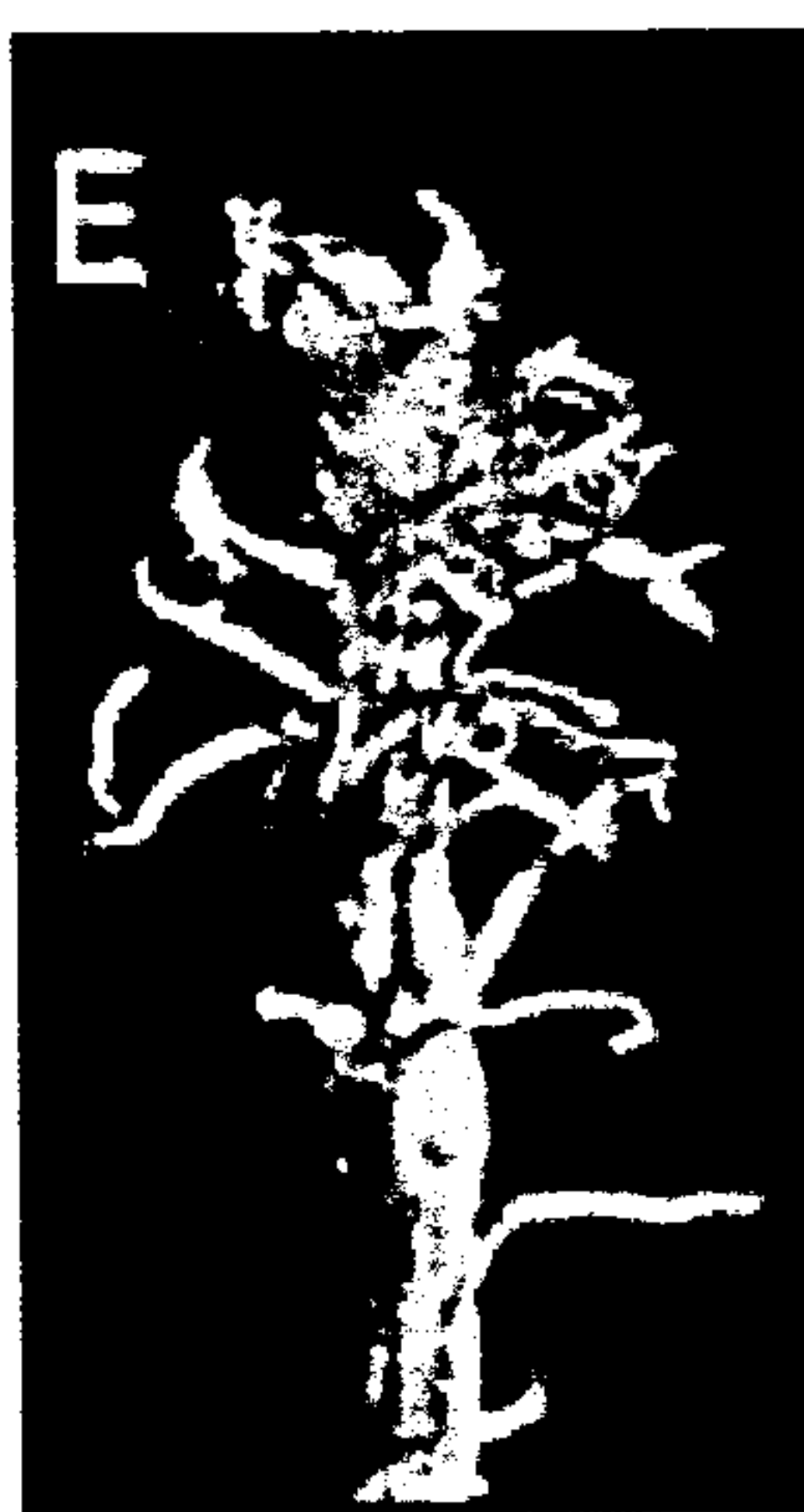
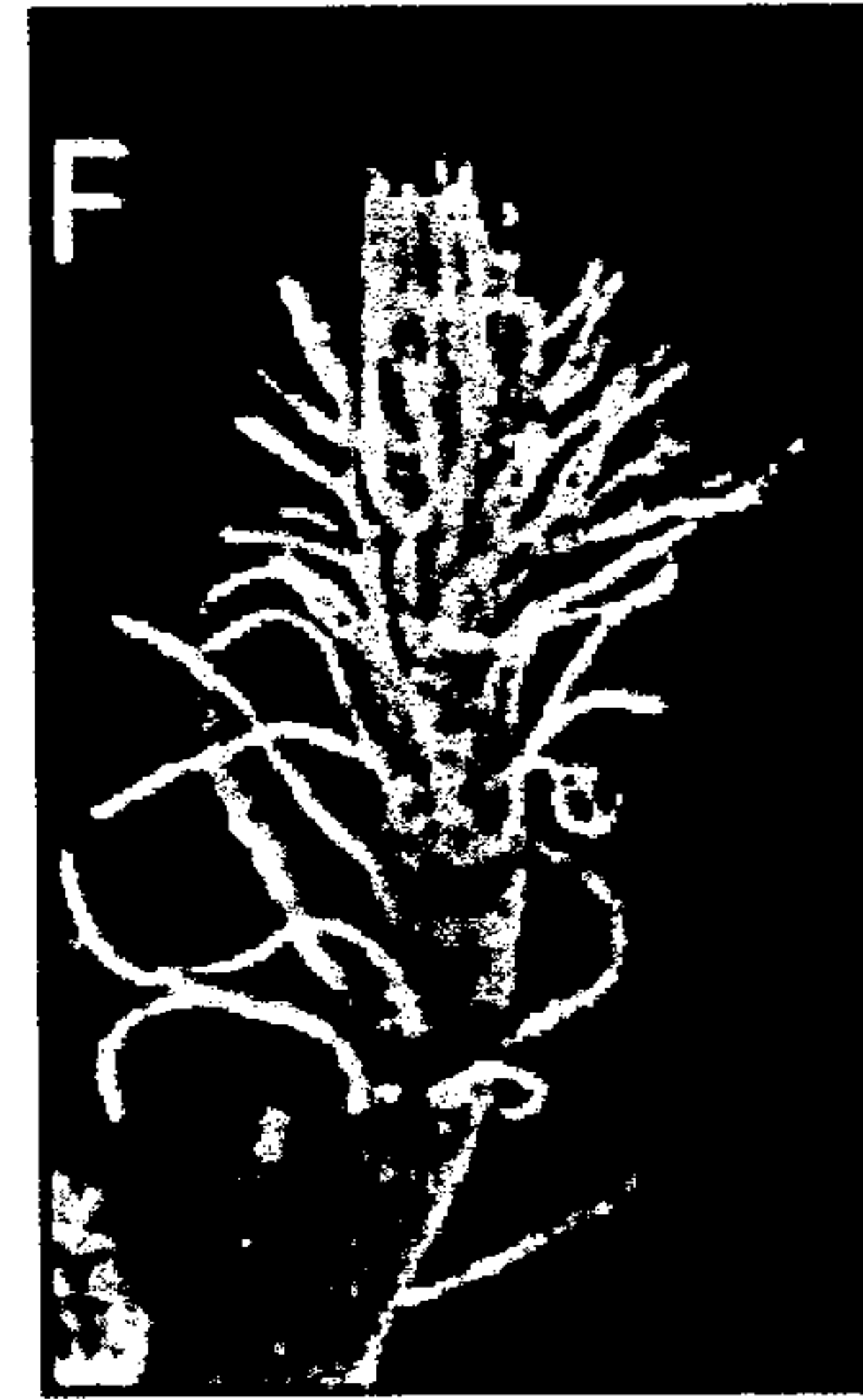
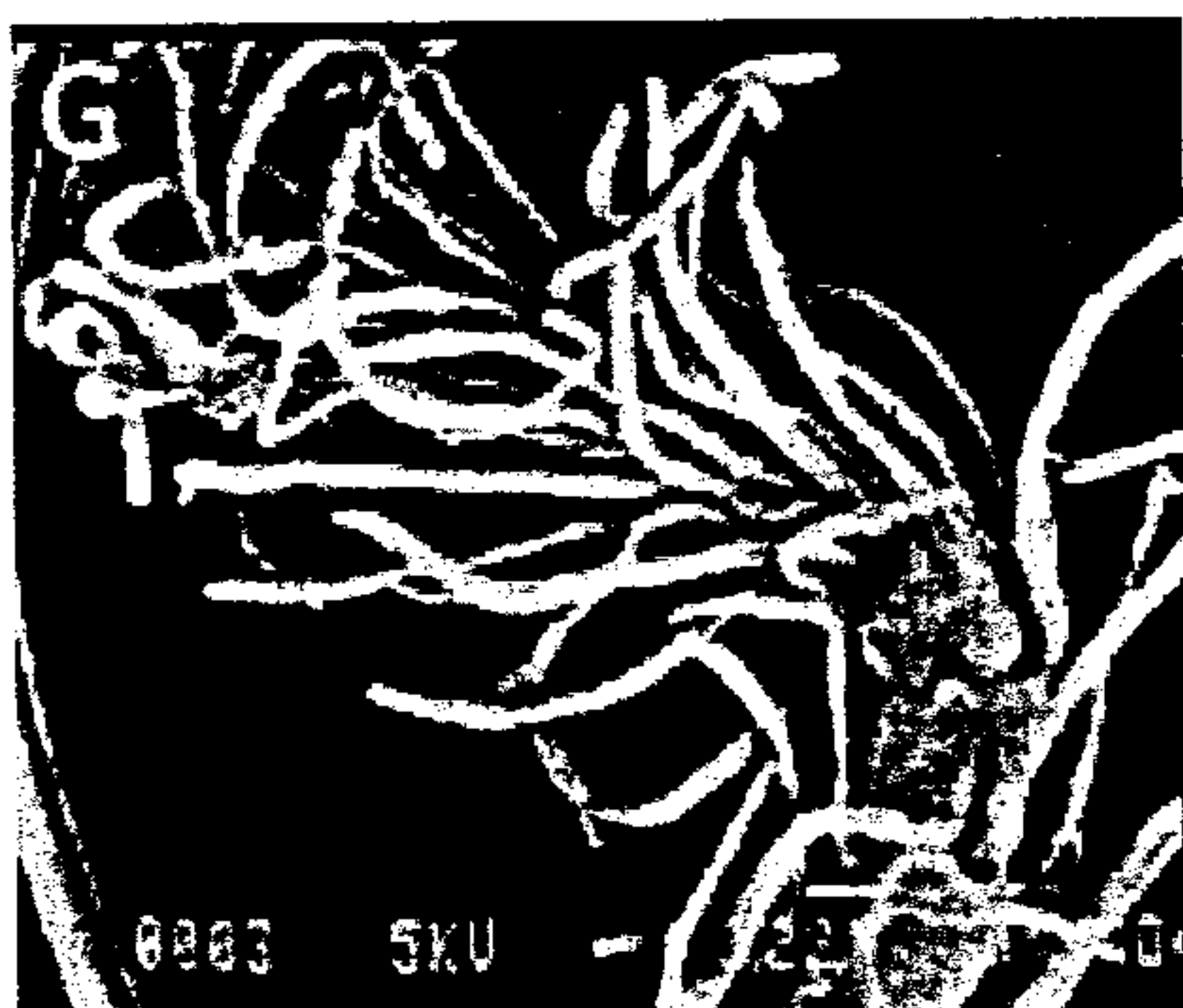
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*Fig. 7I.**Fig. 7J.**Fig. 7K.**Fig. 7L.**Fig. 7M.**Fig. 7N.**Fig. 7O.**Fig. 7P.**Fig. 7Q.**Fig. 7R.**Fig. 7S.*

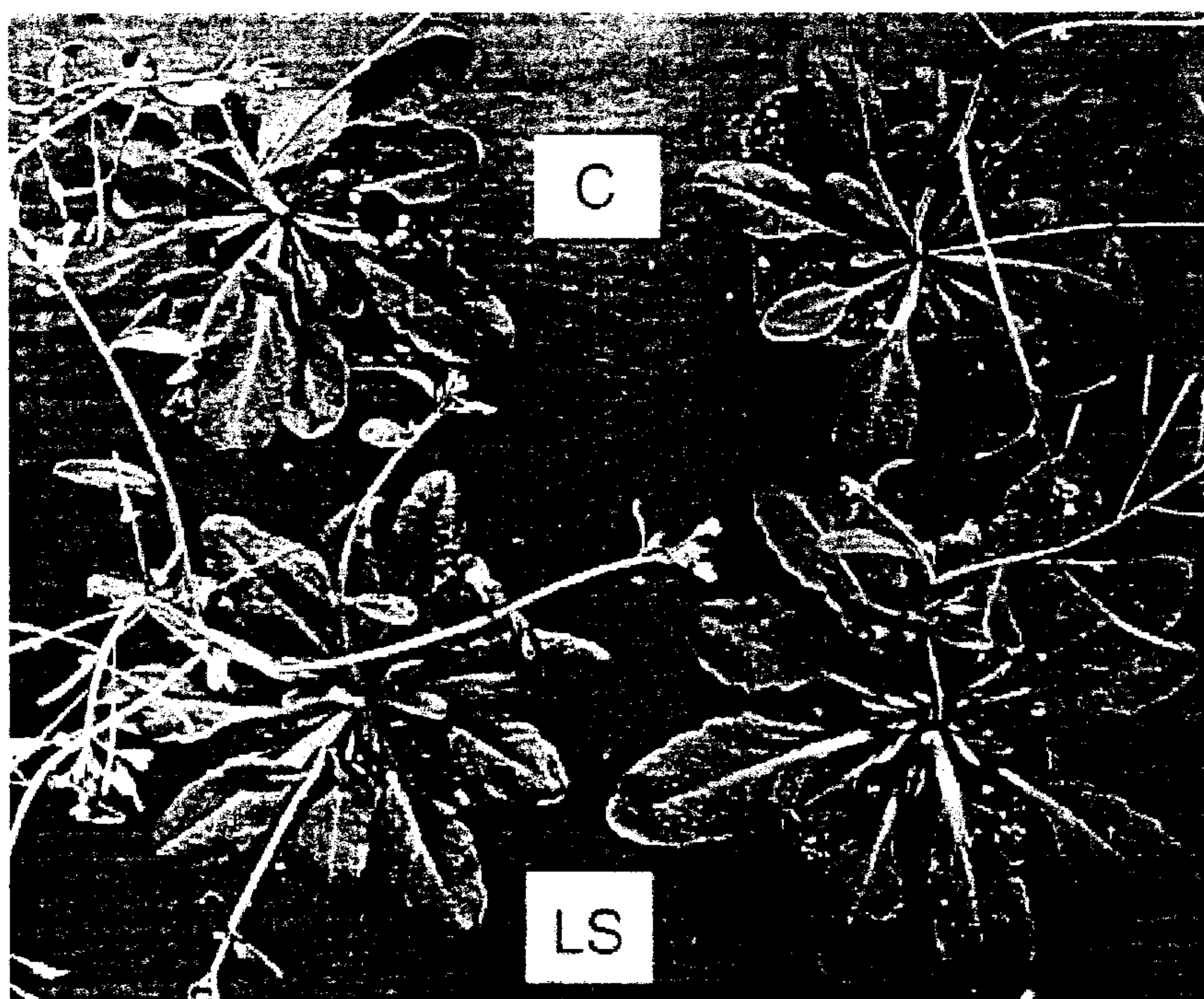
12/14

*Fig. 8A.**Fig. 8B.**Fig. 8C.**Fig. 8D.**Fig. 8E.**Fig. 8F.**Fig. 8G.**Fig. 8H.**Fig. 8I.*

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*Fig. 9A.**Fig. 9B.**Fig. 9C.**Fig. 9D.**Fig. 9E.**Fig. 9F.**Fig. 9G.**Fig. 9H.**Fig. 9I.*

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*Fig.10.**Fig.11.**Fig.12.*

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