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(54) **Titre : SEQUENCE D'ACIDE NUCLEIQUE DE LA PHOTOMORPHOGENESE 1 CONSTITUTIVE (COP1) DE Zea mays ET SON UTILISATION**

(54) **Title: CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) NUCLEIC ACID SEQUENCE FROM Zea mays AND ITS USE THEREOF**

(57) **Abrégé/Abstract:**
The present invention relates to an isolated COP1 nucleic acid sequence from a maize plant and the isolated COP1 nucleic acid sequence is named as ZmCOP1. The present invention also relates to a method of using the ZmCOP1 nucleic acid sequence to control the shade avoidance response of a crop plant for high density farming and yield enhancement.

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(54) Title: CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) NUCLEIC ACID SEQUENCE FROM *ZEA MAYS* AND ITS USE THEREOF

(57) Abstract: The present invention relates to an isolated COP1 nucleic acid sequence from a maize plant and the isolated COP1 nucleic acid sequence is named as ZmCOP1. The present invention also relates to a method of using the ZmCOP1 nucleic acid sequence to control the shade avoidance response of a crop plant for high density farming and yield enhancement.

CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) NUCLEIC ACID SEQUENCE FROM *Zea mays* AND ITS USE THEREOF

The present invention is in the field of plant molecular biology. More specifically the present invention relates to an isolated nucleic acid molecule, a protein and fragments of the protein that the isolated nucleic acid molecule encodes. Most specifically, the present invention relates to a constitutive photomorphogenesis 1 (COP1) nucleic acid sequence from *Zea mays* that encodes a COP1 protein and fragments of the COP1 protein associated with plant photomorphogenesis. The present invention also relates to a method of using the isolated COP1 nucleic acid molecules, the COP1 proteins and fragments of the COP1 proteins for molecular manipulation of shade avoidance responses of crop plants to light for improving their

density tolerance and thereafter for enhancing their yield when planted at a high population density.

Background of the Invention

Plant growth is a highly malleable process that is strongly influenced by environmental factors, especially light. Light plays a vital role in plants' photomorphogenesis and affects almost all aspects of plant growth and development. The effects of light on plant development are especially prominent at the seedling stage. Under normal light conditions with unobstructed direct light, a plant seedling develops according to a characteristic photomorphogenic pattern, that is, it has open, expanded cotyledons and a short hypocotyl. This developmental pattern rapidly establishes the seedling as a photoautotrophic organism, and most of the plant's energy is devoted to cotyledon and leaf development while longitudinal extension growth is minimized. A seedling growing in darkness, however, will etiolate, displaying elongated hypocotyls and closed and unexpanded cotyledons. Under low light conditions where light quality and intensity are reduced by shading, obstruction or high population density, a seedling develops according to a different pattern as a shade-avoiding seedling that displays reduced cotyledon expansion relative to the seedling grown in unobstructed light, and hypocotyl extension is greatly increased. During this developmental response of the seedling to the low light conditions, the hypocotyl is elongated which couples with reduction in cotyledon and leaf expansion.

Thus, a significant problem for crop farming is created when crop plants are grown at high population density as it often results in a low light level for each individual plant. To compete for this light, plants have to re-distribute their energy and nutrition towards height extension, often called a shade avoidance response, resulting in an accelerated stem elongation and thin stems. This shade avoidance response to poor light conditions in a populated environment often results in crop yield loss. For example, in maize plants, accumulating evidence suggests that the stem elongation process itself may be linked to suppression of ear development. Corn prolificacy and ear establishment are sensitive to light intensity. High population density may cause abortion of ear development at lower nodes, even at all nodes. High density leads to most of the red and blue spectra of the sunlight being absorbed

by the upper leaves, leaving the far-red light filtered or reflected to the lower canopy. The red/far-red ratio is a function of canopy density. If the density is high, the red/far-red ratio is low. This low ratio triggers the shade avoidance response, in which the plants distribute resources for stem elongation in a competition for sunlight (Quail et al, Science 268, 675-680, 1995). Reduction or elimination of the shade avoidance response has been shown to improve harvest index or yield (Maliakal et al, Critic. Rev. Plant Sci. 17, 465-539,1999; Thiele et al, Plant Physiol. 120, 73-81, 1999; Robson et al, Nature Tech. 14, 995-998, 1996). Thus, the shade avoidance response is relevant to the harvest index, for example at high population density.

Various attempts have been made to overcome the shade avoidance problem in crop farming. Breeding efforts usually result in shorter plants and, in the case of corn, smaller tassels to save energy and nutrition for kernel development (Duvick and Cassman, Crop Sci. 39, 1622-1630, 1999; Chapman and Edmeades, Crop Sci. 39, 1315-1324, 1999). Molecular and biotechnological approaches have also been tried to identify a gene or a set of genes that manipulate the photomorphogenesis pathway in a manner modifying the plant architecture to have shorter internodes. Such a plant, when growing in a dense population, would have the ability to respond to low light environment without extending its stem, thereby minimizing the shade avoidance response and enhancing yield (see, for example, Smith, US Patent No. 5,945,579; Hershey and Keller, US Patent No. 5,268,526; Deng et al., PCT Application WO00/18940).

In recent decades, many genes or gene mutants in light-signal transduction and shade avoidance response pathways have been identified and studied (Chory, Plant Cell 9: 1225-1234, 1997; Chory et al., Cell 58: 991-999, 1989; Deng et al., Genes Dev. 5: 1172-1182, 1991; Karlin-Neumann et al., Plant Physiol. 88: 1323-1331, 1988; Lissemore and Quail, Mol. Cell Biol. 8: 4840-4850, 1988; US Pat. No: 5,945,579; McNellis and Deng, Plant Cell 7: 1749-1761, 1995; Nagatani et al., Plant Physiol.102: 269-277, 1993; Osterlund et al., Trends Cell Bio. 9: 113-118, 1999; Parks and Quail, Plant Cell 5: 39-48, 1993). Among these genes, a constitutive photomorphogenesis 1 gene (COP1) from *Arabidopsis* has been studied and demonstrated to be regulated by light during plant development in response to different light conditions (Osterlund et al., Trends Cell Bio. 9: 113-118, 1999; Deng et al., Cell 71: 791-801, 1992; McNellis et al.. Plant Cell 6: 1391-1400, 1994; McNellis et al., Plant Cell 8: 1491-1503, 1996;

Osterlund and Deng, Plant Journal 16 (2): 201-208, 1998; Stacey et al., Plant Cell 11: 349-363, 1999; Torii et al., EMBO 17: 5577-5587, 1998; von Arnim and Deng, Cell 79: 1035-1045, 1998; Yamamoto et al., Plant Cell 10: 1083-1094, 1998; Deng et al., PCT Application WO00/18940). The COP1 gene was initially identified through recessive loss-of-function mutations in *Arabidopsis* that display a constitutively photomorphogenic phenotype regardless of light conditions (Deng et al., Genes Dev. 5: 1172-1182, 1991). The constitutively photomorphogenic phenotype and recessive nature of *cop1* mutations indicate that COP1 may act as a negative regulator, or light-inactivated repressor, of photomorphogenesis. The COP1 gene in *Arabidopsis* encodes a protein that contains three recognizable domains: a ring finger domain (zinc-binding motif), a coiled-coil domain and multiple WD-40 repeats characteristic of the B subunit of trimeric G-proteins (Deng et al., Cell 71: 791-801, 1992; PCT Application WO00/18940). These protein domains have been implicated in protein-protein interactions, and thus COP1 might interact with multiple partners via these interactive domains to regulate plant morphogenic development and the shade avoidance response. Overexpression of a full-length COP1 results in quantitative hypersuppression of photomorphogenic development (McNellis et al., Plant Cell 6: 1391-1400, 1995), which suggests that COP1 plays a role in a regulatory step in mediating the repression of photomorphogenic development (Osterlund et al., Trends Cell Bio. 9: 113-118, 1999; Deng et al., PCT Application WO 00/18940). The wild-type COP1 protein normally acts to repress the photomorphogenic pathway in darkness and light reverses this repression. COP1 appears to be a downstream light-signaling component (Deng et al., Cell 71: 791-801, 1992; PCT Application WO 00/18940). Overexpression of a fragment of COP1 in *Arabidopsis* is hypothesized to down regulate native COP1, this has also resulted in shorter stems of transgenic plants growing under low light conditions in comparison with those of wild-type plants (see, Deng et al., PCT Application WO 0018940).

Thus, the COP1 proteins in plants growing at low light conditions such as in a highly populated environment will act to repress normal photomorphogenic development of these plants and help activate shade avoidance response pathway to stimulate stem elongation. Therefore, reducing the level of functional COP1 proteins in plants might produce a phenotype typical of plants growing at high light intensity

conditions even when the plants are under low light conditions. This phenotype could include well developed leaves, more chloroplasts, shorter and thicker stems.

Although some studies have been done to understand the role of COP1 proteins in plant morphogenesis and development, there is little reported effort on utilizing COP1 to deal with an unsolved, common problem in crop farming; that is the shade avoidance response of plants. Deng and his colleagues (Deng et al, PCT application WO 00/18940) disclosed an isolated COP1 nucleic acid from *Arabidopsis* and use of said COP1. Their publication was directed to improved seedling emergence characteristics and not to a solution to shade avoidance related problems in crop plants grown at high population density.

Thus, there exists a need in the field for a new and different approach to reduce or diminish the shade avoiding response of crop plants growing at high population density. There exists a need, through use of a different light transduction component, i.e., COP1 gene, to improve some of crop plants' agronomic traits such as reduced stem length and increased shade tolerance that are closely associated with crop yield.

Summary of the Invention

Therefore, the present invention, in one aspect, relates to an isolated nucleic acid molecule from a maize plant (*Zea mays*) comprising a full-length nucleic acid sequence from a cDNA identified as ZmCOP1 and having the function of improving crop plants' agronomic traits that are associated with the crop yield. ZmCOP1 comprises 2230 nucleotides coding a polypeptide with 693 amino acid residues. The sequence of ZmCOP1 comprises SEQ ID NO: 12.

The present invention, in another aspect, provides an isolated nucleic acid from *Zea mays* comprising a nucleotide sequence, wherein the nucleotide sequence is defined as follows: (1) the nucleotide sequence has at least 80% sequence identity to a sequence comprising SEQ ID NO: 12; (2) the nucleotide sequence hybridizes under stringent conditions to the complement of a second isolated nucleic acid, wherein the nucleotide sequence of the second isolated nucleic acid comprising SEQ ID NO: 12; or (3) the nucleotide sequence is complementary to (1) or (2).

The present invention, in still another aspect, provides an isolated nucleic acid from *Zea mays* comprising a nucleotide sequence, wherein the nucleotide sequence is

defined as follows: (1) the nucleotide sequence encodes a polypeptide having an amino acid sequence that has at least 90% sequence identity to a sequence comprising SEQ ID NO: 13; (2) the nucleotide sequence hybridizes under stringent conditions to the complement of a second isolated nucleic acid, wherein the nucleotide sequence of the second isolated nucleic acid encodes a polypeptide having an amino acid sequence comprising SEQ ID NO: 13; or (3) the nucleotide sequence is complementary to (1) or (2).

The present invention, in yet another aspect, also relates to a recombinant DNA construct for producing high-density tolerant crop plants. The construct comprises a light inducible promoter, a COP1 structural nucleic acid sequence that comprises a sequence at least 80% identical to SEQ ID NO: 12 or a fragment thereof, and a transcription terminator. The recombinant DNA construct causes reduction of the indigenous COP1 protein level upon its transformation into a crop plant through introduction of the COP1 structural nucleic acid sequence in an antisense orientation wherein an antisense COP1 mRNA is transcribed and base-paired with the indigenous COP1 mRNA. The recombinant DNA construct also causes the reduction of the indigenous COP1 protein level upon its transformation into a crop plant by overexpressing a full length or a fragment of the COP1 protein that binds to a native COP1 protein and makes the COP1 protein complex non-functional. With the reduction of the native COP1 protein level in the crop plants the density tolerance of the crop plants is improved and the crop plants may be overplanted at a high population density to achieve enhanced yield.

The light inducible promoter used in the recombinant DNA construct may be, but may not be limited to, a *cab* promoter, an ATHB-2 promoter, or a far-red light inducible promoter for the antisense approach or overexpression of the COP1 nucleic acid sequence or a fragment thereof.

The present invention, in yet another aspect, also relates to transgenic crop plants that demonstrate a high-density tolerant trait. These transgenic crop plants contain exogenous COP1 nucleic acid sequences that may be in an "antisense" orientation or may be overexpressed. The exogenous COP1 nucleic acid sequences are at least 80% identical to SEQ ID NO: 12 or fragments thereof. In a preferred emdoiment, the crop plants contain a full-length ZmCOP1 nucleic acid sequence having SEQ ID NO: 12 or a fragment thereof. In one example of the present

invention when a fragment is considered, the fragment contains about 1233 nucleotides from the 5' end of the ZmCOP1 having SEQ ID NO: 14. In another example of the present invention, the fragment contains about 906 nucleotides from the 5' end having SEQ ID NO: 16. The transgenic crop plants have reduced levels of the native COP1 proteins from their native COP1 nucleic acid sequences. The transgenic crop plants also demonstrate a number of other desirable agronomic traits over wild-type crop plants in that they have shorter stems and more sturdy architecture.

The present invention, in yet still another aspect, also provides a method of overplanting crop plants at a high population density for yield enhancement by producing the transgenic crop plants with reduced COP1 protein level in comparison to that of the wild crop plants. Through reduction of the COP1 protein levels in the transgenic crop plants the architecture of the transgenic plants is modified and their shade avoidance responses to light are minimized. In a preferred embodiment, the levels of the functional endogenous COP1 proteins in the transgenic crop plants may be reduced by binding an endogenous COP1 mRNA with an antisense ZmCOP1 sequence that comprises the full-length ZmCOP1 nucleic acid sequence of the present invention encoding SEQ ID NO: 13. The levels of the functional endogenous COP1 proteins in the transgenic plants may also be reduced by binding the endogenous COP1 mRNA with an antisense ZmCOP1 sequence that only comprises a fragment of the COP1 nucleic acid sequence. The level of the functional endogenous COP1 proteins may also be reduced by overexpressing a full length unaltered or mutated ZmCOP1 protein or a fragment thereof with binding domains that binds to a native endogenous COP1 protein and thus rendering the endogenous COP1 protein complex non-functional. The fragment of the ZmCOP1 protein used in the present invention as an example may comprise 411 amino acid residues from the N terminal end having SEQ ID NO: 15 that comprises a protein-binding domain. The fragment of the COP1 protein in another example may also comprise 301 amino acid residues from the N terminal end having SEQ ID NO: 17 that comprises a protein-binding domain.

Brief Description of the Drawings

Figure 1. Nucleotide sequence alignment of *Arabidopsis* COP1 (AtCOP1_gi-402684) and maize (*Zea mays*) COP1 (ZmCOP1). The homology comparison indicates that these two nucleotide sequences have a 50% sequence identity.

Figure 2. Nucleotide sequence alignment of the rice (*Oryza sativa*) COP1 (OsCOP1, gi7592844) and maize (*Zea mays*) COP1 (ZmCOP1). The homology comparison indicates that these two nucleotide sequences have a 77% sequence identity.

Figure 3. Peptide sequence alignment of maize (*Zea mays*) COP1 (ZmCOP1) and selected COP1 peptide sequences from other plants. ZmCOP1 is from maize (*Zea mays*), PsCOP1 from pea (*Pisum sativum*), At COP1 from *Arabidopsis thaliana*, COP1 from Japanese morning glory (*Ipomoea nil*), OsCOP1 from rice (*Oryza sativa*), and LeCOP1 from tomato (*Lycopersicon esculentum*).

Figure 4. .A plasmid map of pMON47118. The coding sequence of the N-terminal end 301 amino acid residues of ZmCOP1 was placed under the control of a *cab* promoter.

Figure 5. A plasmid map of pMON47119. The coding sequence of the N-terminal end 411 amino acid residues of ZmCOP1 was placed under the control of a *cab* promoter

Figure 6. A plasmid map of pMON47120. ZmCOP1 was cloned in the vector in reverse orientation and placed under the control of a *cab* promoter.

Figure 7. A plasmid map of pMON47130. The coding sequence of the N-terminal end 301 amino acid residues of ZmCOP1 was placed under the control of a rice-actin promoter (RACT) promoter.

Figure 8. A plasmid map of pMON47131. The coding sequence of the full length ZmCOP1 was cloned in the vector in reverse orientation and was placed under the control of a rice-actin promoter (RACT) promoter.

Figure 9. The height comparison of the transformed R1 plants with the wild type plants at maturity. The average height and growth rate of some R1 transformed plants from pMON47118 in several events were lower compared to those of the wild type plants growing nearby. LH172 is a wild type inbred used for transformation; ZM is a transformed R1 plant; LH172/ZM represents a F1 plant obtained by crossing LH172 plant with a transformed R0 plant. Each number under each bar in the figure, e.g., ZM_S35321, represents one event.

Figure 10. Western analysis of YAA plants is shown.

Figure 11. Plant height of some YAA events at V11 stage are shown.

Figure 12. Western analysis of Kyle plants grown under normal (full green house) light and weak light.

Figure 13. Western blot analysis to determine protein expression levels in Kyle 17 and Kyle 50 events. Both Kyle positive (Pos) and negative (Neg) events are shown at different growth stages. Stage 1 represents the V3-V4 stage, 2 represents the V5-V7 stage, and 3 the VT stage. P is a positive control.

Figure 14. Mesocotyl lengths of Kyle seedlings grown under 1 micromole per meter squared of light.

Figure 15. Plant height of 5 Kyle events four weeks after transplanting.

Figure 16. Picture of positive (on both sides) and negative (center) lines from the Kyle 77 event.

Figure 17. Height of plants from the Kurt R1/F1 plants grown in the field. Shown below is the expression level of their transgene by Western analysis.

Detailed Description

Provided below are the following definitions to aid those skilled in the art in understanding the detailed description of the present invention.

As used herein, “antisense technology” refers to a method to introduce into cells a RNA or single-stranded DNA molecule that is complementary to the mRNA of the target gene. This antisense molecule may base-pair with the endogenous mRNA, preventing translation of the mRNA into a protein.

As used herein, a "coding sequence", “structural nucleotide sequence” or “structural gene” is a nucleotide sequence that is translated into a polypeptide, usually via mRNA, when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence may include, but may not be limited to, genomic DNA, cDNA, and recombinant nucleotide sequences.

As used herein, a constitutive photomorphogenesis 1 nucleic acid, or “COP1 nucleic acid”, refers to a nucleic acid encoding all or part of a specific constitutive photomorphogenesis 1 protein, or “COP1 protein”. A COP1 nucleic acid may be defined functionally by its ability to confer a modulated photomorphogenic response

upon transformation into a plant. The COP1 nucleic acids may include any COP1 nucleic acids from any source. The exemplary COP1 nucleic acid is the COP1 nucleic acid as disclosed in the present invention.

As used herein, a "C-terminal region" refers to the region of a peptide, polypeptide, or protein chain from the middle thereof to the end that carries the amino acid having a free carboxyl group. A "N-terminal region" refers to the region of a peptide, polypeptide, or protein chain from the amino acid having a free amino group to the middle of the chain.

As used herein, "expression" refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid of the invention. Expression may also refer to translation of mRNA into a polypeptide. Also as used herein, "overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

As used herein, a "genotype" refers to the genetic constitution, latent or expressed, of a plant, the sum total of all genes present in an individual. As used herein, a "phenotype" of a plant is any of one or more characteristics of a plant (e.g. male sterility, yield, quality improvements, etc.), as contrasted with the genotype. A change in genotype or phenotype may be transient or permanent.

As used herein, a "homolog" of a nucleotide sequence refers to an isolated nucleic acid sequence which is substantially the same as the COP1 nucleic acid sequence of the present invention or its complementary nucleotide sequence. A "homolog" of the COP1 nucleic acid sequence is a polynucleotide sequence from a plant species that encodes a polypeptide that is functionally similar to COP1 and that preferably has substantial amino acid sequence identity or similarity to COP1 from maize.

Planting or population density varies from a crop to a crop, from a growing region to another region and from a year to another year. As used herein, the term "high population density" is defined as a density at least 10% higher than the average prevailing density for a given crop in a given growing region. Preferably, the high population density is at least 10% higher, more preferably at least 40% higher, more preferably at least 70% higher, and most preferably at least 100% higher than the average prevailing density for the given crop in the given growing region. The

"average prevailing density" is defined as the average of the planting density used by the majority of farmers in a region. Taken corn as an example, the average prevailing density is about 20,000 plants per acre in Missouri, USA. The higher population density is preferably at least 22,000 plants per acre, more preferably at least 28,000 plants per acre, more preferably at least 34,000 plants per acre, and most preferably at least 40,000 plants per acre.

The average prevailing densities of a few crop plants in the USA in 2000 are listed below (Table 1). The examplery crop species are just examples and, therefore, may not be construed as limitations to the scope of the present invention. Similarly, the country selected above, i.e., USA, is also an example in which the average prevailing densities of these few crop plants can be demonstrated. It may not be construed as a limitation of the present invention.

Table 1. The average prevailing densities of a few crop plants in the USA (per acre)

Crop Name	Density	Crop Name	Density
Corn	20,000 – 25,000	Canola	260,000-350,000
Wheat	1,000,000-1,500,000	Sunflower	17,000-23,000
Rice	650,000-900,000	Cotton	28,000-55,000
soybean	150,000-200,000		

As used herein, "hybridization" refers to the ability of a strand of nucleic acid to join with a complementary strand via base pairing. Hybridization occurs when complementary sequences in the two nucleic acid strands bind to one another.

As used herein, "identical" nucleotide or protein sequences are determined by using programs such as a BLAST program (Altschul et al., Nucleic Acids Res. 25:3389-3402; 1997) using the default parameters (Expectation value (E): blank; Alignment view options: pairwise; Filter query sequence: no; Cost to open a gap: 0; Cost to extend a gap: 0; X dropoff value for gapped alignment: 0; Show GI's in deflines: no; Penalty for a nucleotide mismatch: -3; Reward for a nucleotide match: 1; Threshold for extending hits: 0; Perfom gapped alignment: yes; Query Genetic code to use: standard; DB Genetic code: standard; Believe the query defline: no;

Matrix: BLOSUM62; Word size: 0; Effective length of the database: 0; Query strand Use: both).

As used herein, an "isolated" nucleic acid is one that has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, by conventional nucleic acid-purification methods. The term also embraces recombinant nucleic acids and chemically synthesized nucleic acids.

The term "polypeptide" or "protein", as used herein, refers to a polymer composed of amino acids connected by peptide bonds. The term "polypeptide" or "protein" also applies to any amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to any naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, that protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring amino acids. It is well known in the art that proteins or polypeptides may undergo modification, including but not limited to, disulfide bond formation, gamma-carboxylation of glutamic acid residues, glycosylation, lipid attachment, phosphorylation, oligomerization, hydroxylation and ADP-ribosylation. Exemplary modifications are described in most basic texts, such as, for example, Proteins - Structure and Molecular Properties, 2nd ed. (Creighton, Freeman and Company, N. Y., 1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold (In: Post-translational Covalent Modification of Proteins, Johnson, Academic Press, N. Y., pp. 1-12, 1983), Seifter et al. (*Meth. Enzymol.* 182: 626, 1990) and Rattan et al. (*Ann. N.Y. Acad. Sci.* 663: 48-62, 1992). Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli* or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the polypeptide, a methionine residue at the NH_2

terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine containing and the methionine-less amino terminal variants of the protein of the invention. Thus, as used herein, the term "protein" or "polypeptide" includes any protein or polypeptide that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer to all naturally occurring amino acids and, unless otherwise limited, known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

As used herein, the term "isolated polypeptide" refers primarily to a polypeptide produced by expression of an isolated nucleic acid molecule of the present invention or by chemically synthesizing process. Alternatively, this term may refer to a polypeptide which has been sufficiently separated from other polypeptides or proteins with which it would naturally be associated, so as to exist in substantially pure form. Also as used herein, a "functionally equivalent fragment" of the isolated polypeptide refers to a polypeptide that lacks at least one residue a native full length COP1 polypeptide. Such a fragment retains COP1 activity when expressed in a transgenic plant or possesses a characteristic functional domain or an immunological determinant characteristic of a native COP1 polypeptide. Immunologically active fragments typically have a minimum size of 7 or 17 or more amino acids. Preferably, COP1 fragments are at least 10 amino acids in length.

As used herein, the term "native" refers to a naturally occurring ("wild type") nucleic acid or polypeptide.

As used herein, a "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. 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 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. The percentage of sequence identity may be determined by using programs such as a BLAST program (Altschul et al., Nucleic Acids Res. 25:3389-3402; 1997) using the default parameters.

As used herein, a "promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the later elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". Promoters which cause conditional expression of a structural nucleotide sequence under the influence of changing environmental conditions or developmental conditions are commonly referred to as "inducible promoter".

"Promoter" refers to a DNA sequence that binds an RNA polymerase (and often other transcription factors as well) and promotes transcription of a downstream DNA sequence. Said sequence can be an RNA that has function, such as rRNA (ribosomal RNA) or tRNA (transfer RNA). Often, the RNA produced is a hetero-nuclear (hn) RNA that has introns which are spliced out to produce an mRNA (messenger RNA). A "plant promoter" is a native or non-native promoter that is functional in plant cells. Constitutive promoters are functional in most or all tissues of a plant throughout plant development. Tissue-, organ- or cell-specific promoters are expressed only or predominantly in a particular tissue, organ, or cell type, respectively "Specifically" expressed and "enhanced" expression are not distinguishable and are used inter-changeably herein. Often, a promoter discussed as "specifically" expressed in one paper or patent is found to only offer "enhanced" expression in that tissue as the number of tissues studied for expression is increased, or more sensitive techniques are used to study expression in the same tissues. "Enhanced expression" is used herein to refer to any promoter that provides an increased expression in a single tissue or developmental stage, or under a particular

environmental condition, but causes expression, even significant expression, in other tissue(s), or developmental stage(s), or environmental condition(s).

Temporally regulated promoters are functional only or predominantly during certain periods of plant development or at certain times of day, as in the case of genes associated with circadian rhythm, for example. Inducible promoters selectively express an operably linked DNA sequence in response to the presence of an endogenous or exogenous stimulus, for example by chemical compounds (chemical inducers) or in response to environmental, hormonal, chemical, and/or developmental signals. Inducible or regulated promoters include, for example, promoters regulated by light, heat, stress, flooding or drought, phytohormones, wounding, cold, or chemicals such as ethanol, jasmonate, salicylic acid, or safeners.

Any plant promoter can be used as a 5' regulatory sequence for modulation expression of a particular gene or genes. One preferred promoter would be a plant RNA polymerase II promoter. Plant RNA polymerase II promoters, like those of other higher eukaryotes, have complex structures and are comprised of several distinct elements. One such element is the TATA box or Goldberg-Hogness box, which is required for correct expression of eukaryotic genes in vitro and accurate, efficient initiation of transcription in vivo. The TATA box is typically positioned at approximately -25 to -35, that is, at 25 to 35 basepairs (bp) upstream (5') of the transcription initiation site, or cap site, which is defined as position +1 (Breathnach and Chambon, Ann. Rev. Biochem. 50:349-383, 1981; Messing et al., In: Genetic Engineering of Plants, Kosuge et al., eds., pp. 211-227, 1983). Another common element, the CCAAT box, is located between -70 and -100 bp. In plants, the CCAAT box may have a different consensus sequence than the functionally analogous sequence of mammalian promoters (the plant analogue has been termed the "AGGA box" to differentiate it from its animal counterpart; Messing et al., In: Genetic Engineering of Plants, Kosuge et al., eds., pp. 211-227, 1983). In addition, virtually all promoters include additional upstream activating sequences or enhancers (Benoist and Chambon, nature 290:304-310, 1981; Gruss et al., Proc. Nat. Acad. Sci. USA 78:943-947, 1981; and Khoury and Gruss, Cell 27:313-314, 1983) extending from around 100 bp to 1,000 bp or more upstream of the transcription initiation site.

When fused to heterologous DNA sequences, such promoters typically cause the fused sequence to be transcribed in a manner that is similar to that of the gene

sequence with which the promoter is normally associated. Promoter fragments that include regulatory sequences can be added (for example, fused to the 5' end of, or inserted within, an active promoter having its own partial or complete regulatory sequences (Fluhr, et al., Science 232:1106-1112, 1986; Ellis et al., EMBO J. 6:11-16, 1987; Strittmatter and Chua, Proc. Nat. Acad. Sci. USA 84:8986-8990, 1987; Poulsen and Chua, Mol. Gen. Genet. 214:16-23, 1988; Comai et al., Plant Mol. Biol. 15:373-381, 1991). Alternatively, heterologous regulatory sequences can be added to the 5' upstream region of an inactive, truncated promoter, e.g., a promoter including only the core TATA and, sometimes, the CCAAT elements (Fluhr, et al., Science 232:1106-1112, 1986; Strittmatter and Chua, Proc. Nat. Acad. Sci. USA 84:8986-8990, 1987; Aryan et al., Mol. Gen. Genet. 225:65-71, 1991).

Promoters are typically comprised of multiple distinct "*cis*-acting transcriptional regulatory elements," or simply "*cis*-elements," each of which can confer a different aspect of the overall control of gene expression (Strittmatter and Chua, Proc. Nat. Acad. Sci. USA 84:8986-8990, 1987; Ellis et al., EMBO J. 6:11-16, 1987; Benfey et al., EMBO J. 9:1677-1684, 1990). "*cis* elements" bind *trans*-acting protein factors that regulate transcription. Some *cis* elements bind more than one factor, and *trans*-acting transcription factors may interact with different affinities with more than one *cis* element (Johnson and McKnight, Ann. Rev. Biochem. 58:799-839, 1989). Plant transcription factors, corresponding *cis* elements, and analysis of their interaction are discussed, for example, in: Martin, Curr. Opinions Biotech. 7:130-138, 1996; Murai, In: Methods in Plant Biochemistry and Molecular Biology, Dashek, ed., CRC Press, 1997, pp. 397-422; and Methods in Plant Molecular Biology, Maliga et al., eds., Cold Spring Harbor Press, 1995, pp. 233-300. The promoter sequences of the present invention can contain "*cis* elements" which can modulate gene expression. *Cis* elements can be part of the promoter, or can be upstream or downstream of said promoter. *Cis* elements (or groups thereof) acting at a distance from a promoter are often referred to as repressors or enhancers. Enhancers act to upregulate the transcriptional initiation rate of RNA polymerase at a promoter, repressors act to decrease said rate. In some cases the same elements can be found in a promoter and an enhancer or repressor. *Cis* elements are generally sites where transcription factors bind to the DNA and modulate the rate at which RNA polymerase binds to the promoter.

The term "constitutive promoter" means a regulatory sequence that causes

expression of a structural nucleotide sequence in most cells or tissues at most times. Constitutive promoters are active under many environmental conditions and states of development or cell differentiation. A variety of constitutive promoters are well known in the art. Examples of constitutive promoters that are active in plant cells include but are not limited to the nopaline synthase (NOS) promoters; the cauliflower mosaic virus (CaMV) 19S and 35S (sometimes called 35S herein, or a derivative of which is called e35S {US patents 5,359,142, 5,196,525, 5,322,938, 5,164,316, and 5,424,200}); the tobacco mosaic virus promoter; the figwort mosaic virus promoters; and actin promoters, such as the *Arabidopsis* actin gene promoter (see, *e.g.*, Huang *et al.*, *Plant Mol. Biol. 33*:125-139 (1997).

The term "tissue-specific promoter" means a regulatory sequence that causes an enhancement of transcription from a downstream gene in specific cells or tissues at specific times during plant development, such as in vegetative tissues or reproductive tissues. Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only (or primarily only) in certain tissues, such as vegetative tissues, e.g., roots, leaves or stems, or reproductive tissues, such as fruit, ovules, seeds, pollen, pistols, flowers, or any embryonic tissue. Reproductive tissue specific promoters may be, e.g., ovule-specific, embryo-specific, endosperm-specific, integument-specific, seed coat-specific, pollen-specific, petal-specific, sepal-specific, or some combination thereof. One skilled in the art will recognize that a tissue-specific promoter may drive expression of operably linked sequences in tissues other than the target tissue. Thus, as used herein a tissue-specific promoter is one that drives expression preferentially in the target tissue, but may also lead to expression in other tissues as well.

Suitable seed-specific (inclusive of seed enhanced promoters) can be derived from the following genes: MAC1 from maize (Sheridan *et al.*, *Genetics 142*:1009-1020 (1996); Cat3 from maize (GenBank No. L05934, Abler *et al.*, *Plant Mol. Biol. 22*:10131-1038 (1993); vivparous-1 from *Arabidopsis* (Genbank No. U93215); Atimyc1 from *Arabidopsis* (Urao *et al., Plant Mol. Biol. 32*:571-57 (1996); Conceicao *et al., Plant 5*:493-505 (1994), herein incorporated by reference in their entireties); napA from *Brassica napus* (GenBank No. J02798); the napin gene family from *Brassica napus* (Sjodahl *et al.*, *Planta 197*:264-271 (1995)). Seed specific promoters are an integral part of the current invention. It should be noted that a seed specific

promoter can often cause the expression of a gene in more than just seeds, or in more than one portion or tissue of a seed. Thus seed specific can be read as seed enhanced and is meant to be inclusive of any promoter that preferentially drives expression in any tissue in seed.

Promoters derived from genes encoding embryonic storage proteins, which includes the gene encoding the 2S storage protein from *Brassica napus* (Dasgupta et al, Gene 133:301-302 (1993); the 2s seed storage protein gene family from *Arabidopsis*; the gene encoding oleosin 20kD from Brassica napus (GenBank No. M63985); the genes encoding oleosin A (GenBank No. U09118) and oleosin B (GenBank No. U09119) from soybean; the gene encoding oleosin from *Arabidopsis* (GenBank No. Z17657); the gene encoding oleosin 18kD from maize (GenBank No. J05212, Lee, Plant Mol. Biol. 26:1981-1987 (1994)); and the gene encoding low molecular weight sulphur rich protein from soybean (Choi et al., Mol. Gen. Genet. 246:266-268 (1995)), can also be used.

Promoters can also be induceable under particular environmental conditions. For example a promoter could be upregulated, or even turned on, by far-red light, cold, heat, drought, blue light (or any other mix of wavelengths), day length, or myriad other environmental conditions. These promoters could be isolated by the use of general molecular biology techniques including transcription profiling of possible genes, and then isolation of the promoters of those genes through cloning and PCR.

As noted above, the present invention provides a recombinant DNA construct or expression vector that facilitates the expression of the COP1 nucleic acid sequence discussed herein in plants. As used herein, the term "recombinant DNA construct" refer to assemblies of DNA fragments through genetic engineering operatively linked in a functional manner that direct the expression of the COP1 nucleic acid sequence discussed herein, as well as any additional sequence(s) or gene(s) of interest in the plants.

As used herein, "regeneration" refers to the process of growing a plant from a plant cell or tissue (e.g., plant protoplast or explant).

As used herein, "sequence homology" refers to nucleic acid or polypeptide sequence that has certain percentage of nucleotide or amino acid similarity, as used in the present invention, to a native COP1 nucleic acid or polypeptide sequence or COP1 nucleic acid or polypeptide sequence. Ordinarily, if a COP1 nucleic acid or

polypeptide sequence encompassed by the present invention has at least about 70% nucleotide or amino acid similarity to a native COP1 nucleic acid or polypeptide sequence or to a COP1 nucleic acid, preferably at least 80%, more preferably at least about 90%, and most preferably at least about 95% similarity, such sequence homology is considered to be substantial homology.

As used herein, the term "sequence identity" refers to amino acid or nucleic acid sequences that when compared using the *local homology* algorithm of Smith and Waterman in the BestFit program (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI, 1981) are exactly alike.

As used herein, the term "sequence similarity" refers to amino acid sequences that when compared using the *local homology* algorithm of Smith and Waterman in the BestFit program (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI, 1981) match when conservative amino acid substitutions are considered.

As used herein, "shade avoidance responses" refer to plants that, when growing at a high density condition or other shading environments, will compete for light by elongating their stems unlimitedly. These plants will usually be taller with thinner stems and have reduced photosynthesis rate and reduced allocation of resource to fruits.

As used herein, a "stringent condition" is functionally defined with regard to hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic acid sequence of interest) by the specific hybridization procedure discussed in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Hobart, 1989, at 9.52-9.55). Regarding the amplification of a target nucleic acid sequence (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize substantially only to the target nucleic acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind so as to produce a unique amplification product. For hybridization of a probe or primer to a polynucleotide of another plant species in order to identify homologs, preferred hybridization and washing conditions are as discussed in Sambrook et al.(supra, at 9.47-9.57, wherein "high stringent conditions" include hybridization at 65^0C in a hybridization solution that includes 6x SSC and washing for 1 hour at 65^0C

in a wash solution that include 0.5x SSC, 0.5% SDS. "Moderate stringency" conditions are similar except that the temperature for the hybridization and washing steps are performed at a lower temperature at which the probe is specific for a target sequence, preferably at least 42^0C, more preferably at least 50^0C, more preferably at least 55^0C, and more preferably at least 60^0C.

As used herein, a "tissue sample" is any sample that comprises more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g., derived from a leaf, an ear or a stem, etc.).

As used herein, a "3′ untranslated region" or "3' untranslated nucleic acid sequence" refers to a piece of transcribed but untranslated nucleic acid sequence at the 3' end that functions in a plant cell to cause transcriptional termination and the addition of polyadenylate nucleotides to the 3′ end of said RNA sequence. Typically, a DNA sequence located from four to a few hundred base pairs downstream of the polyadenylation site serves to terminate transcription. The region is required for efficient polyadenylation of transcribed messenger RNA (mRNA). RNA polymerase transcribes a coding DNA sequence through a site where polyadenylation occurs.

As used herein, "transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism such as a host plant, resulting in genetically stable inheritance. Host plants containing the transformed nucleic acid fragments are referred to as "transgenic plants".

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described in detail in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).

<u>Constitutive photomorphogenesis 1 (COP1) gene and protein</u>

The present invention is directed to an isolated constitutive photomorphogenesis 1 (COP1) nucleic acid that encodes a COP1 protein. As

disclosed in the present invention, the COP1 nucleic acid disclosed herein is isolated from a maize plant and is a full length COP1 cDNA sequence comprising 2230 nucleotides. Its COP1 protein comprises 693 amino acid residues.

In a preferred embodiment, an isolated nucleic acid molecule of the present invention may comprise a nucleotide sequence or complement thereof, wherein the nucleotide sequence encodes a polypeptide having an amino acid sequence that has at least 90% sequence identity to SEQ ID NO: 13.

In a further preferred embodiment, an isolated nucleic acid molecule of the present invention may comprise a nucleotide sequence or complement thereof, wherein the nucleotide sequence encodes a polypeptide having an amino acid sequence that has at least 93% sequence identity to SEQ ID NO: 13.

In a more preferred embodiment, an isolated nucleic acid molecule of the present invention may comprise a nucleotide sequence or complement thereof, wherein the nucleotide sequence encodes a polypeptide having an amino acid sequence that has at least 96% sequence identity to SEQ ID NO: 13.

In a most preferred embodiment, an isolated nucleic acid molecule of the present invention may comprise a nucleotide sequence or complement thereof, wherein the nucleotide sequence encodes a polypeptide having an amino acid sequence that has at least 98% sequence identity to SEQ ID NO: 13.

The isolated nucleic acid of the present invention may also comprise a nucleotide sequence or complement thereof, wherein the nucleotide sequence encodes a polypeptide having an amino acid sequence set forth in SEQ ID NO: 13 with conservative amino acid substitutions.

The present invention is directed to a method for manipulating COP1 gene expression in transgenic plants to overcome shade avoidance responses when they grow in a highly populated environment. For this purpose, the COP1 nucleic acid used in the present invention is not necessarily the maize COP1 nucleic acid disclosed herein. It can be any COP1 nucleic acids available in the art and these COP1 nucleic acids may include the sequences from *Arabidopsis* (Deng et al., Cell 27, 791-801, 1992), rice (gi7592844), tomato (gi4090943), pea (Zhao et al., Biochimica et Biophysica Acta-Gene Structure and Expression 1395, 326-328, 1998) and Japanese morning glory (*Ipomoea nil*). The species provided herein are just a few examples of COP1 sequences that can be readily available for use in the present invention and thus

should not be interpreted in any way to limit the scope of the present invention. The COP1 nucleotide sequence used in the present invention can be a full length or a fragment of any of the COP1 nucleotide sequences from any species. Those skilled in the art will be able to identify other COP1 sequences from different species and alterations that can be made to the COP1 sequences and method disclosed herein while not departing from the scope of the present invention.

Preparation of cDNA libraries for isolation of COP1 gene

Complementary DNA (cDNA) libraries from a plant may be prepared and screened for COP1 nucleic acids. Using a maize plant as an example herein and throughout the detailed descriptions of the preferred embodiments, cDNA libraries from the maize plant may be prepared according to standard techniques known to those skilled in the art, for instance, in Sambrook et al., Molecular Cloning – A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1989). Using conventional methodologies, cDNA libraries can be constructed from the mRNA of a given tissue sample or an organ using poly dT primers and reverse transcriptase (Efstratiadis et al., Cell 7:279-288, 1976; Higuchi et al., Proc. Natl. Acad. Sci. (U.S.A.) 73:3146-3150, 1976; Maniatis et al., Cell 8:163, 1976; Land et al., Nucleic Acids Res. 9:2251-2266, 1981; Okayama et al., Mol. Cell. Biol. 2:161-170, 1982; Gubler et al., Gene 25:263, 1983). Several methods may be employed to obtain full-length cDNA constructs. For example, terminal transferase can be used to add homopolymeric tails of dC residues to the free 3' hydroxyl groups (Land, et al., Nucleic Acids Res. 9:2251-2266, 1981). This tail can then be hybridized by a poly dG oligo which can act as a primer for the synthesis of full length second strand cDNA. A simplified method has been developed by using synthetic primer-adapters that have both homopolymeric tails for priming the synthesis of the first and second strands and restriction sites for cloning into plasmids (Coleclough et al., Gene 34:305-314, 1985) and bacteriophage vectors (Krawinkel et al., Nucleic Acids Res. 14:1913, 1986; and Han et al., Nucleic Acids Res. 15: 6304, 1987).

A method to enrich preparations of mRNA is to fractionate by size. One such method is to fractionate by electrophoresis through an agarose gel (Pennica et al., Nature 301:214-221, 1983). Another such method employs sucrose gradient centrifugation in the presence of an agent, such as methylmercuric hydroxide, that

denatures secondary structure in RNA (Schweinfest, et al., Proc. Natl. Acad. Sci. (U.S.A.) 79:4997-5000, 1982).

In one of the preferred embodiments, preparation of appropriately enriched cDNA libraries from tissue of interest such as a tissue sample from the stem or ear of the maize plant may be described as below. The maize plants may be grown in a greenhouse and, when they reach a desired developmental stage, they may be used for collection of the tissue samples. The cDNA library may be constructed using techniques known to those skilled in the art. Briefly, mRNA from the tissue sample may be isolated and cDNA prepared. Short chains of oligo d-T nucleotides may be hybridized with the poly-A tails of the mRNA and serve as a primer for the enzyme, reverse transcriptase, which synthesizes a complementary DNA (cDNA) strand. The cDNA may be enriched for the desired sequences using subtraction hybridization procedures following Davis et al. (Proc. Natl. Acad. Sci. USA 81: 2194-2198, 1984). The quality of the cDNA library may be determined by examining the cDNA insert size, and also by sequence analysis of a random selection of an appropriate number of clones from the library.

Amplification of the COP1 gene from the cDNA library

As described herein, COP1 nucleic acid molecules from the cDNA from the maize plant may be amplified through use of many available methods. The most preferred method of achieving such a goal may employ the polymerase chain reaction, i.e., "PCR" (Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273, 1986; Erlich et al., European Patent Application 50,424; European Patent Application 84,796, European Patent Application 258,017, European Patent Application 237,362; Mullis, European Patent Application 201,184; Mullis et al., U.S. Patent No. 4,683,202; Erlich., U.S. Patent No. 4,582,788; and Saiki et al., U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define the COP1 nucleic acid of the cDNA library in its double-stranded form.

The COP1 nucleic acid molecules may also be amplified by alternative methods, such as the "Ligase Chain Reaction", i.e., LCR (Barany, Proc. Natl. Acad. Sci. (U.S.A.) 88:189-193, 1991). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of

oligonucleotides are selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, oligonucleotide ligation assay, or isothermal amplification methods may also be used to amplify and analyze the COP1 nucleic acid molecules from the cDNA library of a plant such as the maize plant (Malek et al., U.S. Patent 5,130,238; Davey et al., European Patent Application 329,822; Schuster et al., U.S. Patent 5,169,766; Miller et al., PCT Application WO 89/06700; Kwoh et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173-1177, 1989; Landegren et al., Science 241: 1077-1080, 1988; Gingeras et al., PCT Application WO 88/10315; Walker et al., Proc. Natl. Acad. Sci. (U.S.A.) 89:392-396, 1992).

Sequencing of the COP1 nucleic acid from the cDNA library

The COP1 nucleic acid molecule of the cDNA library from the maize plant may be sequenced after its amplification through use of many available methods. The most preferred method of achieving such a goal may employ the polymerase chain reaction ("PCR"), as described above, using primer pairs that are capable of hybridizing to the proximal sequences that define the COP1 cDNA library in its double-stranded form.

Antibody production

In one of the preferred embodiments, antibodies to the maize COP1 of the present invention may be produced using standard immunological techniques for production of polyclonal antisera and, if desired, immortalizing the antibody-producing cells of the immunized host for sources of monoclonal antibody production. Techniques for producing antibodies to any substance of interest are well known, e.g., as in Harlow and Lane (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988) and as in Goding (Monoclonal Antibodies: Principles and Practice, 2nd eds, Academic Press, NY, 1986). The

antibodies produced in the present invention are useful in immunoassays for determining the amount or presence of the COP1 protein. Such assays are also useful in quality controlled production of compositions containing COP1 of the present invention. In addition, the antibodies can be used to assess the efficacy of recombinant production of the COP1, as well as for screening expression libraries for the presence of COP1 encoding gene. They may also be used as affinity ligands for purifying and/or isolating the COP1 proteins. The COP1 antigens may be obtained by over expressing the full or partial length of the COP1 gene.

Promoter selection and vector construction

Exogenous genetic material such as the wild type COP1 nucleic acid or its fragment thereof may be transferred into a plant cell by use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (See, Plant Molecular Biology: A Laboratory Manual, Clark eds, Springer, New York, 1997).

In one of the preferred embodiments, the construct may be an antisense construct comprising the COP1 nucleic acid that is complementary to, and is capable of pairing, with the native COP1 mRNA and thus prevent translation of the native COP1 mRNA. See Mol et al. (FEBS Lett. 268: 427-430, 1990) and Green et al. (Annu. Rev. Biochem. 55: 569-597, 1986) for general description of the technique. An antisense vector may be constructed by standard procedures and introduced into cells.

In another preferred embodiment, the construct may be a regular transformation vector and the process involves a "dominant-negative" approach to reduce the functions of native COP1 proteins. In such a method, part or all of the COP1 normal nucleic acid sequence is placed under the control of a promoter so that a partial or whole sequence of a protein similar to the targeted native protein is produced in small or large quantity. These partial or whole sequence of the expressed COP1 proteins may interact with the native COP1 proteins in such a way that the expression level and function of the native COP1 proteins be reduced. Because of the dominant-negative response of the endogenous cop1 alleles, this process will modify the shade-avoidance response to cause production of dominant-negative transgenic plants.

A construct or vector may include a plant promoter to express a COP1 nucleic acid or a fragment thereof. Promoters which are known or found to cause transcription of nucleic acid molecules can be used for DNA transcription in the maize plants. Such promoters may be obtained from a variety of sources such as plants and plant viruses. The promoter selected should not cause any potential problems for plant's growth and development. For example, the promoter selected should not cause any seed germination problems. A number of promoters which are active in plant cells have been described in the literature and have been used to create DNA constructs which have been expressed in plants (see, e.g., PCT publication WO 84/02913). For the purpose of the present invention, it is preferred that the particular promoter selected should be a light-inducible promoter. This light-inducible promoter should be, in the case of overexpressing the COP1 binding domains, capable of causing sufficient expression of the exogenous COP1 so that the exogenous COP1 proteins that include the protein-binding domains will be produced at a higher level to cause the binding activities in the transformants to result in the inactivity of the indigenous COP1 proteins. This light-inducible promoter should be, in the case of using the antisense technology, capable of producing an effective amount of mRNA from the exogenous COP1. Thus the effective amount of mRNA so produced will bind to the indigenous mRNA in the "antisense" orientation and cause suppression of the COP1. In either of the above two events, since the indigenous COP1 expression is suppressed, the desired phenotype will in expectation have shorter internodes. The promoters suitable for the present invention may include a *cab* promoter, an ATHB-2 promoter, a rice HB-2 promoter and a corn HB-2 promoter. In one of the preferred embodiments, the promoter may be the *cab* promoter. The methods for identifying and isolating a light-inducible promoter for the present invention can be readily available (e.g., Sheen, Plant Cell 2: 1027-1038, 1990).

In addition to promoters which are known to cause transcription of COP1 in plants as described above, other promoters may be identified for use in the present invention by screening a plant cDNA library for nucleic acids which are selectively or preferably expressed in the target tissues or cells.

The vector or construct may also include a structural gene or a fragment of the structural gene thereof. The "structural gene" or a fragment of the "structural gene" as used herein in the present invention comprises the COP1 gene or a fragment of the

COP1 gene. The COP1 gene may be operatively linked downstream to a promoter as described above. In one of the preferred embodiments, the COP1 gene may be a wild type COP1 nucleic acid or a portion of the COP1 nucleic acid from any source. The COP1 nucleic acid may be from a maize plant and may be the ZmCOP1 nucleic acid as disclosed in the present invention having SEQ ID NO: 12.

The vector or construct may also include, within the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. For example, such sequences that have been isolated include the Tr7 3' sequence and the nos 3' sequence (Ingelbrecht et al., The Plant Cell 1:671-680, 1989; Bevan et al., Nucleic Acids Res. 11:369-385, 1983) or the like.

The vector or construct may also include regulatory elements. Examples of such regulatory elements may include the Adh intron 1 (Callis et al., Genes and Develop. 1:1183-1200, 1987), the sucrose synthase intron (Vasil et al., Plant Physiol. 91:1575-1579, 1989), the TMV omega element (Gallie et al., The Plant Cell 1:301-311, 1989), and maize heat shock protein 70 (hsp70) intron (Brown and Santino, PCT Application WO93/19189). These and other regulatory elements may be included when appropriate.

The vector or construct may also include a selectable marker, a screenable marker and other elements as appropriate. Examples of these elements and markers mentioned herein are known in the art and may be readily used in the present invention. Those of the skilled in the art should refer to the following art for details (for selectable markers, see Potrykus et al., Mol. Gen. Genet. 199:183-188, 1985; Hinchee et al., Bio. Technology 6:915-922, 1988; Stalker et al., J. Biol. Chem. 263:6310-6314, 1988; European Patent Application 154,204; Thillet et al., J. Biol. Chem. 263:12500-12508, 1988; for screenable markers see, Jefferson, Plant Mol. Biol, Rep. 5: 387-405, 1987; Jefferson et al., EMBO J. 6: 3901-3907, 1987; Sutcliffe et al., Proc. Natl. Acad. Sci. (U.S.A.) 75: 3737-3741, 1978; Ow et al., Science 234: 856-859, 1986; Ikatu et al., Bio. Technol. 8: 241-242, 1990; and for other elements see, European Patent Application Publication Number 0218571; Koziel et al., Plant Mol. Biol. 32: 393-405; 1996).

Methods and compositions for transforming bacteria and other microorganisms are known in the art (see, for example, Sambrook et al., Molecular

Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

Plant transformation

The COP1 nucleic acid molecules of the present invention may be transferred into a plant cell and the plant cell regenerated into a whole plant. The COP1 nucleic acid molecules may be from any source, whether naturally occurring or otherwise obtained through methodologies in the field that are readily known to those skilled in the art, that are capable of being inserted into any plant cells. The COP1 nucleic acid molecules may be transferred into either monocotyledonous and dicotyledonous plants (See specifically, Chistou, Particle Bombardment for Genetic Engineering of Plants, Pp. 63-69 (*Zea mays*), Biotechnology Intelligence Unit. Academic Press, San Diego, California, 1996), and generally Chistou, Particle Bombardment for Genetic Engineering of Plants, Biotechnology Intelligence Unit. Academic Press, San Diego, California, 1996). As an example, the COP1 gene may be transformed into the maize plant using any of the methods as described herein.

There are many methods for transforming the COP1 nucleic acid molecules into plant cells such as the maize plant cells. Suitable methods are believed to include virtually any methods by which nucleic acid molecules may be introduced into the cells, such as by Agrobacterium infection or direct delivery of nucleic acid molecules that may include PEG-mediated transformation, electroporation and acceleration of DNA coated particles, etc. (Potrykus, Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:205-225, 1991; Vasil, Plant Mol. Biol. 25: 925-937, 1994). For example, electroporation has been used to transform *Zea mays* protoplasts (Fromm et al., Nature 312:791-793, 1986). In general, the following are four most commonly used general methods for delivering a gene into cells: (1) chemical methods (Graham and van der Eb, Virology, 54:536-539, 1973); (2) physical methods such as microinjection (Capecchi, Cell 22:479-488, 1980), electroporation (Wong and Neumann, Biochem. Biophys. Res. Commun. 107:584-587, 1982); Fromm et al., Proc. Natl. Acad. Sci. (USA) 82:5824-5828, 1985); U. S. Patent No. 5,384,253; and the gene gun (Johnston and Tang, Methods Cell Biol. 43:353-365, 1994); (3) viral vectors (Clapp, Clin. Perinatol. 20:155-168, 1993; Lu et al., J. Exp. Med. 178:2089-2096, 1993; Eglitis and Anderson, Biotechniques 6:608-614, 1988); and (4) receptor-mediated mechanisms

(Curiel et al., Hum. Gen. Ther. 3: 147-154, 1992; Wagner et al., Proc. Natl. Acad. Sci. (USA) 89: 6099-6103, 1992).

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments. See for example (Potrykus et al., Mol. Gen. Genet., 205:193-200, 1986; Lorz et al., Mol. Gen. Genet., 199:178, 1985; Fromm et al., Nature, 319:791, 1986; Uchimiya et al., Mol. Gen. Genet.:204:204, 1986; Callis et al., Genes and Development, 1183, 1987; Marcotte et al., Nature, 335:454, 1988). Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Among them are the methods for corn (U. S. Patent No. 5,569,834, U. S. Patent No. 5,416,011; McCabe et al., Biotechnology 6:923, 1988; Christou et al., Plant Physiol., 87:671-674, 1988). Illustrative methods for the regeneration of cereals from protoplasts are also described (Fujimura et al., Plant Tissue Culture Letters, 2:74, 1985; Toriyama et al., Theor. Appl. Genet. 205:34, 1986; Yamada et al., Plant Cell Rep.4: 85, 1986; Abdullah et al., Biotechnology, 4:1087, 1986).

In one of the preferred embodiments, the present invention employs the Agrobacterium-mediated transformation technology to introduce the COP1 nucleic acid into the maize plant and to achieve a desired result. Agrobacterium-mediated transfer is a widely applicable system for introducing genes such as the COP1 gene into plant cells because the gene such as the COP1 gene can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of Agrobacterium-mediated plant integrating vectors to introduce a nucleic acid into plant cells is well known in the art. See, for example, Fraley et al. (Biotechnology 3:629-635, 1985), Hiei et al. (U. S. Patent No. 5591616), and Rogers et al. (Meth. In Enzymol 153: 253-277, 1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of the COP1 nucleic acid to be transferred is defined by the border sequences and is usually inserted into the plant genome as described in Spielmann et al. (Mol. Gen. Genet., 205:34, 1986).

A transgenic plant such as a transgenic maize plant formed using Agrobacterium transformation methods typically contains a single added COP1 gene on one chromosome. Such a transgenic plant can be referred to as being heterozygous

for the added COP1 gene. More preferred is a transgenic plant that is homozygous for the added COP1 gene; i.e., a transgenic plant that contains two added COP1 genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregated transgenic plant that contains a single added COP1 gene, germinating some of the seeds produced and analyzing the resulting plants produced for the COP1 gene.

It is understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous COP1 genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous COP1 genes that encode a COP1 polypeptide. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Regeneration of the transformed plants

The regeneration, development, and cultivation of plants such as the maize plants from transformants or from various transformed explants are well known in the art (Weissbach and Weissbach, In: Methods for Plant Molecular Biology, Eds., Academic Press, Inc. San Diego, CA, 1988). This regeneration and growth process may typically include the steps of selection of transformed cells containing exogenous COP1 genes, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. As described in the present invention, the regenerated plants such as the regenerated maize plants that contain the COP1 nucleic acids, either wild type or chemically synthesized, that encode for the COP1 proteins, may be preferably self-pollinated to provide homozygous transgenic maize plants, as discussed before. Otherwise, pollen obtained from the regenerated maize plants may be crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate

regenerated plants. A transgenic maize plant of the present invention may be cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated. Monocotyledonous plants, or monocot plants, may be transformed with a COP1 nucleic acid and then regenerated. Transformation of monocot plants using electroporation, particle bombardment, and Agrobacterium has also been reported. Transformation and plant regeneration have been achieved in many monocot plants that include maize, asparagus, barley and wheat, etc. Dicotyledonous plants, or dicot plants, may also be transformed with COP1 nucleic acid and regenerated. Methods for transforming dicot plants, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants have been published. Among them are the methods for soybean, cotton, and other dicot plants.

Monocot and dicot plants to which the present invention may be applied may include those agronomic and horticultural crop plants. Examples of agronomic crop plants may include cereals such as maize, wheat, rye, barley, oats, buckwheat, sorghum and rice; non-cereals such as sunflower, canola, peas, beans, soybeans, cotton and linseed; vegetables such cauliflower, asparagus, lettuce, tobacco and mustard; and root crops such as sugarbeet, potato, sweet potato, carrot and turnip. Horticultural crops may include celery, tomato, egg plant, cucumber and squash. Fruit crops may include apple, apricot, peach, pear, plum, orange, blackberry, blueberry, strawberry, cranberry and lemon.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones (see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, 1989; Mailga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press, 1995; Birren et al., Genome Analysis: Analyzing DNA, 1, Cold Spring Harbor, New York, 1997).

The following examples further demonstrate several preferred embodiments of the present invention. Those skilled in the art will recognize numerous equivalents to

the specific embodiments described herein. Such equivalents are intended to be within the scope of the present invention and claims.

Examples

Example 1. Computer-aided sequence analysis

A COP1 protein sequence from *Arabidopsis* was retrieved from GenBank (ID 402685) and used as query to BLAST search sequence databases which identified clones in the databases that share high degree homology to *Arabidopsis* COP1. These clones may be used in cloning the full-length COP1 cDNA from these crop species from which the clones were originally obtained including maize.

Example 2. COP1 cDNA cloning

To obtain the full-length sequence of maize COP1 cDNA, several RT-PCR, 5'race and 3'race polymerase chain reactions (PCR) were performed. One microgram of kernel cDNA, recovered from kernels 15 days after pollination (DAP) was used as template in all PCR reactions. The cDNA library had previously been cloned into pSPORT2. Two micromoles of maize COP1 gene specific forward primer L3062COP1F (5'GTACGGACATTCAGAGGACAC3'; SEQ ID NO: 1) and reverse primer L30623'COP1R (5'GTGTCCTCTGAATGTCCGTAC3'; SEQ ID NO: 2) combined with 0.1 mM dNTPs and 5 Units of Taq DNA polymerase was used in a 50 μL PCR reaction to determine the presence of COP1 in the 15 DAP kernel cDNA pools. PCR cycling conditions and parameters were as follows: 95°C for 5 minutes (min) followed by 28 cycles at 95°C for 30 seconds (sec), 60°C for 30 sec and 72°C for 30 sec. To obtain the most 5' sequences of COP1 cDNA, the complementary sequences of M13 Forward primer (5'CCCAGTCACGACGTTGTAAAACG3'; SEQ ID NO: 3) in pSPORT2 vector and the primer L3062cop1R, combined with 0.1 mM dNTPs and 5 units of HotStart Taq™ polymerase (Qiagen, Valencia, CA), were used in a 50 μL in a 5'-race PCR. PCR cycling conditions and parameters were as follows:95°C for 15 min, then 28 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 3 min. An approximately 2 Kb PCR product was obtained, cloned into PCR-blunt TOPO II cloning vector (Invitrogen, Carlsbad, CA) and sequenced. In the meantime,

COP1 gene specific primer L30624070F (5'AATGAAAAGAACTTTGTTGGC3'; SEQ ID NO: 4) and M13 reverse primer (5'AGCGGATAACAATTTCACACAGG3'; SEQ ID NO: 5) were mixed with 0.1 mM dNTPs and 5 Units Taq DNA polymerasein a 50 μL 3'race PCR to obtain the most 3'sequences of the COP1 cDNA. PCR cycling conditions and parameters were as follows:95°C for 5 min, then 28 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 40 seconds. A 650 base pair PCR product was obtained, cloned into PCR-blunt TOPO II cloning vector, and sequenced. For subcloning purposes, and for obtaining a more reliable cDNA clone, COP1 cDNA was re-amplified from 15 DAP kernel cDNA library using forward primer COP15-6 (5'CTGCGCCATGGGCGACTCCTCGGTGG3'; SEQ ID NO: 6) containing NcoI site at the start codon of the COP1 open reading frame and reverse primer L30623'COP1R (SEQ ID NO: 2). A 50 μL of PCR cocktail was made as follows: 1 μg of 15 DAP kernel cDNA, 2 μM of Cop15-6 and L30623'Cop1R primers, 0.1 mM dNTPs, 5% DMSO, and 1 x PCR reaction buffer (Mg++). Manual hot start PCR cyclings were initiated at 95°C for 15 min. Then 2 units of Expand™ High-Fidelity DNA Polymerase (Roche, 173264) was added and PCR reaction was carried out under the following conditions and parameters: at 95°C for 30 seconds, 60°C for 30 sec and 68°C for 6 min for a total of 28 cycles. A 2230 base pair maize COP1 cDNA sequence was obtained, and cloned into PCR-Blunt TOPO II cloning vector. The full-length COP1 cDNA sequence was confirmed by sequencing six independent clones. The sequence was named as ZmCOP1 (SEQ ID NO: 12). ZmCOP1 represents a full length cDNA sequence containing a 388 base pair 5' UTR, a coding region that encodes 694 amino acids (see translated amino acid sequence; SEQ ID NO: 13) and a 141 base pair 3' UTR. Based upon sequence homology analysis, the isolated maize COP1 nucleotide sequence has 50% and 71% identity with those of COP1 from *Arabidopsis* (gi7446130, Deng et al., Cell 27, 791-801, 1992) and rice (gi7592844), respectively (Figures 1 and 2). The maize COP1 protein sequence is also aligned with other COP1 protein sequences from other species and shows 68% sequence identity to that of pea (gi3121867, Zhao et al., Biochimica et Biophysica Acta-Gene Structure and Expression 1395, 326-328, 1998), 69% to Japanese morning glory (gi11127996), 70% to *Arabidopsis* (gi7446130, Deng et al., Cell 27, 791-801, 1992), 71% to tomato (gi4090943), and 89% to rice (gi7592844), respectively (Figure 3). The identity levels

were determined by BLAST program (Altschul et al., Nucleic Acids Res. 25: 3389-3402, 1997) with default parameters (Expectation value (E): blank; Alignment view options: pairwise; Filter query sequence: no; Cost to open a gap: 0; Cost to extend a gap: 0; X dropoff value for gapped alignment: 0; Show GI's in deflines: no; Penalty for a nucleotide mismatch: -3; Reward for a nucleotide match: 1; Threshold for extending hits: 0; Perfom gapped alignment: yes; Query Genetic code to use: standard; DB Genetic code: standard; Believe the query defline: no; Matrix: BLOSUM62; Word size: 0; Effective length of the database: 0; Query strand Use: both).

Example 3. Northern Blot analysis of COP1 from the cDNA library

Northern Blot analysis of the expression of maize COP1 during ear development was performed. Total RNA (15 μg each) from different tissues were mixed with one volume of RNA Sample Loading Buffer (Sigma, R-4268), heated at 65^0C for 10 min., chilled on ice for one min. and loaded on a 1% formaldehyde agarose gel. The RNA was separated on the gel under constant voltage at 65v for 4 to 5 hours and then transferred onto a piece of nylon membrane at 4°C overnight using a Schleicher & Schuell transfer system (Keene, NH, USA 03431). A 157 base pair 3'UTR fragment of COP1 was amplified by PCR using forward primer 3'COP1 (5'TGCTCCTTGATGTTATGG3'; SEQ ID NO: 7) and reverse primer L30623'COP1R. A 910 base pair5' fragment was also amplified by PCR using forward primer COP15-6 and COP301 (5'GATGAATTCATCAAGGAGGATCAGAAGAAG3'; SEQ ID NO:8). Purified PCR products (25 ng each) were labeled with p32dCTP using Random Primed DNA Labeling Kit (Boehringer Mannheim, Cat. # 1004760). The membrane was hybridized with both labeled 3'UTR and 5'COP1 probes.

Example 4. Construction of maize transformation vectors

In an effort to identify an efficient way for reducing the endogenous COP1 mRNA and protein level, three sequences of the COP1 gene with different lengths were created and dominant negative and antisense strategies were employed. The dominant negative strategy employed a fragment of the 5' nucleotide sequence encoding a protein binding domain was cloned into an overexpression vector. Two

sequences employing COP1 were created for this purpose, one containing the first 1233 nucleotide residues (SEQ ID NO: 14) encoding 411 amino acids (SEQ ID NO: 15) in pMON47119 (Figure 4), and another containing the first 906 nucleotide residues (SEQ ID NO: 16) encoding 301 amino acids (SEQ ID NO: 17) in pMON47118 (Figure 5). A full length COP1 was used in the antisense approach. The construct was pMON47120 and the detail of the vector was shown in Figure 6.

Forward primer of COPantisense5'-end (5'GATGAATTCCTGCGGCATGGGCGAC3'; SEQ ID NO: 9) and reverse primer of COPantisense3'-end (5'AACCATGGACTGAACCTCTTGAACG3'; SEQ ID NO: 10) were used to sub-clone full-length maize COP1 for the antisense strategy. Primers COP15-6 and COP301 were used to sub-clone N-terminal 301 (N301) amino acid protein domain of maize COP1. This maize N301 protein domain was equivalent to N 282 protein dimerization domain of *Arabidopsis* COP1 (Deng et al., PCT Application WO 00/18940). Forward primer of COP15-6 and reverse primer of COP411 (5'GATGAATTCATCATTTCGAGACTCCAGC3'; SEQ ID NO: 11) with two stop codons (TGA) near EcoRI cleavage site were used to sub-clone the N-terminal 411 (N411) amino acid protein domain of the maize COP1. This N411 fragment was equivalent to the N392 fragment COP1 in *Arabidopsis* that contained the protein dimerization domain and core sequences required for COP1 protein translocation from cytoplasm to nucleus (PCT Application WO 00/18940). PCR mixture (50 μL) was made as follows: 100 ng of PCR-Blune-COP1 plasmid DNA, 2 μ M of primers, 0.1 mM dNTPs, 5% DMSO, 1X PCR buffer, and 5U Expand High Fidelity DNA polymerase. PCR cycling conditions and parameters were as follows: 95°C, 5 min., followed by 25 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 68° C, 6 min. for antisense COP1 amplification, 2 min. for COPaa301 amplification, and 3 min for COPaa411 amplification. The PCR products were gel purified, cloned into PCR-Blunt TOPO II cloning vector, and full-length sequenced.

To construct maize *Agrobacterium*-mediated transformation vectors, pMON32502 plasmid DNA was digested with HindIII, EcoRI and NcoI. A 3135 base pair HindIII and EcoRI vector backbone fragment was isolated. Plasmid DNA of pMON24037 was digested with HindIII and NcoI. A 1689 base pair of the HindIII and NcoI fragment containing the promoter of maize chlorophyll a/b binding protein and the hsp70 intron was obtained. PCR-Blunt vector containing antisense COP1,

COP1aa301, and COP1aa411 were digested respectively with NcoI and EcoRI to obtain the NcoI and EcoRI fragments of antisense COP1, COP1aa301 and COP1aa411. The HindIII and EcoRI vector backbone fragment was ligated with the HindIII and NcoI *cab* promoter fragment, and NcoI and EcoRI antisense COP1, or COPaa301, or COPaa411, respectively, to form pMON47120, pMON47118, and pMON47119. The three constructs were then transformed individually into *Agrobacterium* strain ABI. All these gene constructs were under the control of *cab* promoter (light inducible) and were designed to reduce the functional ZmCOP1 protein level in the above-ground part of corn plants. The vector pMON47118 contained the coding sequence for N-terminal 301 amino acid residues of the ZmCOP1 gene that covered the dimmerization domain. The vector pMON47119 contained the nucleotide sequence coding for the 411 amino acid residues. The N-terminus of ZmCOP1 carried the dimerization and nuclear localization domains. pMON47120 contained the full-length antisense ZmCOP1 gene.

To serve as a check for evaluation of ZmCOP1 functions in corn, two additional ZmCOP1 constructs, i.e., pMON47130 and pMON47131, were made for transformation. A fragment of ZmCOP1 gene containing 903 nucleotide residues was driven by a constitutive promoter, i.e., a rice-actin (RACT) promoter. In order to construct pMON47130 transformation vector, a plasmid DNA of pMON47123 was digested with NcoI and XhoI restriction enzymes. A 1404 base-pair XhoI and NcoI fragment which contained rice-actin promoter (RACT) was obtained from pMON47123. Plasmid DNA of pMON47118 was also digested with NcoI and XhoI restriction enzymes. A fragment containing 8458 bp from 4379 bp XhoI site to 2719 bp NcoI site was obtained from pMON47118. Then, the 1404 bp fragment was ligated to the 8458 bp fragment and pMON47130 construct was made (Figure 7). In order to make pMON47131 transformation vector, a plasmid DNA of pMON47120 was digested with NcoI and XhoI restriction enzymes. A fragment containing 9567 bp from 5594 bp XhoI site to 3934 bp NcoI site on pMON47120 was obtained. The RACT promoter fragment obtained from pMON47123 as described above was ligated to the 9567 bp fragment and the construct, i.e., pMON47131, was made (Figure 8). The vector contained the full length ZmCOP1 coding sequence in reverse orientation.

Example 5. Overexpression of ZmCOP1 N301 domain for antibody production

In order to make polyclonal antibody for maize COP1 protein, plasmid DNA of pMON47118 and pET30(a) (Novagen, Madison, WI) were digested with NcoI and EcoRI . The NcoI and EcoRI COPaa301 fragment was directionally cloned into pET30(a) vector under the control of the IPTG inducible T7 polymerase promoter and was transformed into *E. coli* BL21(DE3) competent cells. *E. coli* BL21 (DE3) cells containing pET30(a)-COPaa301 construct were induced with 3 mM IPTG overnight at room temperature. Proteins were purified under denaturing conditions using Ni-NTA Superflow resin (QIAGEN, Valencia, CA) as described in the manufacturer's protocol. An about 38kDa His-tag COP301 protein was purified and confirmed by Western Blot analysis using monoclonal antibody against S-tag protein. About 1 mg purified COPaa301 protein was used to inoculate two rabbits for antibody production following the standard protocol of Pocono Rabbit Farm & Laboratory Inc. (Canadesis, PA). The preimmune serum from these two rabbits showed no reactivity with COPaa301 protein.

Example 6. Transformation of corn plant with the vectors

1) Plant materials

Ears from LH172 were obtained mostly from greenhouses and were usually harvested about 10 to 11 days post pollination. Before isolation, ears were stored from 0 to 5 days at 4°C. Ears were sterilized for 20 min in 50%(v/v) commercial bleach (Clorox^{R}, with 5.25% sodium hypochlorite) followed by 3 rinses with sterile water.

Table 2. Media used in corn transformation (per liter).

Component	½ MS VI	½ MS PL	Co-culture medium	LH172 MS	MS/BAP	MSOD
MS salts	.1 g	0.1 g	2.2 g	4.4 g	4.4 g	.1 g
Sucrose	20 g	0.1 g	20 g	30 g	30 g	--
Maltose	--	--	--	--	--	40 g
Glucose	10 g	36 g	10 g	--	--	20 g
l-Proline	0.115 g	0.115 g	0.115	1.36 g	1.36 g	--
Casamino Acids	--	--	--	0.05 g	0.05 g	--
Glycine	2 mg	2 mg	2 mg	--	--	--
l-Asparagine	--	--	--	--	--	150 mg
myo-Inositol	100 mg	100 mg	100 mg	--	--	100 mg
Nicotinic Acid	0.5 mg	0.5 mg	0.5 mg	0.65 mg	0.65 mg	0.65 mg
Pyridoxine·HCl	0.5 mg	0.5 mg	0.5 mg	0.125 mg	0.125 mg	0.125 mg
Thiamine·HCl	0.1 mg	0.1 mg	0.6 mg	0.125 mg	0.125 mg	0.125 mg
Ca Pantothenate	--	--	--	0.125 mg	0.125 mg	0.125 mg

2,4-D	--	--	3 mg	0.5 mg	0.5mg	--
Picloram	--	--	--	2.2 mg	.1 mg	--
Silver Nitrate	--	--	--	3.4 mg	--	--
Na-Thiosulfate	--	--	--	--	--	--
Phytagar	--	--	--	7.0 g	7.0 g	7.0 g
Low EEO agarose	--	--	5.5 g	--	--	--

2) *Agrobacterium* induction and inoculation

Agrobacterium tumefaciens (ABI strain) was grown in LB liquid medium (50 mL medium per 250 mL flask) containing 100 mg/L kanamycin and 50 mg/L spectinomycin for an initial overnight propagation (on a rotary shaker at 150 to 160 rpm) at 27°C. Ten mL of the overnight *Agrobacterium* suspension was transferred to 50 mL of fresh LB in a 250 mL flask (same medium additives and culture conditions as stated above) andgrown for approximately 8 hours. Suspension was centrifuged around 3500 rpm and pellet resuspended in AB minimal medium (now containing ½ the level of spectinomycin and kanamycin used for LB) containing 100 μM acetosyringone (AS) for a final concentration of 0.2 X 10^9 cfu/mL (or an OD of 0.2 at 660 nm). These *Agrobacterium* cultures were allowed to incubate as described above for approximately 15 to 16 hours. The *Agrobacterium* suspension was harvested via centrifugation and washed in ½ MS VI medium (Table 2) containing AS. The suspension was then centrifuged again before being brought up in the appropriate amount of ½ MS PL (Table 2) (also containing AS) so that the final concentration of *Agrobacterium* was 1 X 10^9 cfu/mL (which is equal to an OD of 1.0 at 660 nm). Immature embryos from each ear of a LH172 plant (1.5 mm to 2.0 mm long) were directly dissected into a 1.5-mL eppendorf tube with ½ MS PL containing *Agrobacterium* at an OD of 1.0. The eppendorf tube was capped tightly and inverted 3 times so that embryos inside were fully immersed. About half of the solution in the tube was drained by using a sterile SAMCO transfer pipette. The rest of the solution together with the embryos were poured into 2-3 layers of sterile Baxter filter paper (5.5cm in diameter). Embryos were removed from the filter paper by flipping the filter paper over and slightly pressing it against the co-culture medium (Table 2) with the addition of 20 μM silver thiosulfate in the petri dish. The embryos were cultured at 23°C for 1 day and then were transferred to the first selection medium (LH172MS; Table 2).

3) Callus induction and selection (in dark)

Selection was performed in LH172 medium with 500mg/L carbenicillin and 100 mg/L paromomycin. Three transfers to new plates containing the same medium were made every two weeks.

4) Regeneration (in light)

Paromomycin resistant callus was first moved to MS/BAP medium (Table 2) with with 3.5 mg/L 6-BA for 5 to 7 days. After the 6-BA pulse, callus with green shoot tips were moved to MSOD (Table 2) with 100 mg/L paromomycin plates and were cultured for another 10 to 12 days. After this stage, green shoots started to grow out as well as white roots. Those small plantlets were transferred to phytatray (1 event per phytatray) containing MSOD media with 100 mg/L paromomycin. After 2 to 3 weeks, plants were ready to be transplanted into soil. Plants were acclimated in the growth chamber for 1 week and then moved to the greenhouse for hardening. Plants were screened for the presence of *nptII* after 3 to 5 days of the hardening process. Only *nptII* positive plants were considered for further experimentation.

Example 7. R0 and R1 transgenic plants

A total of 30 events from pMON47118 construct were selected from 137 R0 plants (79 events) based on the expression levels of gene of interest (GOI) by Northern and Western Blot analyses. Among the 30 events, 8 events had only F1 seeds and 22 events had R1 seeds. Twenty R1 or F1 seeds of each of these events were planted in a greenhouse. Phenotype observations were conducted weekly including germination, leaf color, plant height, growth rate, tassel morphology, ear morphology and ear number. The average height and growth rate of several events were lower compared to the wild type plants growing nearby (Figure 9). In comparison of wild type and transformed adult plants, several transformed R1 plants in the event S35307 showed better ear growth, i.e., more and larger ears at third and fourth nodes, and distinct ear morphology, i.e., longer husk leaves. These phenotypic changes may be attributed to the COP1 transgene. Variations among individual plants in an event will be examined for correlation to the presence and expression level of the COP1 transgene. Cells transformed with a full length or a fragment of ZmCOP1 is used to produce young corn plants using standard protocols. These plants are called R0 generation plants. R0 plants are generated from many transformation events. These plants are grown in greenhouse and screened for the presence of ZmCOP1

transgene by PCR. The messenger RNA and protein expression level of the transgene in R0 plants are examined by Northern and Western blotting techniques. Events are selected based on the presence and expression level of the transgene in the R0 plants. The selected events are planted as R1 plants. R1 plants are examined for the presence of the transgene, the transgene expression level and the expected phenotypic traits such as short internodes and better ear development. All the data are used in selecting R1 plants for R2 evaluation. Those R1 plants that show a moderate to high level of transgene expression and a desired phenotype (shorter internodes and better ear development at high density) are chosen. R2 plants are planted in field as pedigree lines. The zygosity of each line is determined by the presence of the transgene in each plant in the line and by the positive/negative segregation ratio of its R1 predecessor. A few lines are selected based on their phenotype, transgene expression and homozygosity. These lines are crossed with another one or more inbred lines to make hybrid seeds. The hybrid seeds are planted at different density (20,000, 30,000 and 40,000 plants/acre) side by side with a control hybrid that is a best yield performer. The lines that give the best hybrid yield and the best density regimes are selected for further yield testing. The best line or lines proven in these yield trails are bulked up and grown in a large scale. For example, grow the new hybrid at 30,000 plants/acre, resulting in a 10% increase in biomass. Because the ZmCOP1 transgene is able to reduce shade avoidance response and hold harvest index the same, this results in a 10% yield gain. In some selected lines, reduced shade avoidance response also enhances harvest index; this increases yield even more.

Example 8. Constitutive expression of ZmCOP1 in corn

A construct harboring a rice actin promoter and ZmCOP1aa301 gene fragment (pMON47130, Figure 7) was transformed into corn and the events named YAA. Western blot analysis on the R0 plants showed that some YAA events have quite high expression of the ZmCOP1aa301 protein (Figure 10).

F1 seeds from eight events were selected for a dark and a light experiment. The dark experiment was conducted in a growth chamber with optimal conditions for corn seedling growth except that no light was provided. At day 5 after shoot emerging

from soil, mesocotyl lengths of each seedling were measured. Positive and negative segregants were identified by Western blotting. Mesocotyl length data did not show a significant difference between positive and negative segregants. This agrees with the results of a similar experiment Arabidopsis. Eight events of YAA plants were also grown in a growth chamber with 500 mmol.m^{-2}.s^{-1} white lights for 14 hours, and dark for 10 hours daily. Positive and negative plants were identified by Western blot analysis of ZmCOP1aa301 protein. Plant heights were measured weekly. The comparisons of positive and negative plant height are shown in Figure 2. The results indicated that expression of ZmCOP1aa301 in maize using rice actin (RACT) promoter may have resulted shorter plants; while 7 out of 8 event showed a trend of shorter stature in the positive plants at V11 stage, the difference in two of these events was statistically significant at p=0.05 and p=0.01 level this sample size and in this growth conditions (Figure 11).

Shorter stature in Kyle R3 plants

Kyle events are the transformants from construct pMON47118, which contains a light inducible CAB promoter and ZmCOP1aa301 gene fragment. Western analysis has shown that the ZmCOP1aa301 fragment was expressed in the Kyle plants under normal light (Figure 12 and 13) but not in dark or weak light (Figure 12). Also, the gene was expressed at high level at different growth and developmental stages.

Seeds from some Kyle events were germinated and seedlings grown in dark and under 1 mmol/m2.s dim light and under normal green house light. Mesocotyl and coleoptile length were measured on day 10. Leaf samples were taken from each plant for DNA amplification (PCR) of the gene of interest. Seedlings grown under full green house light had virtually no mesocotyl, and their coleoptile length is short and uniform among Kyle positive, negative and wildtype plants. When seedlings were grown under 1 mmol/m2.s light, their coleoptile and mesocotyl were much longer, but no statistically significant difference was detected between positive and negative seedlings (Figure 5).

A greenhouse experiment using R3 homozygous lines of Kyle events was carried out. Five homozygous positive lines and 5 of their corresponding negative lines were chosen and

100 seeds of each were planted in the Jerseyville greenhouse. PCR results on 12 plants of each line were used to confirm homozygosity. Thirty-six plants were transplanted for each line and the positions in the rows were randomized. Plant height data is being recorded on a weekly basis since transplanting. Positive plants were shorter compared to their corresponding negative lines in all events tested up to VT stage. The difference is statistically significant (Table 1). However, only event Kyle50 maintained this difference at maturity.

Table 3. Plant height and t-test of 5 Kyle events

	Height (cm)			P-value of comparison	
Event	POS	NEG	LH172	to NEG	to LH172
Kyle15	55.19	71.22*	85.83	$3.7x10^{-09}$*	$6.5x10^{-12}$
Kyle15	63.22	71.22*	85.83	$8.1x10^{-05}$*	$5.5x10^{-12}$
Kyle17	48.13	69.73	85.83	$1.8x10^{-09}$	$1.9x10^{-15}$
Kyle50	36.24	65.09	85.83	$1.1x10^{-13}$	$1.4x10^{-17}$
Kyle72	63.08	74.21	85.83	$2.1x10^{-05}$	$7.4x10^{-12}$
Kyle77	64.78	79.78	85.83	$3.3x10^{-09}$	$2.6x10^{-11}$

* Compared to the average of all negative events

Plant height of Kurt R1

Kurt events are the transformants from construct pMON47119, which contains a light inducible CAB promoter and ZmCOP1aa411 gene fragment. Western analysis of some Kurt events has shown that the ZmCOP1aa411 fragment was expressed at high level in many of the events tested (Figure 8). Some R1 and F1 Kurt events were grown in the field. Positive plants were identified by PCR. The height of both positive and negative plants was measured on a weekly basis up to VT stage. Figure 8 summarizes data obtained at V10 stage, showing that the positive plants were shorter than their negatives in many events.

In summary, the above describes the present invention. It will be understood by those skilled in the art that, without departing from the scope and spirit of the present invention and without undue experimentation, the present invention can be performed within a wide range of equivalent parameters. While the present invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. The present invention covers any uses, variations, or adaptations of the invention following the principles of the invention in general. Various permutations and combination of the elements provided in all the claims that follow are possible and fall within the scope of this invention.

SEQUENCE LISTING

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<140>
<141> August 28, 2002

<150> PCT/US02/27391
<151> August 28, 2002

<150> US60/315,593
<151> August 29, 2001

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

1. A nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17 wherein said polypeptide binds to a constitutive photomorphogenesis 1 (COP1) protein and acts dominant-negative with respect to native COP1, wherein the nucleic acid molecule is operably linked to a heterologous promoter.

2. The nucleic acid molecule as in claim 1 wherein the nucleotide sequence has at least 80% sequence identity to SEQ ID NO:16 or its complement.

3. The nucleic acid molecule as in claim 1 wherein the nucleotide sequence has at least 85% sequence identity to SEQ ID NO:16 or its complement.

4. The nucleic acid molecule as in claim 1 wherein the nucleotide sequence has at least 90% sequence identity to SEQ ID NO:16 or its complement.

5. The nucleic acid molecule as in claim 1 wherein the nucleotide sequence has at least 95% sequence identity to SEQ ID NO:16 or its complement.

6. An isolated nucleic acid molecule comprising a nucleotide sequence that is identical to SEQ ID NO:16 or its complement.

7. An isolated polypeptide having an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17 wherein said polypeptide binds to a constitutive photomorphogenesis (COP1) protein and acts dominant negative with respect to native COP1.

8. The isolated polypeptide as in claim 7 having an amino acid sequence that has at least 96% sequence identity to SEQ ID NO:17.

9. The isolated polypeptide as in claim 7 having an amino acid sequence that has at least 98% sequence identity to SEQ ID NO:17.

10. An isolated polypeptide comprising SEQ ID NO:17.

11. A recombinant DNA construct comprising a nucleic acid molecule that hybridizes to a constitutive photomorphogenesis 1 (COP1) nucleotide sequence encoding a polypeptide having an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17, said nucleic acid molecule being operably linked upstream (5') to a promoter and downstream (3') to a regulatory element, wherein upon its transformation into a plant said construct causes reduction of indigenous COP1 protein level, wherein hybridization conditions include hybridization solution 6 x SSC, wash solution 0.5 x SSC, 0.5% SDS, and temperatures of 42° - 65°C.

12. The recombinant DNA construct as in claim 11, wherein upon its transformation said construct causes overexpression of COP1 proteins that bind to indigenous COP1 proteins wherein binding to the COP1 protein renders said indigenous COP1 proteins non-functional.

13. The recombinant DNA construct as in claim 11, wherein upon its transformation said construct causes expression of an antisense COP1 mRNA that is complementary to indigenous COP1 mRNA in an inverted orientation, that base-pairs to said indigenous COP1 mRNA wherein expression of the antisense COP1 mRNA renders said indigenous COP1 mRNA untranslatable.

14. The recombinant DNA construct as in claim 11, wherein said promoter is a light-inducible promoter.

15. The recombinant DNA construct as in claim 14, wherein said promoter is selected from the group consisting of a cab promoter, an ATHB-2 promoter, and a far red light inducible promoter.

16. The recombinant DNA construct as in claim 14, wherein said promoter is a cab promoter.

17. The recombinant DNA construct as in claim 11, wherein said regulatory element comprises an intron.

18. The recombinant DNA construct as in claim 17, wherein said intron comprises a hsp70 intron.

19. The recombinant DNA construct as in claim 11, wherein said COP1 nucleotide sequence is isolated from a monocot plant.

20. The recombinant DNA construct as in claim 19, wherein said COP1 nucleotide sequence is isolated from a maize plant.

21. A transgenic plant cell comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17 wherein said polypeptide binds to a COP1 protein.

22. The plant cell as in claim 21 wherein said plant cell is a monocot plant cell.

23. The plant cell as in claim 21 wherein said plant cell is a dicot plant cell.

24. The plant cell of claim 21 further defined as being selected from the group consisting of maize, wheat, rye, barley, oats, buckwheat, sorghum, rice, sunflower, canola, peas, beans, soybeans, cotton, linseed, cauliflower, asparagus, lettuce, tobacco, mustard, sugar beet, potato, sweet potato, carrot, turnip, celery, tomato, egg plant, cucumber, squash, apple, apricot, peach, pear, plum, orange, blackberry, blueberry, strawberry, cranberry and lemon.

25. The plant cell of claim 24 wherein said plant cell is a maize plant cell.

26. A method for increasing yield of a crop, comprising the steps of:

a) inserting into the genome of a crop plant a nucleic acid sequence comprising in the 5' to 3' direction,

i) a promoter that functions in the cells of said crop plant, said promoter operably linked to;

ii) a structural nucleic acid sequence that encodes a constitutive photomorphogenesis 1 protein having an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17, comprising a protein binding domain that binds to a COP1 protein and interacts as a dominant-negative with native COP1, said structural nucleic acid sequence operably linked to:

iii) a 3' untranslated nucleic acid sequence;

b) obtaining transformed plant cells containing the nucleic acid sequence of step (a);

c) regenerating from said transformed plant cells a transformed crop plant;

d) obtaining seeds from said transformed crop plant and progeny of said transformed crop plant; and

e) overplanting said seeds at a high population density.

27. The method of claim 26, wherein said structural nucleic acid sequence comprises a sequence that has at least 90% sequence identity to SEQ ID NO:16.

28. The method of claim 26, wherein said structural nucleic acid sequence comprises a sequence of SEQ ID NO:16.

29. The method of claim 26, wherein said structural nucleic acid sequence is transformed into the cells of said crop plant in an antisense orientation and causes the reduction of the native constitutive photomorphogenesis 1 protein level upon its transformation.

30. The method of claim 26, wherein said structural nucleic acid sequence is overexpressed in the cells of said crop plant and proteins so produced bind to native constitutive photomorphogenesis 1 proteins that causes the reduction of the native constitutive photomorphogenesis 1 protein level upon its transformation into the cells of said crop plant.

31. The method of claim 26, wherein said high population density is defined as a density at least 10% to 100% higher than an average prevailing density for said crop in a growing region.

32. The method of claim 31, wherein said high population density is at least 100% higher than the average prevailing density for said crop in said growing region.

33. The method of claim 31, wherein said high population density is at least 70% higher than the average prevailing density for said crop in said growing region.

34. The method of claim 31, wherein said high population density is at least 40% higher than the average prevailing density for said crop in said growing region.

35. The method of claim 31, wherein said high population density is at least 10% higher than the average prevailing density for said crop in said growing region.

36. The method of claim 26, wherein said crop is selected from the group consisting of maize, wheat, rye, barley, oats, buckwheat, sorghum, rice, sunflower, canola, peas, beans, soybeans, cotton, linseed, cauliflower, asparagus, lettuce, tobacco, mustard, sugar beet, potato, sweep potato, carrot, turnip, celery, tomato, egg plant, cucumber, squash, apple, apricot, peach, pear, plum, orange, blackberry, blueberry, strawberry, cranberry and lemon.

37. The method of claim 36, wherein said crop is a maize crop.

38. The method of claim 37, wherein said high population density for said maize crop is at least 50,000 plants per acre.

39. The method of claim 37, wherein said high population density for said maize crop is at least 42,500 plants per acre.

40. The method of claim 37, wherein said high population density for said maize crop is at least 35,000 plants per acre.

41. The method of clam 37, wherein said high population density for said maize crop is at least 27,500 plants per acre.

```
ZmCOP1  ACTTTATGTGACAGCAGACGTGCACTGGCCAGGGGGATCACCATCCGTCGCCCCGGGTGT
AtCOP1  ------------------------------------------------------------

ZmCOP1  CAATAATATCACTCTGTACATCCACAAACAGACGATACGGCTCTCTCTTTTATAGGTGTA
AtCOP1  ------------------------------------------------------------

ZmCOP1  AACCTTAAACTGCCGTACGTATAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGC
AtCOP1  ------------------------------------------------------------

ZmCOP1  CTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGT
AtCOP1  ------------------------------------------------------------

ZmCOP1  AACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGA
AtCOP1  ------------------------------------------------------------

ZmCOP1  CTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGG
AtCOP1  ------------------------------------------------------------

ZmCOP1  ATATCTGCAGAATTCGCCCTTCTGCGCCATGGGCGACTCCTCGGTGGCCGGCGCGCTCGT
AtCOP1  -------CAAAA----ACCAAAATCACAATCGAAGAAATCTT--TTGAAAGCAAAATGGA

ZmCOP1  GCCGTCTGTGCCCAAGCCGGAGCCCGCGCCGTCCGGTGACACCTCCGCGGCGGCCGCGGC
AtCOP1  AGAGATTTCGACGGATCCGGTT---GTTCCAGC-GGTGAAACCTGACCCGAGA-----AC

ZmCOP1  GACTACAGCGGCGCTGGCGATGCCGGAGGAGGCGGGTATGCGCGCGGCGTCGGCGTCGCC
AtCOP1  ATCTTCAGTTGG--TGAAGGTGCTAATCGTCATGAAAATGACGACGGAGGAAGCGGCGGT

ZmCOP1  TCAGGGGCCTGCGGAGGAGGGGGAGGGCCCCGCCGATAGGGACCTTCTCTGCCCGATCTG
AtCOP1  TCTGAGATT---GGAGCACCGGATCTG----G---ATAAAGACTTGCTTTGTCCGATTTG

ZmCOP1  CATGGCCGTCATCAAGGACGCCTTCCTCACCGCATGCGGCCACAGCTTCTGCTACATGTG
AtCOP1  TATGCAGATTATTAAAGATGCTTTCCTCACGGCTTGTGGTCATAGTTTCTGCTATATGTG

ZmCOP1  CATCGTCACGCATCTCAGCAACAAGAGCGACTGCCCCTGCTGCGGCCACTACCTTACCAA
AtCOP1  TATCATCACACATCTTAGGAACAAGAGTGATTGTCCCTGTTGTAGCCAACACCTCACCAA

ZmCOP1  GGCCCAGCTCTACCCCAACTTTCTCCTTGACAAGGTTCTGAAGAAAATATCAGCCCAACA
AtCOP1  TAATCAGCTTTACCCTAATTTCTTGCTCGATAAGCTATTGAAGAAAACTTCAGCTCGGCA

ZmCOP1  AATAGCAAAAACAGCATCGCCGATCGATCAATTTCGATGTGCATTGCAACAGGGAAATGA
AtCOP1  TGTGTCAAAAACTGCATCGCCCTTGGATCAGTTTCGGGAAGCACTACAAAGGGGTTGTGA

ZmCOP1  AATGGGGGTTAAAGAGTTGGATAGCCTTATGACTTTGATTGCTGAGAAGAAGCGGCAAAT
AtCOP1  TGTGTCAATTAAGGAGGTTGATAATCTTCTGACACTTCTTGCGGAAAGGAAGAGAAAAAT

ZmCOP1  GGAACAACAAGAATCAGAGACAAATATGCAAATATTGCTAGTCTTCTTACACTGCCTTAG
AtCOP1  GGAACAGGAAGAAGCTGAGAGGAACATGCAGATACTTTTGGACTTTTTTGCATTGTCTAAG

ZmCOP1  AAAGCAAAAGCTAGAAGAGTTGAATGAGATTCAAACTGATCTACAATACATCAAAGAGGA
AtCOP1  GAAGCAAAAAGTTGATGAACTAAATGAGGTGCAAACTGATCTCCAGTATATTAAAGAAGA

ZmCOP1  TATAAGTTCTGTGGAGAGACATAGGGCAGAATTATATCGCACAAAAGAAAGGTACTCCAT
AtCOP1  TATAAATGCCGTTGAGAGACATAGAATAGATTTATACCGAGCTAGGGACAGATATTCTGT

ZmCOP1  GAAGCTGCGCATGCTTTTAGATGAGCCTACTGCGCAAAAAATG--TGGCCCTCTCCTATA
AtCOP1  AAAGTTGCGGATGCTCGGAGATGATCCAA--GCACAAGAAATGCATGGCCACATGAGA-A

ZmCOP1  GACAAAGCTAGCTGTCGCTTTCTTCCCAACTCTCGGACACCACTTAGTGGATCATGTCCA
AtCOP1  GAACCAGATTGGTTTCAACT----CC-AATTCTCTCAGCATAAGAGGAGGAAATTTTGTA

ZmCOP1  GGAACTTTACAGAATAAGAAGCTTGATTTGAAAGCTCAAGTAAGCCATCAAGGATTTCAA
AtCOP1  GGCAATTATCAAAACAAAAAGGTAGAGGGGAAGGCACAAGGAAGCTCTCATGGGCTACCA
```

Figure 1A

```
ZmCOP1   AGGAGAGATGCTCTAACTTCTTCTGATCCTCCTAACTCCCCTATACAATCGGGTAATGTT
AtCOP1   AAGAAGGATGCGCTGAGTGGGTCAGATTCGCAAAGTTT---GAATCAGTCAACTGTCTCA

ZmCOP1   ATTGCTAGGAAGAGGCGAGTTCAAGCACAGTTCAATGAGCTTCAAGAATACTACCTGCAA
AtCOP1   ATTGCTAGAAAGAAACGGATTCATGCTCAGTTCAATGATTTACAAGAATGTTACCTCCAA

ZmCOP1   AGACGTCGT-ACTGG--AGCACAGGCACGCAGACAGGAAGAAAGAGATATAGTTGCAATG
AtCOP1   AAGCGGCGTCAGTTGGCAGACCAACCAAATAGTAAACAAGAAAATGATAAGAGTGTAGTA

ZmCOP1   AATAGAGAAGGCTATCATGCAGGTCTTCAGGATTTCCAGTCTGTGCTAACAACGTTCACT
AtCOP1   CGGAGGGAAGGCTATAGCAACGGCCTTGCAGATTTTCAATCTGTGTTGACTACCTTCACT

ZmCOP1   CGATACAGTCGTCTACGTGTCATTGCGGAACTAAGACATGGAGACTTGTTTCACTCTGCC
AtCOP1   CGCTACAGTCGTCTAAGAGTTATAGCAGAAATCCGGCATGGGGATATATTTCATTCAGCC

ZmCOP1   AATATTGTATCCAGTATTGAATTTGATCGTGATGATGAACTATTTGCTACCGCTGGAGTC
AtCOP1   AACATTGTATCAAGCATAGAGTTTGATCGTGATGATGAGCTGTTTGCCACTGCTGGTGTT

ZmCOP1   TCGAAACGTATTAAAGTCTTCGAATTTTCCACTGTTGTTAATGAACCATCAGATGTGCAT
AtCOP1   TCTAGATGTATAAAGGTTTTTGACTTCTCTTCGTTTGTAAATGAACCAGCAGATATGCAG

ZmCOP1   TGCCCAGTTGTTGAAATGGCTACCAGATCTAAACTTAGCTGCCTAAGCTGGAACAAGTAC
AtCOP1   TGTCCGATTGTGGAGATGTCAACTCGGTCTAAACTTAGTTGCTTGAGTTGGAATAAGCAT

ZmCOP1   TCAAAAAATATTATTGCAAGCAGTGACTATGAGGGTATAGTAACTGTGTGGGATGTTCAG
AtCOP1   GAAAAAAATCACATAGCAAGCAGTGATTATGAAGGAATAGTAACAGTGTGGGATGTAACT

ZmCOP1   ACCCGTCAGAGTGTGATGGAATATGAAGAGCATGAGAAGAGAGCATGGAGTGTTGATTTT
AtCOP1   ACTAGGCAGAGTCGGATGGAGTATGAAGAGCACGAAAAACGTGCCTGGAGTGTTGACTTT

ZmCOP1   TCTCGCACAGACTCTTCAATGCTAGTATCTGGGAGTGATGATTGCAAGGTGAAAGTGTGG
AtCOP1   TCACGAACAGAACCATCAATGCTTGTATCTGGTAGTGACGACTGCAAGGTTAAAGTTTGG

ZmCOP1   TGCACAAATCAAGAAGCAAGTGTGATCAATATTGATATGAAAGCAAATATTTGCTCGGTT
AtCOP1   TGCACGAGGCAGGAAGCAAGTGTGATTAATATTGATATGAAAGCAAACATATGTTGTGTC

ZmCOP1   AAATATAATCCTGGATCAAGCTTCTACGTTGCAGTCGGATCTGCTGATCACCATATTCAT
AtCOP1   AAGTACAATCCTGGCTCAAGCAACTACATTGCGGTCGGATCAGCTGATCATCACATCCAT

ZmCOP1   TACTTTGATTTACGTAATCCAAGTTCGCCTGTCCATATTTTCGGGGGGCACAAGAAAGCA
AtCOP1   TATTACGATCTAAGAAACATAAGCCAACCACTTCATGTCTTCAGTGGACACAAGAAAGCA

ZmCOP1   GTATCATATGTGAAATTCTTATCTAACAATGAGCTTGCGTCTGCATCAACAGATAGCACA
AtCOP1   GTTTCCTATGTTAAATTTTTGTCCAACAACGAGCTCGCTTCTGCGTCCACAGATAGCACA

ZmCOP1   TTACGCTTATGGGATGTCAAGGATAACTGCCCGGTACGGACATTCAGAGGACACAAAAAT
AtCOP1   CTACGCTTATGGGATGTCAAAGACAACTTGCCAGTTCGAACATTCAGAGGACATACTAAC

ZmCOP1   GAAAAGAACTTTGTTGGCTTGTCTGTGAACAATGAATATATTGCTTGTGGAAGTGAGACA
AtCOP1   GAGAAGAACTTTGTGGGTCTCACAGTGAACAGCGAGTATCTCGCCTGTGGAAGCGAGACA

ZmCOP1   AATGAGGTTTTTGTTTATCACAAGGCTATCTCGAAACCGGCAGCAAGCCATAGATTTGTA
AtCOP1   AACGAAGTATATGTATATCACAAGGAAATCACGAGACCCGTGACATCGCACAGATTTGGA

ZmCOP1   TCTTCTGACCCGGATGATGCCGATGATGATCCTGGTTCTTATTTCATTAGTGCTGTCTGC
AtCOP1   TCGCCAGACATGGACGATGCAGAGGAAGAGGCAGGTTCCTACTTTATTAGTGCGGTTTGC

ZmCOP1   TGGAAGAGTGATAGCCCTACGATGTTAACTGCTAACAGTCAGGGGACCATAAAAGTTCTT
AtCOP1   TGGAAGAGTGATAGTCCCACGATGTTGACTGCGAATAGTCAAGGAACCATCAAAGTTCTG
```

Figure 1B

```
ZmCOP1   GTACTTGCTCCTTGATGTTATGGAGGGCGTTCAAGAGGT-TCACAGTACTGTC---CAGT
AtCOP1   GTACTCGCTGCGTGATTCTA-GTAGACATTACAAAAGATCTTATAGCTTCGTGAATCAAT

ZmCOP1   TG--------TTTCCTTTCGTGTCATTATATTCCCCCAAAATTGGGAACGGGGGCA---T
AtCOP1   AAAAACAAATTTGCCGTCTATGTTCTTTAGTGGGAGTTACATATAGAGAGAGAACAATTT

ZmCOP1   AATTGATCTCCGGTTAG------GGAATGAAGTTTTGCAGATGGTCAGCTGACGTAG---
AtCOP1   ATTAAAAGTAGGGTTCATCATTTGGAAAGCAACTTTGTAT-TATTATGCTTGCCTTGGAA

ZmCOP1   ------------------------------------------------------------
AtCOP1   CACTCCTCAAGAAGAATTTGTATCAGTGATGTAGATATGTCTTACGGTTTCTTAGCTTCT

ZmCOP1   ----------------------------------------
AtCOP1   ACTTTATATAATTAAATGTTAGAATCAAAAAAAAAAAA
```

Figure 1C

```
OsCOP1      ------------------------------------------------------------
ZmCOP1      ACTTTATGTGACAGCAGACGTGCACTGGCCAGGGGGATCACCATCCGTCGCCCCGGGTGT

OsCOP1      ------------------------------------------------------------
ZmCOP1      CAATAATATCACTCTGTACATCCACAAACAGACGATACGGCTCTCTCTTTTATAGGTGTA

OsCOP1      ------------------------------------------------------------
ZmCOP1      AACCTTAAACTGCCGTACGTATAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGC

OsCOP1      ------------------------------------------------------------
ZmCOP1      CTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGT

OsCOP1      ------------------------------------------------------------
ZmCOP1      AACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGA

OsCOP1      ------------TTATTCACGCCCAGTCGCCGCCTCCACC-GCCGCCGCCTGCTCGACTC
ZmCOP1      CTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGG
                         * *   ****  * *  ** * * *  ** ****** *   **

OsCOP1      ACCACCGCAGGGCGGCCTCCTCCTGCCGCATGGGTGACTCGACGGTGGCCGGCGCGCTGG
ZmCOP1      ATATCTGCAGAATT-CGCCCTTCTGCGCCATGGGCGACTCCTCGGTGGCCGGCGCGCTCG
            *    * ****     *  *** ****  ****** *****  ***************** *

OsCOP1      TGCCATCGGTGCCGAAGCAGGAGCAGGCGCCGTCGGGGGACGCGTCCACGGCGGCGTTGG
ZmCOP1      TGCCGTCTGTGCCCAAGCCGGAGCCCGCGCCGTCCGGTGACACCTCCGCGGCGGCCGCGG
            **** ** ***** **** *****  ******** ** *** * *** *******   **

OsCOP1      CG------GTGGCGGGGGAGGGGGAGGAGGATGCGGGGGCGCGCGCCTCCGCGG------
ZmCOP1      CGACTACAGCGGCGCTGGCGATGCCGGAGGAGGCGGGTATGCGCGCGGCGTCGGCGTCGC
            **       * ****  ** *  *  ****** *****    ******  *  ***

OsCOP1      ----GGGGCA--------ACGGGGAGGCCGCGGCCGACAGGGACCTCCTCTGCCCGATCT
ZmCOP1      CTCAGGGGCCTGCGGAGGAGGGGGAGGGCCCCGCCGATAGGGACCTTCTCTGCCCGATCT
                 *****          * ******* * * ***** ******** ***********

OsCOP1      GCATGGCGGTCATCAAGGACGCCTTCCTCACCGCCTGCGGCCACAGCTTCTGCTACATGT
ZmCOP1      GCATGGCCGTCATCAAGGACGCCTTCCTCACCGCATGCGGCCACAGCTTCTGCTACATGT
            ******* **********************************************

OsCOP1      GCATCGTCACGCATCTCAGCCACAAGAGCGACTGCCCCTGCTGCGGCAACTACCTCACCA
ZmCOP1      GCATCGTCACGCATCTCAGCAACAAGAGCGACTGCCCCTGCTGCGGCCACTACCTTACCA
             ******************** ************************ ******* ****

OsCOP1      AGGCGCAGCTCTACCCCAACTTCCTCCTCGACAAGGTCTTGAAGAAAATGTCAGCTCGCC
ZmCOP1      AGGCCCAGCTCTACCCCAACTTTCTCCTTGACAAGGTTCTGAAGAAAATATCAGCCCAAC
            **** ***************** ***** ********  ********** ***** *  *

OsCOP1      AAATTGCGAAGACAGCATCACCGATAGACCAATTTCGATATGCACTGCAACAGGGAAACG
ZmCOP1      AAATAGCAAAAACAGCATCGCCGATCGATCAATTTCGATGTGCATTGCAACAGGGAAATG
            **** ** ** ******** ***** ** ********** **** ************ *

OsCOP1      ATATGGCGGTTAAAGAACTAGATAGTCTTATGACTTTGATCGCGGAGAAGAAGCGGCATA
ZmCOP1      AAATGGGGGTTAAAGAGTTGGATAGCCTTATGACTTTGATTGCTGAGAAGAAGCGGCAAA
            * **** *********  * ***** ************** ** ************** *
```

Figure 2A

```
OsCOP1      TGGAACAGCAAGAGTCAGAAACAAATATGCAAATATTGCTGGTCTTCTTGCATTGCCTCA
ZmCOP1      TGGAACAACAAGAATCAGAGACAAATATGCAAATATTGCTAGTCTTCTTACACTGCCTTA
            ******* ***** ***** ********************** ******** ** ***** *

OsCOP1      GAAAGCAAAAGTTGGAAGAGCTGAATGAGATTCAAACTGACCTACAGTACATCAAAGAAG
ZmCOP1      GAAAGCAAAAGCTAGAAGAGTTGAATGAGATTCAAACTGATCTACAATACATCAAAGAGG
            *********** * ****** ********************** ***** ********** *

OsCOP1      ATATAAGTGCTGTGGAGAGACATAGGTTAGAATTATATCGAACAAAAGAAAGGTACTCAA
ZmCOP1      ATATAAGTTCTGTGGAGAGACATAGGGCAGAATTATATCGCACAAAAGAAAGGTACTCCA
            ******** ******************  ************ ****************** *

OsCOP1      TGAAGCTCCGCATGCTTTTGGATGAACCTGCTGCATCAAAGATGTGGCCTTCACCTATGG
ZmCOP1      TGAAGCTGCGCATGCTTTTAGATGAGCCTACTGCGCAAAAAATGTGGCCCTCTCCTATAG
            ******* *********** ***** *** ****    *** ******** ** ***** *

OsCOP1      ATAAACCTAGTGGTCTCTTTCTTCCCAACTCTCGGGGACCACTTAGTACATCAAATCCAG
ZmCOP1      ACAAAGCTAGCTGTCGCTTTCTTCCCAACTCTCGGACACCACTTAGTGGATCATGTCCAG
            * *** ****   *** ********************  **********  ****  *****

OsCOP1      GGGGTTTACAGAATAAGAAGCTTGACTTGAAAGGTCAAATTAGTCATCAAGGATTTCAAA
ZmCOP1      GAACTTTACAGAATAAGAAGCTTGATTTGAAAGCTCAAGTAAGCCATCAAGGATTTCAAA
            *    ********************** ******* **** * ** ****************

OsCOP1      GGAGAGATGTTCTCACTTGCTCGGATCCTCCTAGTGCCCCTATTCAATCAGGCAACGTTA
ZmCOP1      GGAGAGATGCTCTAACTTCTTCTGATCCTCCTAACTCCCCTATACAATCGGGTAATGTTA
            ********* *** ****  ** **********   ******* ***** ** ** ****

OsCOP1      TTGCTCGGAAGAGGCGAGTTCAAGCTCAGTTTAACGAGCTTCAAGAATACTATCTTCAAA
ZmCOP1      TTGCTAGGAAGAGGCGAGTTCAAGCACAGTTCAATGAGCTTCAAGAATACTACCTGCAAA
            ***** ******************** ***** ** ****************** ** ****

OsCOP1      GACGGCGTACCGGAGCACAATCACGTAGGCTGGAGGAAAGAGACATAGTAACAATAAATA
ZmCOP1      GACGTCGTACTGGAGCACAGGCACGCAGACAGGAAGAAAGAGATATAGTTGCAATGAATA
            **** ***** ********  **** ** * *** ******** *****  **** ****

OsCOP1      AAGAAGGTTATCATGCAGGACTTGAGGATTTCCAGTCTGTGCTAACAACATTCACACGAT
ZmCOP1      GAGAAGGCTATCATGCAGGTCTTCAGGATTTCCAGTCTGTGCTAACAACGTTCACTCGAT
             ****** *********** *** ************************* ***** ****

OsCOP1      ATAGTCGCTTGCGTGTAATTGCGGAGCTAAGACATGGAGATCTGTTTCACTCTGCAAATA
ZmCOP1      ACAGTCGTCTACGTGTCATTGCGGAACTAAGACATGGAGACTTGTTTCACTCTGCCAATA
            * *****  * ***** ******** **************  ************* ****

OsCOP1      TCGTATCAAGTATCGAATTTGACCGTGATGATGAGCTATTTGCTACTGCTGGAGTCTCAA
ZmCOP1      TTGTATCCAGTATTGAATTTGATCGTGATGATGAACTATTTGCTACCGCTGGAGTCTCGA
            * ***** ***** ******** *********** *********** *********** *

OsCOP1      AGCGCATCAAAGTCTTCGAGTTTTCTACAGTTGTTAATGAACCATCAGATGTGCATTGTC
ZmCOP1      AACGTATTAAAGTCTTCGAATTTTCCACTGTTGTTAATGAACCATCAGATGTGCATTGCC
            * ** ** ********** ***** ** ****************************** *

OsCOP1      CAGTTGTTGAAATGGCTACTAGATCTAAACTCAGCTGCCTTAGCTGGAACAAGTACTCAA
ZmCOP1      CAGTTGTTGAAATGGCTACCAGATCTAAACTTAGCTGCCTAAGCTGGAACAAGTACTCAA
            ******************** *********** ******** ********************

OsCOP1      AAAATGTTATAGCAAGCAGCGACTATGAGGGTATAGTAACTGTTTGGGATGTCCAAACCC
ZmCOP1      AAAATATTATTGCAAGCAGTGACTATGAGGGTATAGTAACTGTGTGGGATGTTCAGACCC
            ***** **** ******** ************************ ******** ** ****
```

Figure 2B

```
OsCOP1      GCCAGAGTGTGATGGAGTATGAAGAACATGAAAAGAGAGCATGGAGTGTTGATTTTTCTC
ZmCOP1      GTCAGAGTGTGATGGAATATGAAGAGCATGAGAAGAGAGCATGGAGTGTTGATTTTTCTC
            * ************** ******** ***** ****************************

OsCOP1      GAACAGAACCCTCGATGCTAGTATCTGGGAGTGATGATTGCAAGGTCAAAGTGTGGTGCA
ZmCOP1      GCACAGACTCTTCAATGCTAGTATCTGGGAGTGATGATTGCAAGGTGAAAGTGTGGTGCA
            * *****  * ** ********************************* ************

OsCOP1      CAAAGCAAGAAGCAAGTGCCATCAATATTGATATGAAGGCCAATATTTGCTCTGTCAAAT
ZmCOP1      CAAATCAAGAAGCAAGTGTGATCAATATTGATATGAAAGCAAATATTTGCTCGGTTAAAT
            **** ************  ****************** ** ********** ** ****

OsCOP1      ATAATCCTGGGTCGAGCCACTATGTTGCAGTGGGTTCTGCTGATCACCATATTCATTATT
ZmCOP1      ATAATCCTGGATCAAGCTTCTACGTTGCAGTCGGATCTGCTGATCACCATATTCATTACT
            ********** ** ***  *** ******** ** ************************ *

OsCOP1      TTGATTTGCGAAATCCAAGTGCGCCTGTCCATGTTTTTGGTGGGCACAAGAAAGCTGTTT
ZmCOP1      TTGATTTACGTAATCCAAGTTCGCCTGTCCATATTTTCGGGGGGCACAAGAAAGCAGTAT
            ******* ** ********* ********** **** ** ************** ** *

OsCOP1      CTTATGTGAAGTTCCTGTCCACCAATGAGCTTGCGTCTGCATCAACTGATAGCACATTAC
ZmCOP1      CATATGTGAAATTCTTATCTAACAATGAGCTTGCGTCTGCATCAACAGATAGCACATTAC
            * ******** *** * ** * ************************ ************

OsCOP1      GGTTATGGGATGTCAAAGAAAATTGCCCTGTAAGGACATTCAGAGGGCACAAGAATGAAA
ZmCOP1      GCTTATGGGATGTCAAGGATAACTGCCCGGTACGGACATTCAGAGGACACAAAAATGAAA
            * ************** ** ** ***** *** ************ ***** *******

OsCOP1      AGAACTTTGTTGGGCTGTCTGTAAATAACGAGTACATTGCCTGCGGGAGTGAAACGAATG
ZmCOP1      AGAACTTTGTTGGCTTGTCTGTGAACAATGAATATATTGCTTGTGGAAGTGAGACAAATG
            ************  ******* ** ** ** ** ***** ** ** ***** ** ****

OsCOP1      AGGTTTTTGTTTACCACAAGGCTATCTCAAAACCTGCTGCCAACCACAGATTTGTATCAT
ZmCOP1      AGGTTTTTGTTTATCACAAGGCTATCTCGAAACCGGCAGCAAGCCATAGATTTGTATCTT
            ************ ************** ***** ** ** * *** ********** *

OsCOP1      CTGATCTCGATGATGCAGATGATGATCCTGGCTCTTATTTTATTAGCGCAGTCTGCTGGA
ZmCOP1      CTGACCCGGATGATGCCGATGATGATCCTGGTTCTTATTTCATTAGTGCTGTCTGCTGGA
            **** *  ******** ************** ******** ***** ** **********

OsCOP1      AGAGCGATAGCCCTACCATGTTAACTGCTAACAGTCAGGGCACCATTAAAGTTCTTGTAC
ZmCOP1      AGAGTGATAGCCCTACGATGTTAACTGCTAACAGTCAGGGGACCATAAAAGTTCTTGTAC
            **** ********** ************************ ***** ************

OsCOP1      TTGCTCCTTGATGAAATCAGTGGTTTTCATGAGATCCCTAGATAGCTTGTATATTTGATG
ZmCOP1      TTGCTCCTTGATGTTATGGAGGGCGTTCAAGAGGTTCACAG-TA----C-----------
            ************  **    **  **** *** * *  ** **

OsCOP1      TATACAGTTGTTTCCTTTTCGTGCCATTATA---CCCCAAA--TGGGAGTGGAGGTATTA
ZmCOP1      TGTCCAGTTGTTTCCTTT-CGTGTCATTATATTCCCCCAAAATTGGGAACGGGGGCATAA
            * * ************** **** *******   *******  *****  ** ** ** * *

OsCOP1      CTGATCTCCAACATAGGGCGCAAAGTTTTGAAGGTAATCAGCTGACATAGGGTTTCGAGG
ZmCOP1      TTGATCTCCGGT-TAGGGAATGAAGTTTTGCAGATGGTCAGCTGACGTAG----------
             ********    *****    ******** ** *  ********* ***
```

Figure 2C

```
OsCOP1      GCTCGAAATGTGCATAGTCCAGAATTCTCATGTATAGGTTTAAAGCAGTCAAGTAATTGA
ZmCOP1      ------------------------------------------------------------

OsCOP1      TTATACATATGTAACGTGAGAATTGAGAAATGAACATCAAATAAGCTTGTTTGGTTGCAT
ZmCOP1      ------------------------------------------------------------

OsCOP1       AAAAAAAAAAAAAAAA
ZmCOP1       ----------------
```

Figure 2D

```
          1                                                  51
OsCOP1    --------MGDSTVAGALVPSVPKQEQAPSGDASTAALA..VAGEGEEDAGARASAGG......NGEAAA
ZmCOP1    --------MGDSSVAGALVPSVPKPEPAPSGDTSAAAAATTAALAMPEEAGMRAASASPQGPAEEGEGPA
InCOP1    MGEREGECEGESSMVGAVVPAVKAR.......NAEEPSISH.....RDEATPSGME......PE.....L
LeCOP1    ---------MVESSVGGVVPAVKGEVMRRMGDKEEGGSVTL.....RDEEVGTVTE......WE.....L
AtCOP1    ---------MEEISTDPVVPA..VKPDPRTSSVGEGANRHE.....NDDGGSGGSEIGAP...D.....L
PsCOP1    ---------MEEHSVGPLVPAV.VKPEP.........SKNF.....STDTTAAGTFLLVPTMSD.....L
MmCOP1    -------------VSGSASAGGAVSAGQSRLSCAARPSAGVGGSSSSLGSSSRKRPLLVPLCNGLLNSYE

                    101
OsCOP1    DR..DLLCPICMAVIKDAFLTACGHSFCYMCIVTHLSHKSDCPCCGNYLTK.AQLYPNFLLDKVLKKMSA
ZmCOP1    DR..DLLCPICMAVIKDAFLTACGHSFCYMCIVTHLSNKSDCPCCGHYLTK.AQLYPNFLLDKVLKKISA
InCOP1    DR..ELLCPICMQIIKDAFLTSCGHSFCYMCIVTHLHNKSDCPCCSHYLTT.AQLYPNFLLDKLLKKTSA
LeCOP1    DR..ELLCPICMQIIKDAFLTACGHSFCYMCIVTHLHNKSDCPCCSHYLTT.SQLYPNFLLDKLLKKTSA
AtCOP1    DK..DLLCPICMQIIKDAFLTACGHSFCYMCIITHLRNKSDCPCCSQHLTN.NQLYPNFLLDKLLKKTSA
PsCOP1    DK..DFLCPICMQIIKDAFLTACGHSFCYMCIITHLRNKSDCPCCGHYLTN.SNLFPNFLLDKLLKKTSD
MmCOP1    DKSNDFVCPICFDMIEEAYMTKCGHSFCYKCIHQSLEDNNRCPKCNYVVDNIDHLYPNFLVNELILKQKQ

                  151                                                201
OsCOP1    R......QIAKTASPIDQFRYALQQ...GND...MAVKELDSLMTLIAEKKRHMEQQESETNMQILLVFL
ZmCOP1    Q......QIAKTASPIDQFRCALQQ...GNE...MGVKELDSLMTLIAEKKRQMEQQESETNMQILLVFL
InCOP1    H......QISKTASPVEQFRHSIEQ...GRE...VSIKELDVLLTILAEKKRKLEQEEAERNMQILLEFL
LeCOP1    R......QISKTASPVEQFRHSLEQ...GSE...VSIKELDALLLMLSEKKRKLEQEEAERNMQILLDFL
AtCOP1    R......HVSKTASPLDQFREALQR...GCD...VSIKEVDNLLTLLAERKRKMEQEEAERNMQILLDFL
PsCOP1    R......QISKTASPVEHFRQAVQK...GCE...VTMKELDTLLLLLTEKKRKMEQEEAERNMQILLDFL
MmCOP1    RFEEKRFKLDHSVSSTNGHRWQIFQDLLGTDQDNLDLANVNLMLELLVQKKKQLEAESHAAQLQILMEFL

                                                  251
OsCOP1    HCLRKQKLEELNEIQTDLQYIKEDISAVERHRLELYRTKERYSMKLRMLLDEPAASKMWPSPMDKPSGLF
ZmCOP1    HCLRKQKLEELNEIQTDLQYIKEDISSVERHRAELYRTKERYSMKLRMLLDEPTAQKMWPSPIDKASCRF
InCOP1    HMLKKKKVDELNEVQNDLQYIKEDINAVERHRIDLYRARDRYSMKLRMLADDPLGSKSRSSSVDRNTIGL
LeCOP1
QMLRKQKVDELNEVQHDLQYIKEDLNSVERHRIDLYRARDRYSMKLRMLADDPIGKKPWSSSTDRNFGGL
AtCOP1    HCLRKQKVDELNEVQTDLQYIKEDINAVERHRIDLYRARDRYSVKLRMLGDDP.STRNAWPH.EKNQIGF
PsCOP1    HCLRKQKVDELKEVQTDLQFIKEDIGAVEKHRMDLYRARDRYSVKLRMLDDSG.GRKSRHSSMDLNSSGL
MmCOP1    KVARRNKREQLEQIQKELSVLEEDIKRVEE.MSGLYSPVSEDSTVPQFEAPSP....SHSSIID......

                          301
OsCOP1    LPNSRGPLSTSNPGGLQNKKLDLKGQIS..HQGFQRRDVLTCSDPPSAP.....IQSGNVIARKRRVQAQ
ZmCOP1    LPNSRTPLSGSCPGTLQNKKLDLKAQVS..HQGFQRRDALTSSDPPNSP.....IQSGNVIARKRRVQAQ
InCOP1    FPSSRSAHGGLASGNLMYKKNDG.........GSQRKDVSVTELSLNGSDSQHMNQSGLAVMRKKRVHAQ
LeCOP1    FSTSQNAPGGLPTGNLTFKKVDSKAQISSP..GPQRKDTSISEL.....NSQHMSQSGLAVVRKKRVNAQ
AtCOP1    NSNSLSIRGGNFVGNYQNKKVEGKAQGSS..HGLPKKD......ALSGSDSQSLNQSTVSMARKKRIHAQ
PsCOP1    ASSPLNLRGGLSSGSH.TKKNDGKSQISSHGHGIQRRD......PITGSDSQYINQSGLALVRKKRVHTQ
MmCOP1    .STEYSQPPG.FSGTSQTKK.............................QPWYNSTLA.SRRKRLTAH

                351                                                401
OsCOP1    FNELQEYYLQRRRTGA.QSRRLEERDIVTINKEGYHAGLEDFQSVLTTFTRYSRLRVIAELRH.GDLFHS
ZmCOP1    FNELQEYYLQRRRTGA.QARRQEERDIVAMNREGYHAGLQDFQSVLTTFTRYSRLRVIAELRH.GDLFHS
InCOP1    FNDLQECYLQKRRQLANQLQNKEERDQNVTRREGYSAGLSEFQSVLSTFTRYSRLRVIAELRH.GDIFHS
LeCOP1    FNDLQECYLQKRRQLANKSRVKEEKDADVVQREGYSEGLADFQSVLSTFTRYSRLRVIAELRH.GDLFHS
AtCOP1    FNDLQECYLQKRRQLADQPNSKQENDKSVVRREGYSNGLADFQSVLTTFTRYSRLRVIAEIRH.GDIFHS
PsCOP1    FNDLQECYLQKRRQAADKPHGQQERDTNFISREGYSCGLDDFQSVLTTFTRYSRLRVIAEIRH.GDIFHS
MmCOP1    FEDLEQCYFSTRMSRISD.......DSRTASQ......LDEFQECLSKFTRYNSVRPLATLSYASDLYNG
```

Figure 3A

```
                                                  451
OsCOP1  ANIVSSIEFDRDDELFATAGVSKRIKVFEFSTVVNEPSDVHCPVVEMATRSKLSCLSWNKYSKNVIASSD
ZmCOP1  ANIVSSIEFDRDDELFATAGVSKRIKVFEFSTVVNEPSDVHCPVVEMATRSKLSCLSWNKYSKNIIASSD
InCOP1  ANIVSSIEFDRDDELFATAGVSRRIKVFDFSSVVNEPADAHCPVVEMSTRSKLSCLSWNKYTKNHIASSD
LeCOP1  ANIVSSIEFDRDDELFATAGVSRRIKVFDFSSVVNEPADAHCPVVEMSTRSKLSCLSWNKYTKNHIASSD
AtCOP1  ANIVSSIEFDRDDELFATAGVSRCIKVFDFSSVVNEPADMQCPIVEMSTRSKLSCLSWNKHEKNHIASSD
PsCOP1  ANIVSSIEFDRDDDLFATAGVSRRIKVFDFSAVVNEPTDAHCPVVEMTTRSKLSCLSWNKYAKNQIASSD
MmCOP1  SSIVSSIEFDRDCDYFAIAGVTKKIKVYEYGTVIQDAVDIHYPENEMTCNSKISCISWSSYHKNLLASSD

                501                                                          551
OsCOP1  YEGIVTVWDVQTRQSVMEYEEHEKRAWSVDFSRTEPSMLVSGSDDCKVKVWCTKQEASAINIDMKANICS
ZmCOP1  YEGIVTVWDVQTRQSVMEYEEHEKRAWSVDFSRTDSSMLVSGSDDCKVKVWCTNQEASVINIDMKANICS
InCOP1  YDGIVTVWDVTTRQSVMEYEEHEKRAWSVDFSRTDPSMLVSGSDDCKVKVWCTKQEASALNIDMKANICC
LeCOP1  YDGIVTVWDVTTRQSVMEYEEHEKRAWSVDFSRTEPSMLVSGSDDCKVKVWCTKQEASVLNIDMKANICC
AtCOP1  YEGIVTVWDVTTRQSLMEYEEHEKRAWSVDFSRTEPSMLVSGSDDCKVKVWCTRQEASVINIDMKANICC
PsCOP1  YEGIVTVWTMTTRKSLMEYEEHEKRAWSVDFSRTDPSMLVSGSDDCKVKVWCTNQEASVLNIDMKANICC
MmCOP1  YEGTVILWDGFTGQRSKVYQEHEKRCWSVDFNLMDPKLLASGSDDAKVKLWSTNLDNSVASIEAKANVCC

                                                  601
OsCOP1  VKYNPGSSHYVAVGSADHHIHYFDLRNPSAPVHVFGGHKKAVSYVKFLSTNELASASTDSTLRLWDVKEN
ZmCOP1  VKYNPGSSFYVAVGSADHHIHYFDLRNPSSPVHIFGGHKKAVSYVKFLSNNELASASTDSTLRLWDVKDN
InCOP1  VKYNPGSSFHVAVGSADHHIHYYDLRNTSAPLHIFSGHKKAVSYVKFLSSHELASASTDSTLRLWDVKDN
LeCOP1  VKYNPGSSVHIAVGSADHHIHYYDLRNTSQPVHIFSGHRKAVSYVKFLSNNELASASTDSTLRLWDVKDN
AtCOP1  VKYNPGSSNYIAVGSADHHIHYYDLRNISQPLHVFSGHKKAVSYVKFLSNNELASASTDSTLRLWDVKDN
PsCOP1  VKYNPGSGNYIAVGSADHHIHYYDLRNISRPVHVFTGHKKAVSYVKFLSNDELASASTDSTLRLWDVKQN
MmCOP1  VKFSPSSRYHLAFGCADHCVHYYDLRNTKQPIMVFKGHRKAVSYAKFVSGEEIVSASTDSQLKLWNVGKP

                                  651
OsCOP1  CPVRTFRGHKNEKNFVGLSVNNEYIACGSETNEVFVYHKAISKPAANHRFVS....SDLDDADDDPGSYF
ZmCOP1  CPVRTFRGHKNEKNFVGLSVNNEYIACGSETNEVFVYHKAISKPAASHRFVS....SDPDDADDDPGSYF
InCOP1  SPVRVFRGHTNEKNFVGLSVSNEFISCGSETNEVFVYHKAISKPVTWHRFGS....PDVDEADEDVTSFF
LeCOP1  LPVRTLRGHTNEKNFVGLSVNNEFLSCGSETNEVFVYHKAISKPVTWHRFGS....PDIDEADEDAGSYF
AtCOP1  LPVRTFRGHTNEKNFVGLTVNSEYLACGSETNEVYVYHKEITRPVTSHRFGS....PDMDDAEEEAGSYF
PsCOP1  LPVRTFRGHANEKNFVGLTVRSEYIACGSETNEVFVYHKEISKPLTWHRFGT....LDMEDAEDEAGSYF
MmCOP1  YCLRSFKGHINEKNFVGLASNGDYIACGSENNSLYLYYKGLSKTLLTFKFDTVKSVLDKDRKEDDTNE.F

        701                                 733
OsCOP1  ISAVCWKS....DSPTMLTANSQGTIKVLVLAP
ZmCOP1  ISAVCWKS....DSPTMLTANSQGTIKVLVLAP
InCOP1  ISAVCWKS....DSPTMLAANSQGTIKVLVLAA
LeCOP1  ISAVCWKS....DSPTMLAANSQGTIKVLVLAA
AtCOP1  ISAVCWKS....DSPTMLTANSQGTIKVLVLAA
PsCOP1  ISAVCWKS....DRPTILTANSQGTIKVLVLAA
MmCOP1  VSAVCWRALSDGESNVLIAANSQGTIKVLELV-
```

Figure 3B

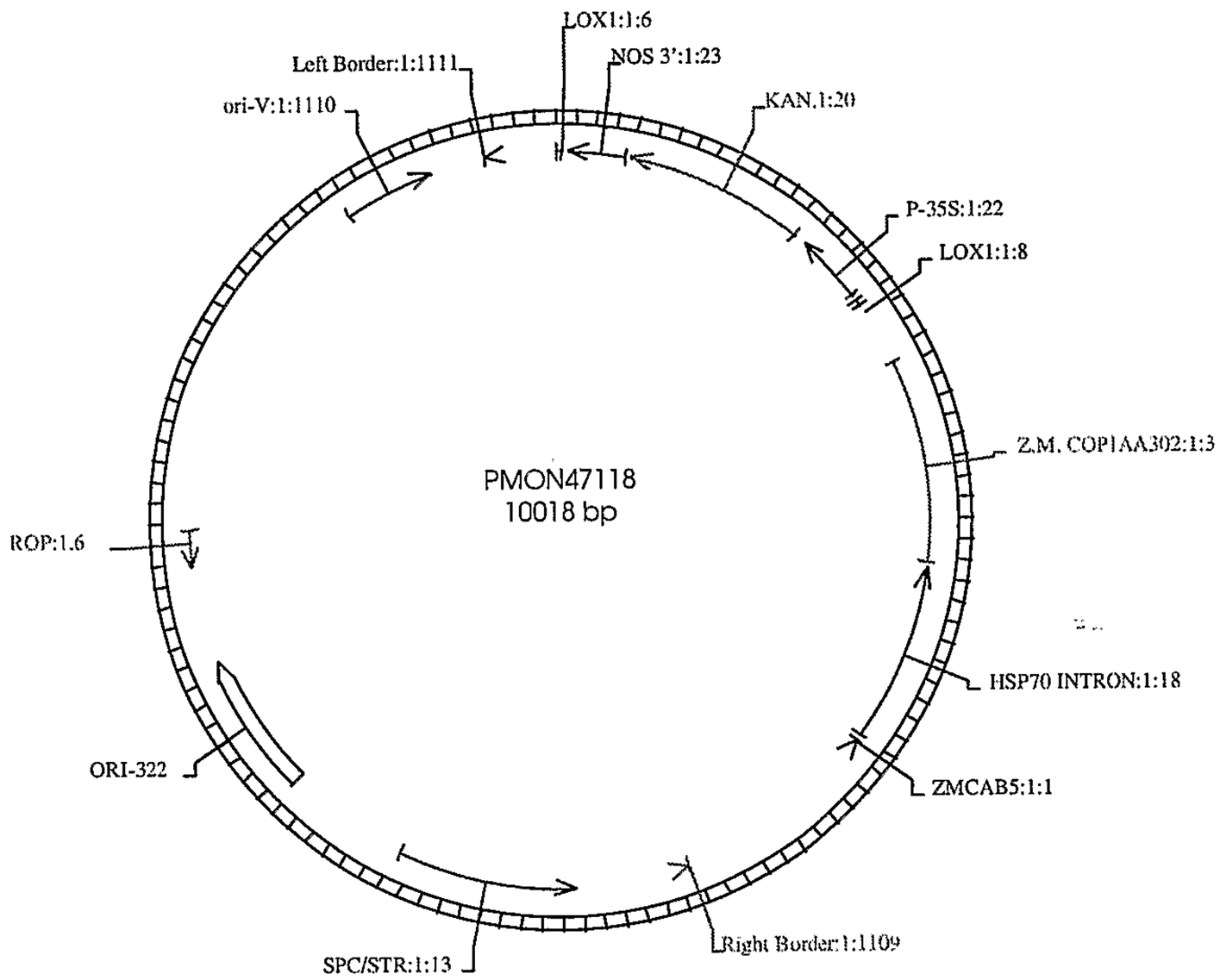

LOX1:1:6
NOS 3':1:23
Left Border:1:1111
ori-V:1:1110
KAN.1:20
P-35S:1:22
LOX1:1:8
Z.M. COP1AA302:1:3
PMON47118
10018 bp
ROP:1.6
HSP70 INTRON:1:18
ZMCAB5:1:1
ORI-322
Right Border:1:1109
SPC/STR:1:13

Figure 4

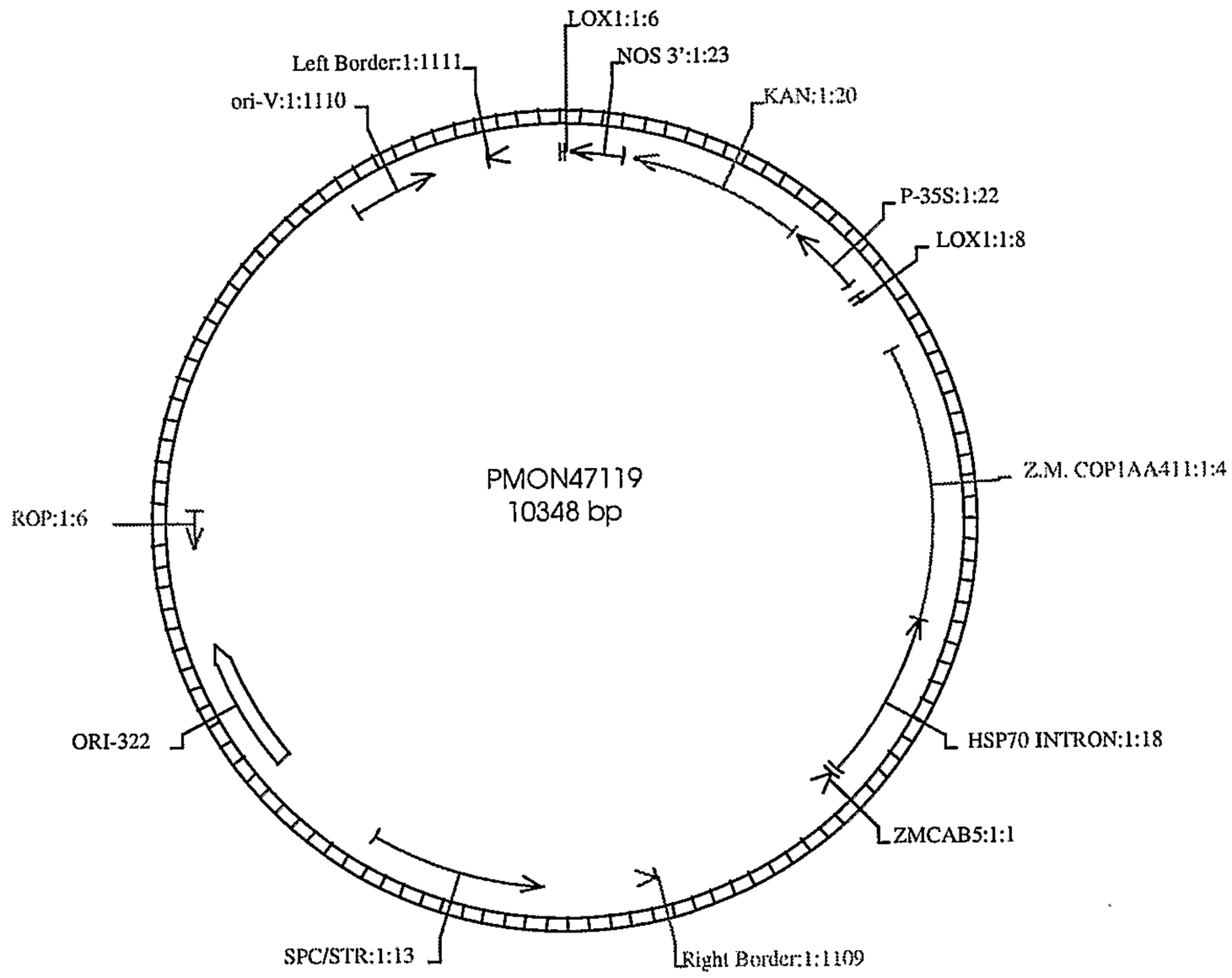

LOX1:1:6
Left Border:1:1111
NOS 3':1:23
ori-V:1:1110
KAN:1:20
P-35S:1:22
LOX1:1:8
PMON47119
10348 bp
Z.M. COP1AA411:1:4
ROP:1:6
HSP70 INTRON:1:18
ORI-322
ZMCAB5:1:1
SPC/STR:1:13
Right Border:1:1109

Figure 5

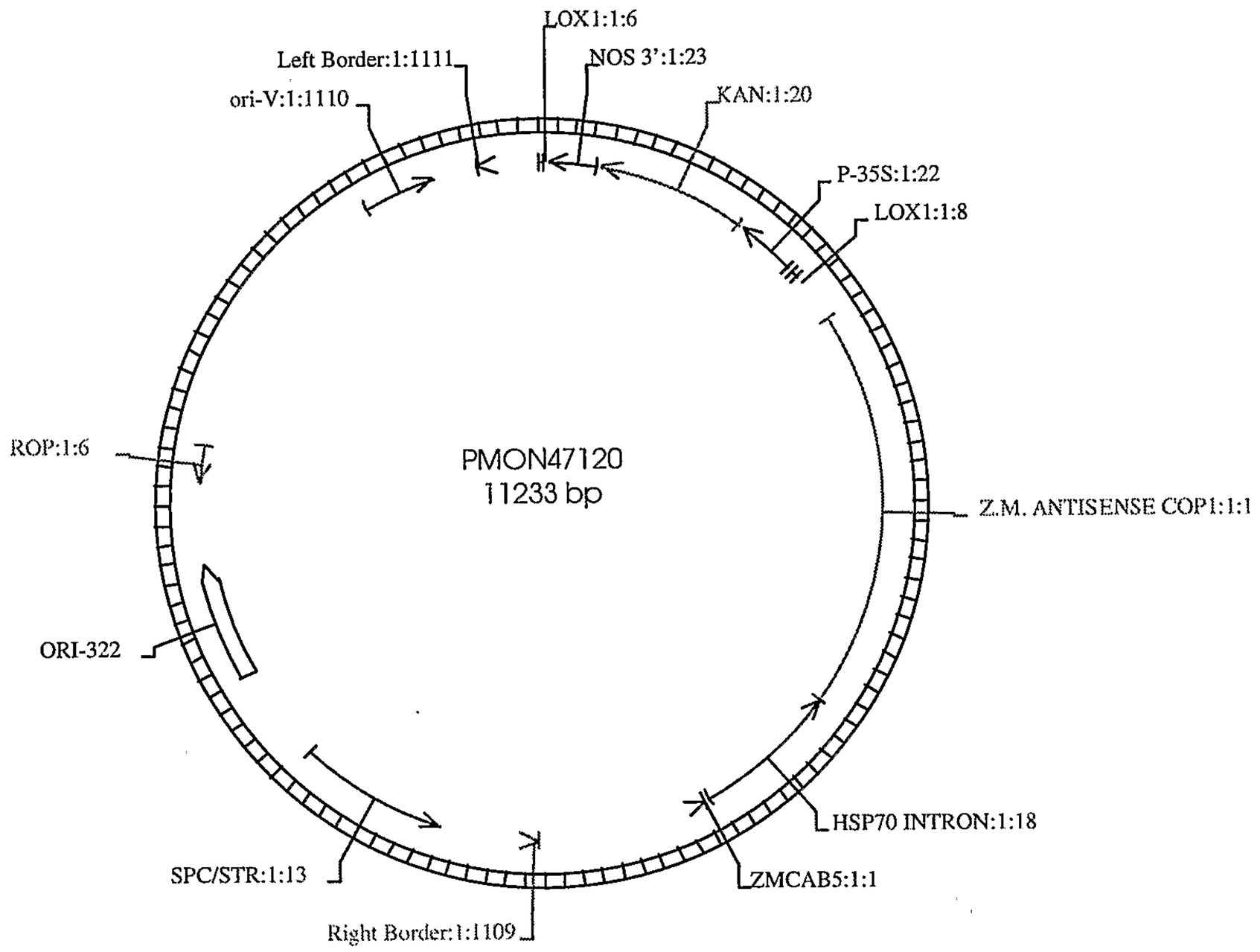

LOX1:1:6
Left Border:1:1111
NOS 3':1:23
ori-V:1:1110
KAN:1:20
P-35S:1:22
LOX1:1:8
ROP:1:6
PMON47120
11233 bp
Z.M. ANTISENSE COP1:1:1
ORI-322
HSP70 INTRON:1:18
SPC/STR:1:13
ZMCAB5:1:1
Right Border:1:1109

Figure 6

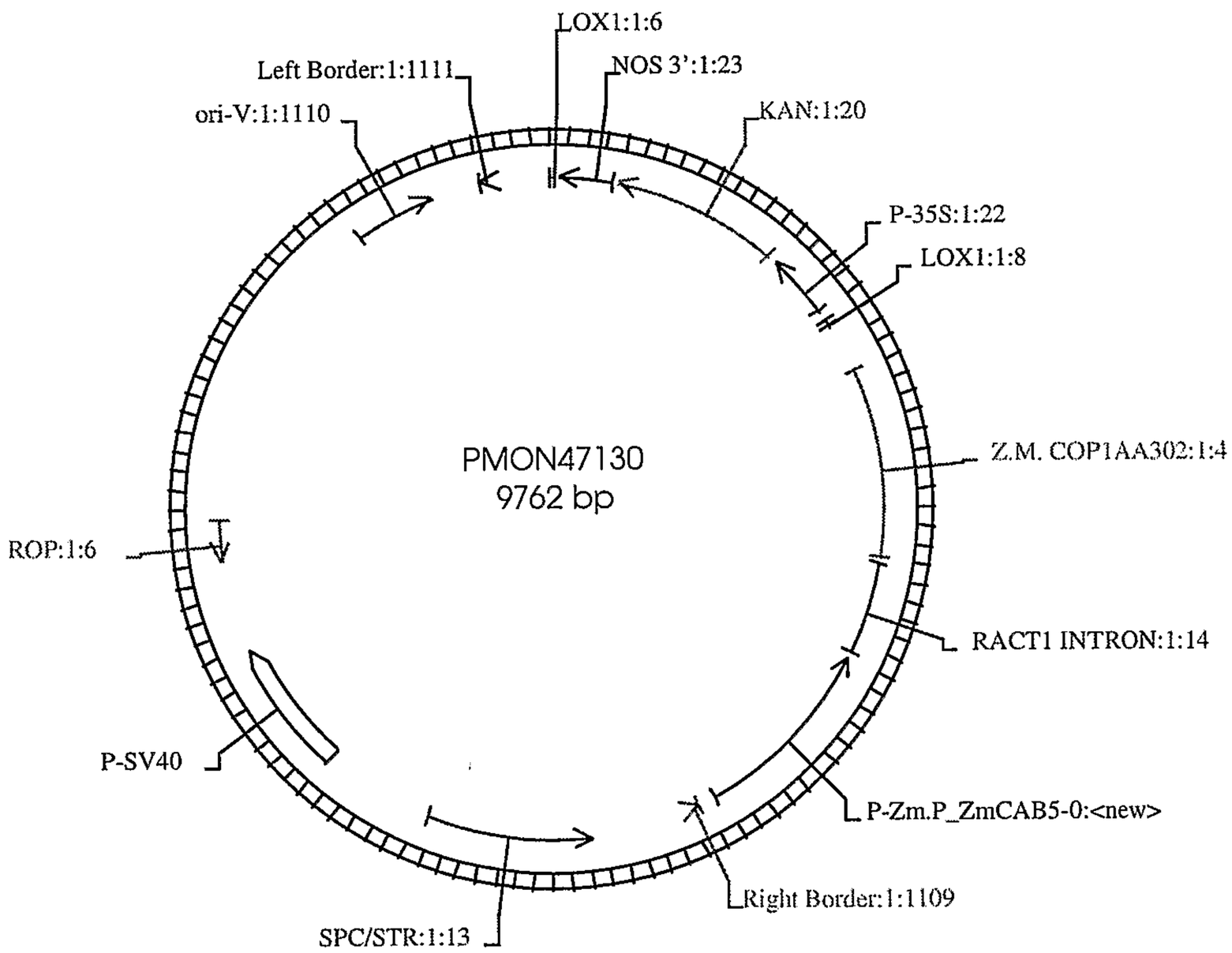

LOX1:1:6
Left Border:1:1111
NOS 3':1:23
ori-V:1:1110
KAN:1:20
P-35S:1:22
LOX1:1:8
Z.M. COP1AA302:1:4
PMON47130
9762 bp
ROP:1:6
RACT1 INTRON:1:14
P-SV40
P-Zm.P_ZmCAB5-0:<new>
Right Border:1:1109
SPC/STR:1:13

Figure 7

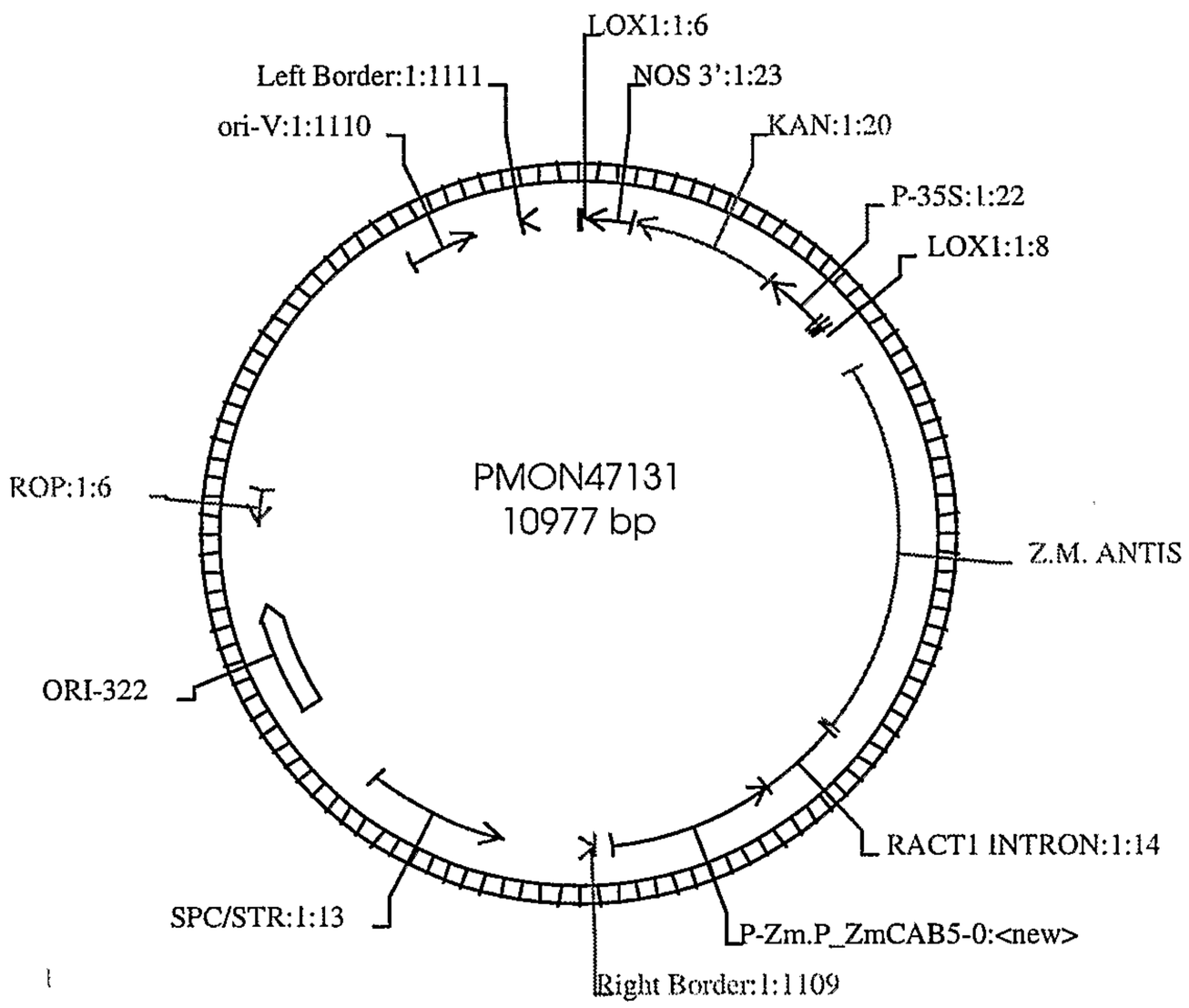

LOX1:1:6
Left Border:1:1111
NOS 3':1:23
ori-V:1:1110
KAN:1:20
P-35S:1:22
LOX1:1:8
ROP:1:6
PMON47131
10977 bp
Z.M. ANTIS
ORI-322
RACT1 INTRON:1:14
SPC/STR:1:13
P-Zm.P_ZmCAB5-0:<new>
Right Border:1:1109

Figure 8

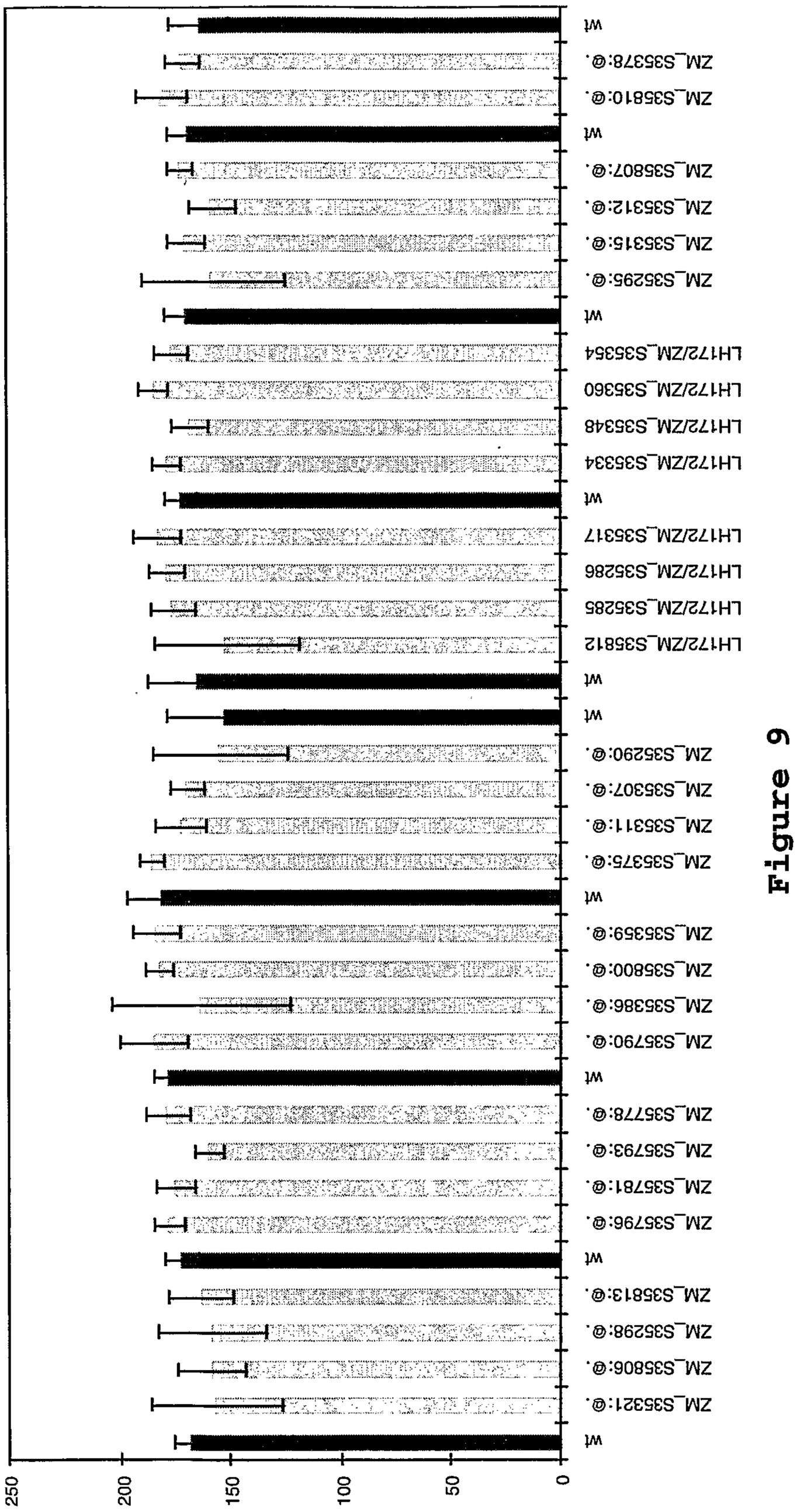

wt
ZM_S35378:@.
ZM_S35810:@.
wt
ZM_S35807:@.
ZM_S35312:@.
ZM_S35315:@.
ZM_S35295:@.
wt
LH172/ZM_S35354
LH172/ZM_S35360
LH172/ZM_S35348
LH172/ZM_S35334
wt
LH172/ZM_S35317
LH172/ZM_S35286
LH172/ZM_S35285
LH172/ZM_S35812
wt
wt
ZM_S35290:@.
ZM_S35307:@.
ZM_S35311:@.
ZM_S35375:@.
wt
ZM_S35359:@.
ZM_S35800:@.
ZM_S35386:@.
ZM_S35790:@.
wt
ZM_S35778:@.
ZM_S35793:@.
ZM_S35781:@.
ZM_S35796:@.
wt
ZM_S35813:@.
ZM_S35298:@.
ZM_S35806:@.
ZM_S35321:@.
wt
250
200
150
100
50
0

Figure 9

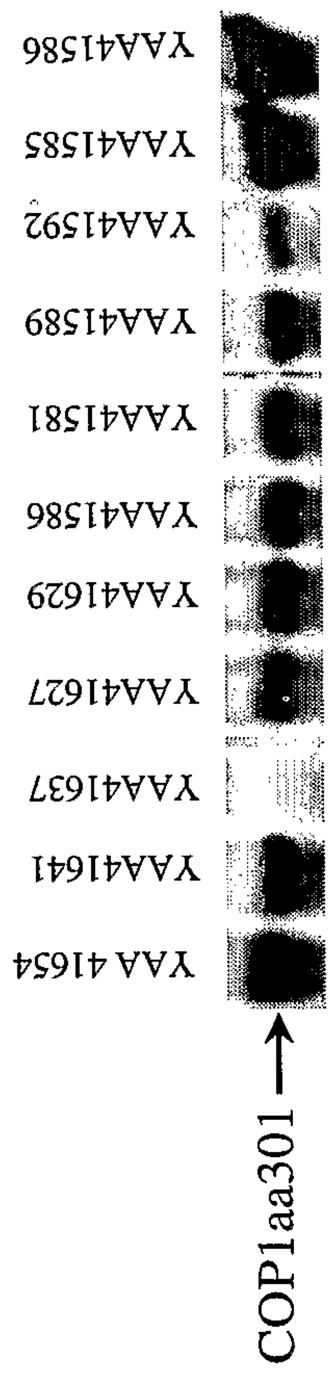

YAA41586
YAA41585
YAA41592
YAA41589
YAA41581
YAA41586
YAA41629
YAA41627
YAA41637
YAA41641
YAA 41654
COP1aa301

Figure 10

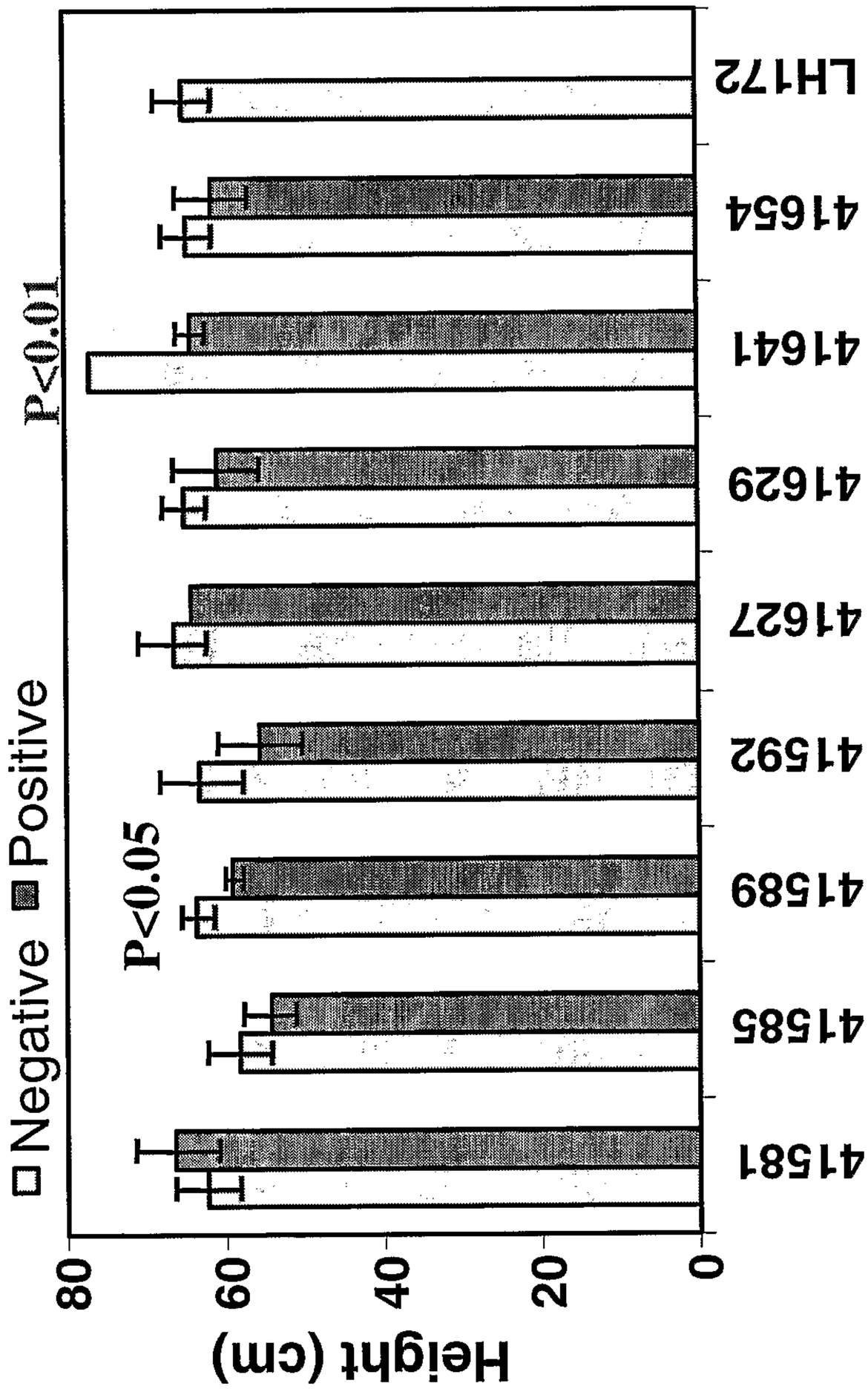

Negative
Positive
P<0.01
P<0.05
Height (cm)
0
20
40
60
80
Events
LH172
41654
41641
41629
41627
41592
41589
41585
41581

Figure 11

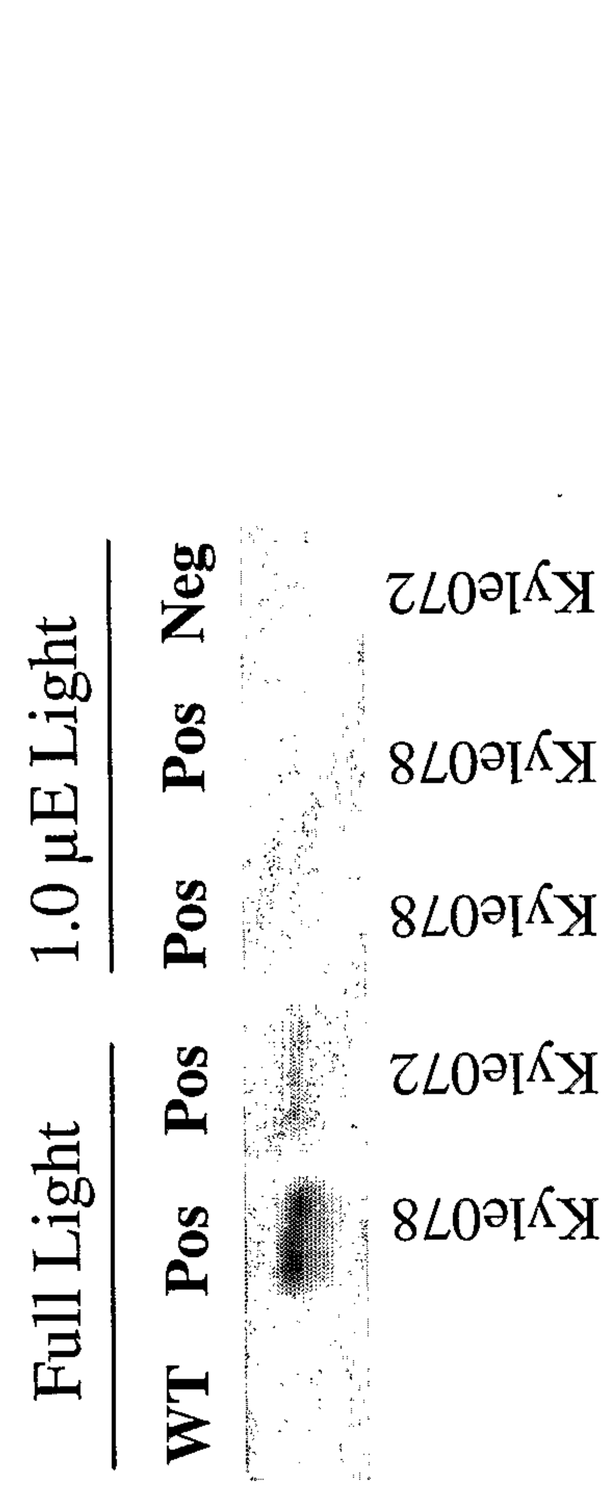

1.0 µE Light
Pos
Pos
Neg
Kyle072
Kyle078
Kyle078
Full Light
WT
Pos
Pos
Kyle072
Kyle078

Figure 12

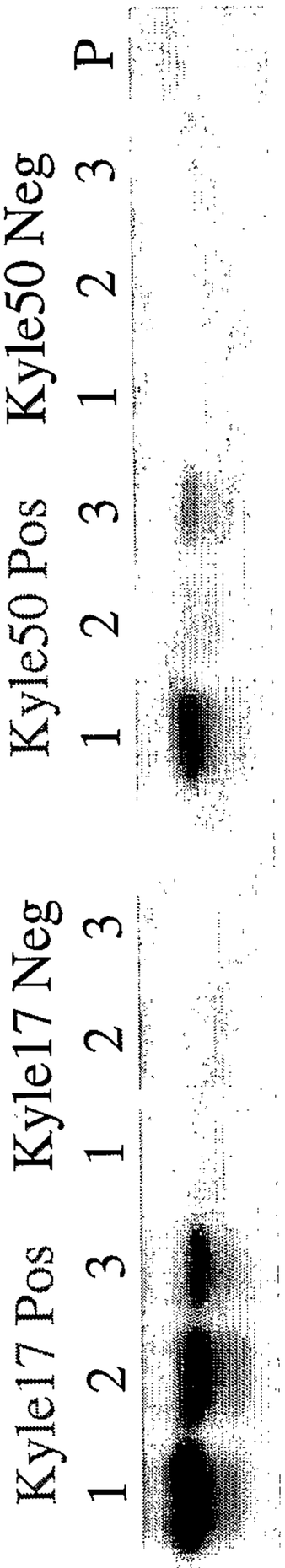


Figure 13

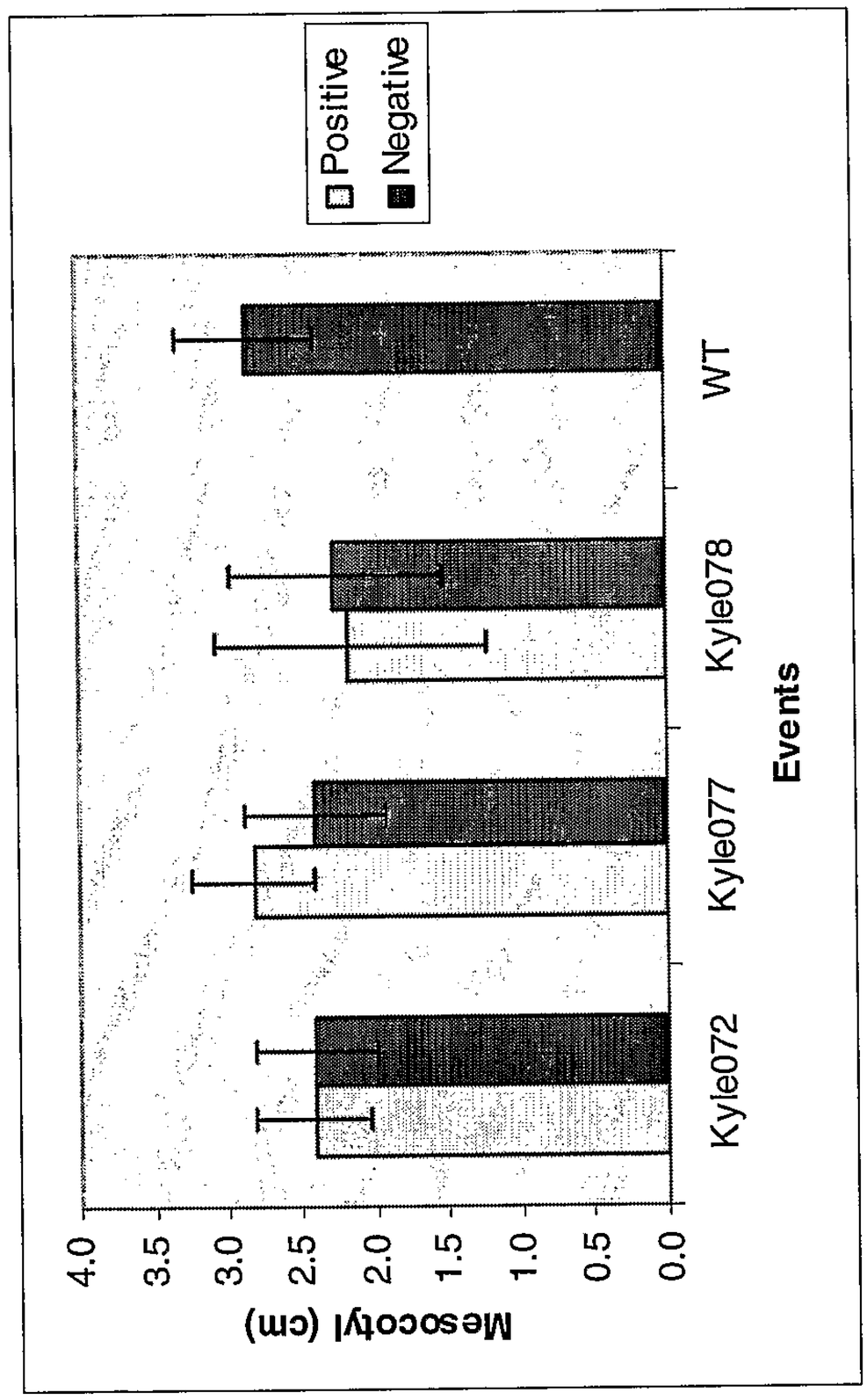

Positive
Negative
WT
Kyle078
Kyle077
Kyle072
Events
Mesocotyl (cm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Figure 14

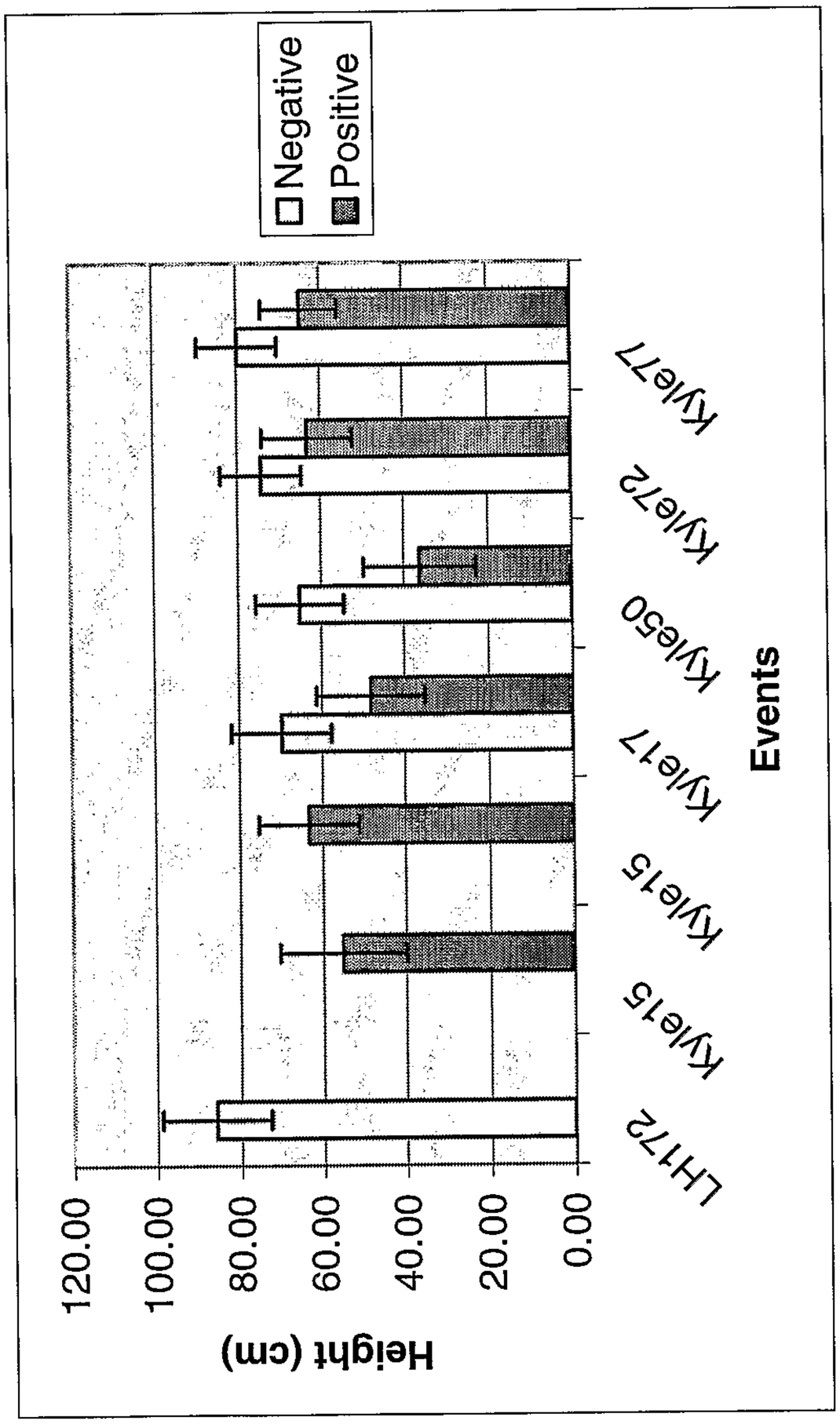

Negative
Positive
Height (cm)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
LH172
Kyle15
Kyle15
Kyle17
Kyle50
Kyle72
Kyle77
Events

Figure 15



Figure 16

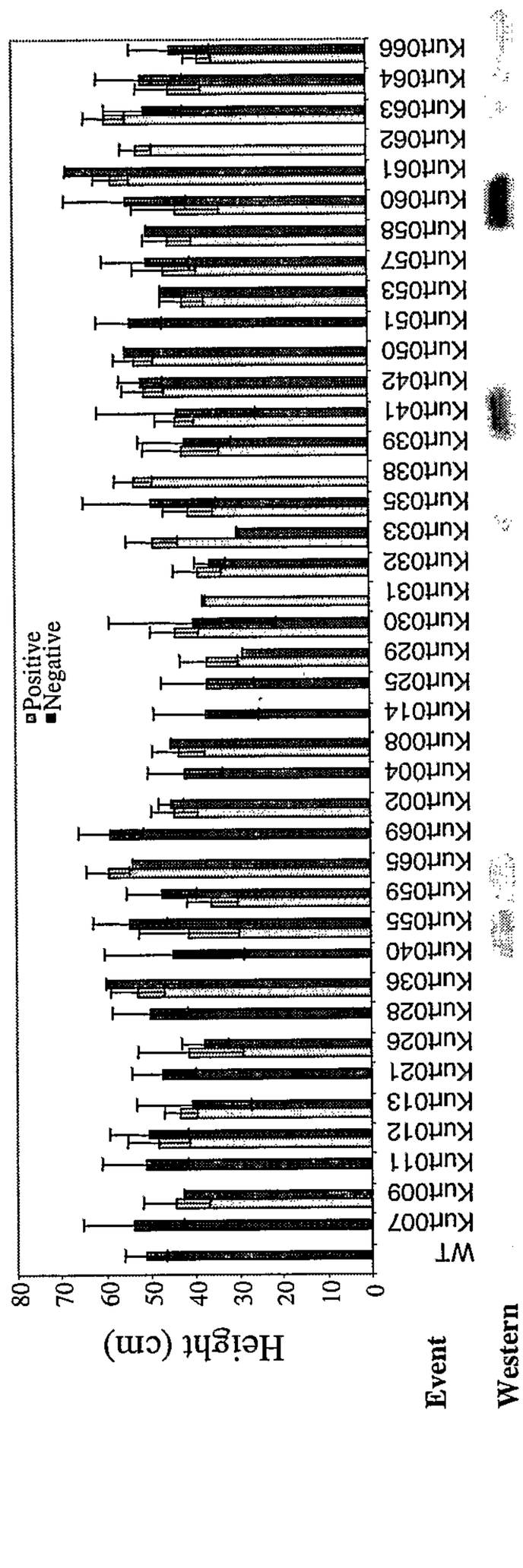

Positive
Negative
Height (cm)
0
10
20
30
40
50
60
70
80
WT
Kurt007
Kurt009
Kurt011
Kurt012
Kurt013
Kurt021
Kurt026
Kurt028
Kurt036
Kurt040
Kurt055
Kurt059
Kurt065
Kurt069
Kurt002
Kurt004
Kurt008
Kurt014
Kurt025
Kurt029
Kurt030
Kurt031
Kurt032
Kurt033
Kurt035
Kurt038
Kurt039
Kurt041
Kurt042
Kurt050
Kurt051
Kurt053
Kurt057
Kurt058
Kurt060
Kurt061
Kurt062
Kurt063
Kurt064
Kurt066
Event
Western

Figure 17