Title: USE OF AUXIN SYNTHASE FOR IMPROVING CROP YIELD

Abstract: Compositions and methods for increasing plant yield are provided. The methods comprise increasing the expression of an auxin synthase gene or coding sequence in the shoot meristem and inflorescence of a plant. The compositions comprise DNA constructs comprising an auxin synthase coding sequence operably linked to a promoter that preferentially drives expression in the shoot meristem and inflorescence of a plant. Also provided are plants, plant cells, plant tissues, and seeds that have been transformed with the DNA construct.
USE OF AUXIN SYNTHASE TO IMPROVE CROP YIELD

FIELD OF THE INVENTION

This invention is in the field of plant genetic engineering. This invention involves a method of obtaining high-yield transgenic plants by expressing indoleacetic acid (IAA) synthase gene in plants. This invention can be applied in the field of crop breeding.

BACKGROUND OF THE INVENTION

Auxin is an important signal chemical that controls plant growth and development. It controls plant cell division, expansion and differentiation, as well as lateral root formation and flowering. (Davies P.J. (2004) *The Plant Hormone: Their Nature*, Kluwer, Dordrecht, the Netherlands). The biosynthetic pathway of auxin at the genetic level has remained unclear. Two major pathways have been proposed: The tryptophan(Try)-independent and Trp-dependent pathways. See, Mashiguchi et al. (2011) *PNAS* 108:18512-18517. There are also auxin synthase genes in microorganisms (Thomashow et al. (1984) *PNAS* 81:5071-5075; Van Onckelen et al. (1986) *FEBS Lett* 198:357-360. Although the research on auxin is very extensive, the synthesis pathways and the function of auxin remain unclear in plants.

In recent years, the auxin synthase gene has been used as a tool for crop improvement. The auxin synthase gene has been expressed in the epithelial cells of cotton ovule resulting in transgenic cotton with traits of high quality and high yield. (Zhang et al. (2011) *Nature Biotechnology* 29: 453-458). The auxin synthase gene was also expressed in grape driven by the ovule-specific promoter resulting in the increase in grape fecundity. (Costantini et al. (2007) *Plant Physiol* 143:1689-1694). However, there are still no effective methods to improve the yield of major crops, such as rice, corn, soybean, wheat, barley, sorghum and sunflower. The impact of auxin on plants is complicated. In fact, excess auxin can be highly harmful to plant. Therefore, methods are needed to utilize auxin to enhance crop yield.

SUMMARY OF INVENTION
Compositions and methods for increasing plant yield are provided. The methods of the invention comprise increasing the expression of an auxin synthase gene or coding sequence in the shoot meristem and inflorescence of a plant. Compositions of the invention comprise DNA constructs comprising an auxin synthase coding sequence operably linked to a promoter that drives expression in the shoot meristem and inflorescence of a plant. Also encompassed are plants, plant cells, plant tissues, and seeds that have been transformed with the DNA construct. The invention recognizes that enhancing the expression of auxin in the shoot meristem and inflorescence of a plant results in an improvement in plant yield, resulting in an increase in crop yield in a field planted with such plants.

Thus, the present invention relates generally to the field of molecular biology and concerns a method for increasing plant yield relative to control plants. More specifically, the present invention concerns a method for increasing plant yield comprising modulating expression of a nucleic acid molecule encoding an auxin synthase gene or a homologue thereof in the shoot meristem and inflorescence of a plant. The present invention also concerns plants having elevated expression of a nucleic acid encoding the auxin synthase gene in shoot meristems and inflorescence, which plants have increased growth and yield relative to control plants. The invention also provides constructs useful in the methods of the invention.

The following embodiments are encompassed by the present invention:

1. A method for increasing plant yield in a plant of interest, said method comprising transforming said plant with a DNA construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence, and selecting plants having a high yield phenotype.

2. The method of embodiment 1, wherein said promoter is a promoter from a plant Mei2-like gene.

3. The method of embodiment 2, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO: 1 - SEQ ID NO: 10.
4. The method of any one of embodiments 1-3, wherein said auxin synthase coding sequence is an indoleacetic acid (IAA) synthase coding sequence from a microorganism.

5. The method of any one of embodiments 1-3, wherein said auxin synthase coding sequence is a plant auxin synthase coding sequence.

6. The method of any one of embodiments 4 or 5, wherein said auxin synthase coding sequence is a synthetic sequence.

7. The method of any one of embodiments 1-3 and 5, wherein said auxin synthase coding sequence is selected from the group consisting of:
   i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
   ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO: 11, 12, or 13;
   iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
   iv) a nucleotide sequence that encodes an amino acid sequence having at least 40% sequence identity to any one of SEQ ID NOs: 14-19.

8. The method of any one of embodiments 1-7, wherein plant is selected from the group consisting of rice, corn, cotton, wheat, barley, soybean, sunflower, canola, and sorghum.

9. An expression cassette comprising a DNA construct, said construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence.

10. The expression cassette of embodiment 9, wherein said promoter is a promoter from a plant Mei2-like gene.
11. The expression cassette of any one of embodiments 9-10, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO: 1 - SEQ ID NO: 10.

12. The expression cassette of any one of embodiments 9-11, wherein said auxin synthase coding sequence is an indoleacetic acid (IAA) synthase coding sequence from a microorganism.

13. The expression cassette of any one of embodiments 9-11, wherein said auxin synthase coding sequence is a plant auxin synthase coding sequence.

14. The expression cassette of any one of embodiments 12 or 13, wherein said auxin synthase coding sequence is a synthetic sequence.

15. The expression cassette of any one of embodiments 9-11 and 13, wherein said auxin synthase coding sequence is selected from the group consisting of:
   i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
   ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO: 11, 12, or 13;
   iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
   iv) a nucleotide sequence that encodes an amino acid sequence having at least 40% sequence identity to any one of SEQ ID NOs: 14-19.

16. A plant transformed with the expression cassette of any one of embodiments 9-12.

17. A transformed seed of the plant of embodiment 16.

18. The method of any one of embodiments 5, wherein said auxin synthase sequence is an endogenous sequence.
19. A transformed plant that exhibits increased expression of auxin synthase in its shoot meristem and inflorescence as compared to a control plant.

20. The transformed plant of embodiment 19, wherein said plant has been transformed with a DNA construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence.

21. The transformed plant of embodiment 19 or 20, wherein said plant wherein said promoter is a promoter from a plant Mei2-like gene.

22. The transformed plant of embodiment 21, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO:1 - SEQ ID NO:10.

23. The transformed plant of any one of embodiments 19-22 wherein said auxin synthase coding sequence is an indoleacetic acid (IAA) synthase coding sequence from a microorganism.

24. The transformed plant of any one of embodiments 19-22, wherein said auxin synthase coding sequence is a plant auxin synthase coding sequence.

25. The transformed plant of any one of embodiments 19-24, wherein said auxin synthase coding sequence is a synthetic sequence.

26. The transformed of any one of embodiments 19-22 and 24-25, wherein said auxin synthase coding sequence is selected from the group consisting of:
   i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
   ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO: 11, 12, or 13;
   iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
iv) a nucleotide sequence that encodes an amino acid sequence having at least
40% sequence identity to any one of SEQ ID NOs: 14-19.

27. The transformed plant of embodiment 24, wherein said auxin synthase coding
sequence is an endogenous sequence.

28. Transformed seed from the plant of any one of embodiments 19-27.

29. The transgenic plant of any one of embodiments 19-27, wherein said plant is
selected from the group consisting of of rice, corn, cotton, wheat, barley, soybean,
sunflower, canola, and sorghum.

30. The plant of any one of embodiments 19-27, wherein said plant is a monocot.

31. The plant of any one of embodiments 19-27, wherein said plant is a dicot.

32. A transformed plant having increased expression of auxin synthase in shoot
meristem or inflorescence and further having increased expression of a TEL sequence.

DETAILED DESCRIPTION

The present invention is drawn to methods for increasing the expression of an
auxin synthase gene or coding sequence in the shoot meristem and inflorescence of plants
or plant cells. By increasing or enhancing the expression of an auxin synthase in the
shoot meristem and inflorescence, the plant exhibits an improvement in plant yield.

Expression in the plant is increased by transforming the plant with a DNA construct
comprising a promoter that drives expression in the shoot meristem and inflorescence of
a plant operably linked to an auxin synthase coding sequence.

An increase in the expression of an auxin synthase sequence in the shoot
meristem and inflorescence of a plant results in an increase in plant growth and yield.

The transformed plant has an increase in yield with no reduction in harvest index.
Transformed plants have increased seed production and larger seed. Some plants may
exhibit increased growth with larger plants resulting. Planting a field of transformed
plants of the invention will result in increased crop yield. By "crop yield" is intended the
amount of a crop that is harvested per unit of land area. Crop yield is the measurement
often used for a cereal, grain, or legume and is normally measured in metric tons per
hectare (or kilograms per hectare). Crop yield also refers to the actual seed generation
from the plant.

By "enhancing or increasing the expression of an auxin synthase gene or coding
sequence" is intended that the expression as measured by the production of mRNA or
auxin synthase protein is increased in the plant of interest as compared to a control plant.

Auxin synthase is increased at least about 0.25-fold, at least about 0.3-fold, at least about
0.5-fold, at least about one-fold, at least about two-fold, at least about three-fold, at least
about four-fold, at least about five-fold, at least about six-fold, at least about seven-fold,
least about eight-fold, at least about nine-fold, at least about 10-fold or greater.

By "control plant" is intended a plant where the expression of auxin synthase
sequence has not been altered or enhanced, i.e., a plant or plant cell genetically identical
to the subject plant or plant cell but which is not exposed to conditions or stimuli that
would increase expression of an auxin synthase sequence.

While not bound by any theory, it is believed that extreme over-production of the
auxin synthase coding sequence may result in plants with undesirable phenotypes.

Therefore, one can control expression by the selection of the promoters used to drive
expression of an auxin synthase sequence in a transformed plant. Tissue-preferred
promoters, particularly, those promoters that are preferentially expressed in the early
stage of flower development are preferred in the invention. Such promoters should not
be strong promoters. Promoters that drive expression in the shoot meristem and
inflorescence are useful in the practice of the invention. Such promoters include Mei2
promoters. The Mei2 promoters provide good results in expressing the recombinant gene
at desired levels and in desired tissues. As discussed below, any promoter that drives
expression in the shoot meristem and inflorescence of a plant may be used. Once a plant
has been transformed, one can select the desired plant based on the phenotype. Thus, the
methods of the invention further comprise selection of the desired phenotype of the
transformed plant. Such desired plants will exhibit increased growth and yield of grain or biomass.

Such desired plants can be grown and crossed with suitable plants to produce seed having the desired phenotype. That is, the recombinant auxin synthase gene that has been inserted into the genome of a plant can be bred into other plants. Such plants will be grown and used to produce a crop with enhanced yield.

By "auxin synthase gene" or auxin synthase sequence" is intended a sequence that encodes the entire amino acid sequence of the auxin synthase protein or variants or truncations of the auxin synthase protein. A number of auxin synthase genes are known in the art and any can be used in the practice of the invention, including fragments and variants of known auxin synthase genes as long as the fragments and variants retain the desired activity of promoting plant growth and increasing yield. Any auxin synthase coding sequence can be used in the practice of the invention. The auxin synthase gene can come from either a microorganism or a plant. For example, the microbial auxin synthase genes include but are not limited are: auxin synthases from Agrobacterium tumefaciens (AAF77123, AAB41874), auxin synthases from Pseudomonas syringae (AAR06971, EF100478, and AAAI7678), auxin synthase from Dickeya dadantii (ADM96599), auxin synthase from Pantoea agglomerans (AAC17187), etc. Auxin synthases from plant may be also used in the practice of the invention. Such auxin synthase sequences include, for example, auxin synthase (CAB79971) from Arabidopsis thaliana and its homologous genes in other plants. Other auxin synthases can be isolated or cloned from other plants and microorganisms using the above identified sequences. Likewise, auxin synthase sequences can be synthesized artificially. The advantage of the synthetic DNA fragment is the amino acid codons usage can be optimized for different target plants. The methods of the invention are not limited to any particular auxin synthase. Thus, other auxin synthases from other organisms can also be utilized for the purpose of this invention.

The auxin synthase or auxin synthase-like proteins include those having at least 40%, at least 50%, at least 58% at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to a known auxin synthase sequence.
A number of auxin synthase genes are disclosed herein and are known in the art and any of these auxin synthase sequences, as well as variants and truncations thereof, can be used in any plant of interest. As discussed below, the sequences herein can be used to isolate other auxin synthase genes that are useful in the practice of the invention.

Nucleotide sequences encoding the auxin synthase proteins of the present invention include the sequences set forth in SEQ ID NOs: 11-13, and variants, fragments, and complements thereof.

Nucleic acid molecules that are fragments of these nucleotide sequences encoding auxin synthase proteins are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence encoding an auxin synthase protein. A fragment of a nucleotide sequence may encode a biologically active portion of an auxin synthase protein, or it may be a fragment that can be used as a hybridization probe or PCR primer useful for isolating other auxin synthase-like sequences. Typically, truncations fragments of the nucleotide sequences of the present invention will encode protein fragments that retain the biological activity of the auxin synthase protein and, hence, retain auxin synthase activity. By "retains activity" is intended that the fragment will have at least about 50%, at least about 70%, 80%, 90%, 95% or higher of the auxin synthase activity of the auxin synthase protein. By "auxin synthase activity" is intended the production of ethylene production and increased plant growth or yield. Those plants exhibiting the desired levels of increased auxin synthase production can be selected based on the phenotype of the regenerated plant. Thus, variants and truncations of the auxin synthase sequence can be tested for activity by transformation of the sequence in a plant of interest and selecting for plants having the desired phenotype.

Variants of the auxin synthase nucleic acid molecules may be made by various methods. These alterations may result in DNA sequences encoding proteins with amino acid sequences different than that encoded by an auxin synthase protein of the present invention. Thus, the protein may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions of one or more amino acids. Methods for such manipulations are generally known in the art.

Preferred auxin synthase proteins of the present invention are encoded by a nucleotide sequence identical or having sequence identity to the nucleotide sequence of
any of the auxin synthase sequences listed herein or contained within the sequence listing.

Variant amino acid or nucleotide sequences having at least about 40%, about 60% or 65% sequence identity, about 70% or 75% sequence identity, about 80% or 85% sequence identity, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or
greater sequence identity compared to a reference auxin synthase sequence using one of
the alignment programs described herein using standard parameters are encompassed by
the invention. Auxin synthase proteins of the present invention include the sequences set
forth in SEQ ID NOs: 14-19, and variants, fragments, and complements thereof. One of
skill in the art will recognize that these values can be appropriately adjusted to determine
the corresponding identity of proteins encoded by two nucleotide sequences by taking into
account codon degeneracy, amino acid similarity, reading frame positioning, and the like.

The determination of percent identity between two sequences can be
accomplished using a mathematical algorithm. A nonlimiting example of a mathematical
algorithm utilized for the comparison of two sequences is the algorithm of Karlin and
BLAST nucleotide searches can be performed with the BLASTN program, score = 100,
wordlength = 12, to obtain nucleotide sequences homologous to auxin synthase-like
nucleic acid molecules of the invention. BLAST protein searches can be performed with
the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences
homologous to auxin synthase protein molecules of the invention. To obtain gapped
alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as
can be used to perform an iterated search that detects distant relationships between
molecules. See Altschul et al. (1997) supra. When utilizing BLAST, Gapped BLAST,
and PSI-Blast programs, the default parameters of the respective programs (e.g.,
BLASTX and BLASTN) can be used. Alignment may also be performed manually by
inspection.

Other mathematical algorithms may be used for the comparison of sequences
including the ClustalW algorithm (Higgins et al. (1994) Nucleic Acids Res. 22:4673-
4680). Unless otherwise stated, GAP Version 10, which uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48(3):443-453, will be used to determine sequence identity or similarity using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity or % similarity for an amino acid sequence using GAP weight of 8 and length weight of 2, and the BLOSUM62 scoring program. Equivalent programs may also be used. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

As indicated, variant auxin synthase nucleic acid molecules may be used in the practice of the invention. "Variants" of the auxin synthase protein encoding nucleotide sequences include those sequences that encode the auxin synthase proteins disclosed herein but that differ conservatively because of the degeneracy of the genetic code as well as those that are sufficiently identical as discussed above. Naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the auxin synthase proteins disclosed in the present invention as discussed below. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, auxin synthase activity.

The skilled artisan will further appreciate that changes can be introduced by mutation of the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded auxin synthase proteins, without altering the biological activity of the proteins. Thus, variant isolated nucleic acid molecules can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis.
and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, conservative amino acid substitutions may be made at one or more, predicted, nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of an auxin synthase protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

Amino acid substitutions may be made in nonconserved regions that retain function. In general, such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity. Examples of residues that are conserved and that may be essential for protein activity include, for example, residues that are identical between all proteins contained in an alignment of similar or related proteins to the sequences of the invention (e.g., residues that are identical in an alignment of homologous proteins). However, one of skill in the art would understand that functional variants may have minor conserved or nonconserved alterations in the conserved residues. In one embodiment, changes in the amino acid sequence will not be made in the conserved motifs or in the region surrounding the motifs as set forth in Figure 2.

Antibodies to the polypeptides of the present invention, or to variants or fragments thereof, are also encompassed. Methods for producing antibodies are well known in the art (see, for example, Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; U.S. Patent No. 4,196,265).

In addition to the auxin synthase proteins listed in this application, this invention also provides methods to clone and utilize new auxin synthase genes from other organisms, including plants, moss, microorganisms, and fungi. For example, by using the sequences provided herein, one can clone new auxin synthase genes using methods such as PCR and nucleic acid hybridization. PCR primers may be designed according to the conservative regions of the DNA sequences of auxin synthase genes. Moreover, the conservative amino acid sequences may be used to design degenerate primers for PCR. A
partially known gene from PCR can be used to clone a full-length gene using various known methods, such as Tail-PCR, 5'RACE, 3'RACE, etc. See, for example, Singer and Burke (2003) Methods Mol Biol 236:241-272; and commercially available kits. As described below, the genes provided in this invention and in other publications can be used to prepare probes to hybridize genomic or cDNA libraries to clone auxin synthase genes.

With the rapid advancement of various sequencing projects, new auxin synthase genes may be identified by searching various databases using the auxin synthase amino acid sequences and/or nucleic sequences provided by this invention. Such databases include but not limited to databases of genome sequence, ETS, and cDNA sequences. BLAST method (Altschul et al. 1990 J. Mol. Biol. 215, 403^10) is a wide used.

In a hybridization method, all or part of an auxin synthase nucleotide sequence disclosed herein can be used to screen cDNA or genomic libraries for additional auxin synthase sequences for use in the invention. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook and Russell, 2001, supra. The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^32P, or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Methods for the preparation of probes for hybridization are generally known in the art and are disclosed in Sambrook and Russell, 2001, supra herein incorporated by reference.

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.
Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the $T_m$ can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5°C + 16.6 \times \log M + 0.41 \times (%GC) - 0.61 \times (% form) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The $T_m$ is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. $T_m$ is reduced by about 1°C for each 1% of mismatching; thus, $T_{m'}$ hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with ≥90% identity are sought, the $T_m$ can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ($T_{m'}$) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point ($T_{m'}$); moderately stringent conditions can utilize a
hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point ($T_m$); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point ($T_m$). Using the equation, hybridization and wash compositions, and desired $T_m$, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a $T_m$ of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel et al., eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

As noted above, methods of the invention comprise transforming a plant of interest with a DNA construct or expression cassette comprising a nucleic acid molecule that encodes an auxin synthase sequence of the invention. The expression cassette will include in the 5′-3′ direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a DNA sequence of the invention, and a translational and transcriptional termination region (i.e., termination region) functional in plants. General methods to introduce and express an auxin synthase gene in a plant and hence crops are currently available. As indicated, an auxin synthase sequence of the invention may be provided in a DNA construct or an expression cassette for expression in a plant of interest. By "plant expression cassette" is intended a DNA construct that is capable of resulting in the expression of a protein from an open reading frame in a plant cell. Typically these contain a promoter and a coding sequence. Often, such constructs will also contain a 3′ untranslated region.

By "plant transformation vector" is intended a DNA molecule that is necessary for efficient transformation of a plant cell. Such a molecule may consist of one or more plant expression cassettes, and may be organized into more than one "vector" DNA molecule. For example, binary vectors are plant transformation vectors that utilize two
non-contiguous DNA vectors to encode all requisite cis- and trans-acting functions for transformation of plant cells (Hellens and Mullineaux (2000) *Trends in Plant Science* 5:446-451). "Vector" refers to a nucleic acid construct designed for transfer between different host cells. "Expression vector" refers to a vector that has the ability to incorporate, integrate and express heterologous DNA sequences or fragments in a foreign cell. The cassette will include 5’ and 3’ regulatory sequences operably linked to a sequence of the invention. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

"Promoter" refers to a nucleic acid sequence that functions to direct transcription of a downstream coding sequence. The promoter together with other transcriptional and translational regulatory nucleic acid sequences (also termed "control sequences") are necessary for the expression of a DNA sequence of interest. Promoters for use in the practice of the invention include those that drive expression in the shoot meristem and inflorescence of a plant. Promoters are of critical importance for this invention. The promoters used by this invention to control the auxin synthase gene are the ones which can regulate the gene express preferentially in plant shoot meristem and inflorescence, and in low to very low expression levels. An example of a promoter for use in the invention includes the promoter from the plant TEL gene. This type of promoter has the following characteristics: a) they are the promoters of plant TEL gene (for instance, NP_594609 and CAA15822); and, b) they regulate the expression specifically in plant shoot meristem and inflorescence. These promoters including the promoter from *Oryza sativa* (SEQ ID NO: 1); from *Zea mays* (SEQ ID NO: 2); from *Glycine max* (SEQ ID NOs:3, 4); from *Arabidopsis thaliana* (SEQ ID NOs: 5-7); and from *Brassica rapa* (SEQ ID NO: 8). Since the function and specificity of expression of a promoter in different plants are normally conservative, the promoter from one plant often has the same or
similar function in another plant. Thus, the promoters provided by this invention may be used in different plants. Promoters to regulate the auxin synthase genes for higher yield can be other promoters, such as the promoters of the FCA gene of *Arabidopsis thaliana* and its homologous promoters from other plants. These promoters including the promoter from Oryza sativa (SEQ ID NO: 9) and from Glycine max (SEQ ID NOs: 10).

Other meristem-and-inflorescence specific promoters can be obtained by general molecular biology methods. For example, by sequencing the mRNA from a plant inflorescence, the genes expressed in the inflorescence can be obtained. The expression specificities of these genes can be determined by gene chip or Northern blot. Genes expressed in the inflorescence, especially in the early inflorescence, may be obtained by general molecular biological techniques. After the genome of a plant was sequenced, the regions of promoters can be determined by molecular informatics methods and cloned. Normally, the promoter of a gene often locates on the 5′ end of the coding region of the protein in the genome, which has a length within about 2-3kb.

The DNA constructs of the invention may further comprise a terminator. The frequently-used terminators include the octopine synthase terminator and nopaline synthase terminator from Agrobacterium. See, for example, Guerineau et al. (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon et al. (1991) *Genes Dev.* 5:141-149; Mogen et al. (1990) *Plant Cell* 2:1261-1272; Munroe et al (1990) *Gene* 91:151-158; Ballas et al. (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi et al. (1987) *Nucleic Acids Res.* 15:9627-9639. Besides, other terminators can also be used in the plant expression cassette. This invention provides an example (Example 2) in which the auxin synthase gene and the terminator were simultaneously synthesized, which simplified the construction procedure of the expression cassette.

The DNA construct or expression cassette is provided with a plurality of restriction sites for insertion of the auxin synthase sequence to be under the transcriptional regulation of the regulatory regions.

Methods of the invention involve introducing a nucleotide construct into a plant. By "introducing" is intended to present to the plant the nucleotide construct in such a manner that the construct gains access to the interior of a cell of the plant. The methods of the invention do not require that a particular method for introducing a nucleotide
construct to a plant is used, only that the nucleotide construct gains access to the interior of at least one cell of the plant. Methods for introducing nucleotide constructs into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

By "plant" is intended whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells, pollen).

"Transgenic plants" or "transformed plants" or "stably transformed" plants or cells or tissues refers to plants that have incorporated or integrated exogenous nucleic acid sequences or DNA fragments into the plant cell. These nucleic acid sequences include those that are exogenous, or not present in the untransformed plant cell, as well as those that may be endogenous, or present in the untransformed plant cell. "Heterologous" generally refers to the nucleic acid sequences that are not endogenous to the cell or part of the native genome in which they are present, and have been added to the cell by infection, transfection, microinjection, electroporation, microprojection, or the like.

In general, plant transformation methods involve transferring heterologous DNA into target plant cells (e.g. immature or mature embryos, suspension cultures, undifferentiated callus, protoplasts, etc.), followed by applying appropriate selection (depending on the selectable marker gene) to recover the transformed plant cells from a group of untransformed cell mass. Explants are typically transferred to a fresh supply of the same medium and cultured routinely. Subsequently, the transformed cells are differentiated into shoots after placing on regeneration medium supplemented with a maximum threshold level of selecting agent. The shoots are then transferred to a selective rooting medium for recovering rooted shoot or plantlet. The transgenic plantlet then grows into a mature plant and produces fertile seeds (e.g. Hiei et al. (1994) *The Plant Journal* 6:271-282; Ishida et al. (1996) *Nature Biotechnology* 14:745-750). Explants are typically transferred to a fresh supply of the same medium and cultured routinely. A general description of the techniques and methods for generating transgenic plants are found in Ayres and Park (1994) *Critical Reviews in Plant Science* 13:219-239 and Bommineni and Jauhar (1997) *Maydica* 42: 107-120. Since the transformed material
contains many cells; both transformed and non-transformed cells are present in any piece
5 of subjected target callus or tissue or group of cells. The ability to kill non-transformed
and allow transformed cells to proliferate results in transformed plant cultures.

Often, the ability to remove non-transformed cells is a limitation to rapid recovery of
transformed plant cells and successful generation of transgenic plants.

Transformation protocols as well as protocols for introducing nucleotide
sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot
10 or dicot, targeted for transformation. Generation of transgenic plants may be performed
by one of several methods, including, but not limited to, microinjection (Crossway et al.
EMBO J. 3:2717 2722), and ballistic particle acceleration (see, for example, U.S. Patent
Nos. 4,945,050; U.S. Patent No. 5,879,918; U.S. Patent No. 5,886,244; and, 5,932,782;

Gamborg and Phillips (Springer-Verlag, Berlin); McCabe et al. (1988) Biotechnology
6:923 926); and Lecl transformation (WO 00/28058). Also see Weissinger et al. (1988)
5,240,855; 5,322,783; 4,945,050; 5,324,646; U.S. Published Application No.
20010026941; 2002015066; and, International Publication No. WO 91/00915.

The cells that have been transformed may be grown into plants in accordance with
conventional ways. See, for example, McCormick et al. (1986) Plant Cell Reports 5:81-

84. These plants may then be grown, and either pollinated with the same transformed
strain or different strains, and the resulting hybrid having constitutive expression of the
5 10 desired phenotypic characteristic identified. Two or more generations may be grown to
ensure that expression of the desired phenotypic characteristic is stably maintained and
inherited and then seeds harvested to ensure expression of the desired phenotypic
characteristic has been achieved. In this manner, the present invention provides
transformed seed (also referred to as "transgenic seed") having a nucleotide construct of
the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

Following introduction of heterologous foreign DNA into plant cells, the transformation or integration of heterologous gene in the plant genome is confirmed by various methods such as analysis of nucleic acids and proteins associated with the integrated gene. Molecular techniques include PCR (Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), Southern blot analysis of genomic DNA, Northern blot analysis and Western blot (Sambrook and Russell, 2001, supra).

A number of selectable markers have been developed for use with plant cells, such as resistance to chloramphenicol, the aminoglycoside G418, hygromycin, or the like. Other genes that encode a product involved in chloroplast metabolism may also be used as selectable markers. For example, genes that provide resistance to plant herbicides such as glyphosate, bromoxynil, or imidazolinone may find particular use. Such genes have been reported (Stalker et al. (1985) J. Biol. Chem. 263:6310-6314 (bromoxynil resistance nitrilase gene); and Sathasivan et al. (1990) Nucl. Acids Res. 18:2188 (AHAS imidazolinone resistance gene). Methods for detecting the presence of a transgene in a plant, plant organ (e.g., leaves, stems, roots, etc.), seed, plant cell, propagule, embryo or progeny of the same are well known in the art.

Fertile plants expressing an auxin synthase protein may be tested for auxin synthase activity, and the plants showing the desired phenotype selected for further breeding.

The methods of the invention can be used with other genes and methods for increasing yield. In one embodiment, the methods of the invention can be used in combination with methods to increase a TEL sequence for enhanced yield in the modified plants. Methods to increase plant growth and yield by increasing expression of a TEL sequence are set forth in PCT Application No. PCT/CN2012/087069, filed 20 December 2012, entitled "Methods for Improving Crop Yield." The disclosure of which is herein incorporated by reference. Any method to increase the expression of a TEL sequence in the plant and to increase the expression of auxin synthase in the shoot meristem and inflorescence can be used. In one embodiment, a plant that has been modified to have
increased expression of a TEL sequence can be transformed with a construct comprising an auxin synthase coding sequence operably linked to a promoter that drives expression in the shoot meristem and inflorescence and plants with the desired phenotype selected after transformation and selection.

In another embodiment, a plant that has increased expression of auxin synthase in shoot meristem and inflorescence can be modified to increase the expression of a TEL sequence. Expression of the TEL sequence can be increased by transforming the plant with a construct comprising a TEL coding sequence operably linked with a promoter that drives expression in a plant cell, or by transforming the plant with an enhancer that increases the expression of the endogenous TEL coding sequence. In another embodiment, a plant that has increased expression of auxin synthase in the shoot meristem and inflorescence can be crossed with a plant that has increased expression of a TEL sequence and plants selected that retain the increased expression of a TEL sequence and the increased expression of auxin synthase in shoot meristem and inflorescence.

The methods of the invention may be used in any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (maize), sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape, Brassica sp., alfalfa, rye, millet, safflower, peanuts, sweet potato, cassava, coffee, coconut, pineapple, citrus trees, cocoa, tea, banana, avocado, fig, guava, mango, olive, papaya, cashew, macadamia, almond, oats, vegetables, ornamentals, and conifers.

Vegetables include, but are not limited to, tomatoes, lettuce, green beans, lima beans, peas, and members of the genus Curcumin such as cucumber, cantaloupe, and musk melon. Ornamentals include, but are not limited to, azalea, hydrangea, hibiscus, roses, tulips, daffodils, petunias, carnation, poinsettia, and chrysanthemum. Preferably, plants of the present invention are crop plants (for example, maize, sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, oilseed rape, Miscanthus, switchgrass, Jatropha, etc.) and conifers.

The following examples are offered by way of illustration and not by way of limitation.
EXPERIMENTAL

Example 1

Cloning of the promoters of Mei2-like genes from rice, corn, soybean, canola and Arabidopsis thaliana.

(1) Cloning of promoter pOsTE1 of Mei2-like gene from rice

The 5’ end sequence of OsTE1 was amplified by PCR using the designed primers pOsTE1-F (5’-AAGCTTGAACCTAGTACTAGACATTACTCTTCCAATGCA) and pOsTE1-R (5’-AGAGGATCTGAGAGACCTACCA).

The PCR conditions were: 95 °C for 5 min, then 30 cycles at 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 2 Kb in length was cloned into T-vector pMD19 (TaKaRa).

Further, the BamHI site inside the promoter was removed by point mutation using primers OsTE-PRO-DELF (5’-AGATCCGAGCAAAAAACAGGGCC) and OsTE-PRO-DEL (5’-TCTATAGCGATAGAACTGTTTGATCTGG-GTAGC). Then, the resulting promoter was digested using HindIII and BamHI from the T-vector, and its nucleotide sequence (SEQ ID NO: 1) was confirmed by standard sequencing procedures.

(2) Cloning of promoter pZmTE1 of Mei2-like gene from corn

The 5’ end sequence of ZmTE1 was amplified by PCR using the designed primers pZmTE1-F (5’-AGAAAGCTTAGTGCCAATCACTGCGTGAGAACCGA) and pZmTE1-R (5’-AGGGATCTGCGAGCAGCCTACCTACCCCTACCTACCA). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 2 Kb in length was cloned into T-vector pMD19 (TaKaRa). Since there’s a BamHI site in this DNA fragment, the 1.4 kb promoter was obtained by a HindIII digestion following with a partial digestion of BamHI. The nucleotide sequence of the promoter ( SEQ ID NO: 2) was confirmed by DNA sequencing using standard procedures.
(3) Cloning of promoter pGmTEl of Mei2-like gene from soybean

The 5' end sequence of GmTEl was amplified by PCR using the designed primers pGmTEl-F (5' - AGGAAGCTTGAAGAGTCCGTTACCTTAAATGATTG) and pGmTEl-R (5' - AGGGGATCTAATACCTTCTTAAATGATTG). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 2.5 Kb in size was cloned into T-vector pMD19 (TaKaRa). The 2.5 kb promoter was digested by HindIII and BamHI from the T-vector, and its nucleotide sequence (SEQ ID NO: 3) was confirmed by sequencing using standard procedures.

(4) Cloning of promoter pAtTE1 of Mei2-like gene from Arabidopsis thaliana

The 5' end sequence of AtTE 1 was amplified by PCR using the designed primers pAtTE1-F (5' - AGGAAGCTTACCTTCTGTTAACAAGAG) and pAtTE1-R (5' - AGGGGATCTAATACCTTTCCCTTTCTTTCA). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 1.7 Kb in size was cloned into T-vector pMD19 (TaKaRa). The 1.7 kb promoter was digested by HindIII and BamHI from the T-vector, and its nucleotide sequence (SEQ ID NO: 5) was confirmed by sequencing using standard procedures.

(5) Cloning of promoter pAtML1 of Mei2-like gene from Arabidopsis thaliana

The 5' end sequence of AtML1 was amplified by PCR using the designed primers pAtML1-F (5' - AGAAAGCTTACCTAGCCCTATGAGCTTAAC) and pAtML1-R (5' - AGAGGATCCAACCTAAATGCCCTCTG). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 3.2 Kb in size was cloned into T-vector pMD19 (TaKaRa). The 3.2 kb promoter was digested by HindIII and BamHI from the T-vector, and its nucleotide sequence (SEQ ID NO: 7) was confirmed by sequencing using standard procedures.
(6) Cloning of promoter pBrTEl of Mei2-like gene from Brassica rupa

The 5' end sequence of BrTEl was amplified by PCR using the designed primers pBrTEl-F (5’- AAGCTTCCCCAATTTAATCGAACC) and pBrTEl-R (5’-GGATCCCTTTAATTATTTTCTTACGGA). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 2.2 Kb fragment was cloned into T-vector pMD19 (TaKaRa). The 2.2 kb promoter was digested by HindIII and BamHI from the T-vector, and its nucleotide sequence (SEQ ID NO: 8) was confirmed by sequencing using standard procedures.

(7) Cloning of promoter pOsFCA of FCA homologous gene from rice

The 5' end sequence of OsFCA was amplified by PCR using the designed primers pOsFCA-F (5’- AAGCTTGGGATGTTAGTCTCGA) and pOsFCA-R (5’-GGATCCGTGGGTGGAGGGGGAGGTGGG). The PCR conditions were: 95 °C for 5 min, then 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then extend at 72 °C for 5 min. The resulting PCR product of about 2.2 Kb fragment was cloned into T-vector pMD19 (TaKaRa). Since there's a HindIII site in this DNA fragment, the 2.2 Kb promoter was obtained by a BamHI digestion following with a partial digestion of HindIII. The nucleotide sequence of the promoter (SEQ ID NO: 9) was confirmed by DNA sequencing using standard procedures.

Example 2

Artificial synthesis of the auxin synthase genes

The coding sequences and terminators of the auxin synthase genes were synthesized artificially by Shanghai Sangon Limited, China. The genes from Agrobacterium tumefaciens and Pseudomonas syringae are Ps-iaaM (SEQ ID NO: 11) and Ag-iaaM (SEQ ID NO: 12). The encoded amino acid sequences of these auxin synthase genes are listed in SEQ ID NOs: 14 and 15 respectively. The restriction sites of BamHI and HindIII were added to its 5' and 3' end of the gene respectively. The two designed genes (SEQ ID NO: 11 and SEQ ID NO: 12) were then synthesized artificially.
Similarly, gene Os-YC from the key enzyme of auxin synthesis was also obtained by gene synthesis, and has a BamHl site in the 5' end and a KpnI and a HindIII site on the 3' end. The Os-YC's nucleotide sequence is SEQ ID NO: 13 (including the terminator).

**Example 3**

Construction of vectors harboring T-DNA with the expression cassettes of the auxin synthase genes

pCambia 1300 was used to construct Agrobacterium-mediated transformation vectors for plants. The promoter was digested with HindIII and BamHl, and then ligated using a 3-way ligation reaction with the auxin synthase genes predigested with BamHl and KpnI, and the pCambia 1300 predigested with HindIII and KpnI. The promoter fragments are pOsTEl, pZmTEl, pGmTEl, pAtTEl, pAtMLl and pBrTEl. The auxin synthase gene fragments are Ag-iaaM and Ps-iaaM. The resulted T-DNA constructs were separately named as:

1) pCambial 300-pOsTE 1-Ag-iaaM
2) pCambial 300-pOsTE 1-Ps-iaaM
3) pCambial300-pZmTEl-Ag-iaaM
4) pCambial300-pZmTEl-Ps-iaaM
5) pCambial 300-pGmTE 1-Ag-iaaM
6) pCambial 300-pGmTE 1-Ps-iaaM
7) pCambial 300-pAtTE 1-Ag-iaaM
8) pCambial 300-pAtTE 1-Ps-iaaM
9) pCambial300- pAtMLl -Ag-iaaM
10) pCambial 300- pAtMLl -Ps-iaaM
11) pCambial 300- pBrTE 1-Ag-iaaM
12) pCambial 300- pBrTEl -Ps-iaaM

**Example 4**
Rice transformation

Rice transformation via Agrobacterium-mediated method is well established in the art (Lu & Gong (1998) Chinese Bulletin of Life Sciences 10: 125-131 and Liu et al. (2003) Molecular Plant Breeding 1: 108-115). The cultivar Oryza sativa (japonica) "Xiushui 134" was used in transformation. The single clones of Agrobacterium strain LBA4404 containing the binary vectors of pCambial300-pOsTEl-Ag-iaaM, pCambial300-pOsTEl-Ps-iaaM, pCambial300-pZmTEl-Ag-iaaM and pCambial300-pZmTEl-Ps-iaaM, respectively, were separately cultured for infecting calli. The prepared calli were immersed in the bacteria cell suspension (OD595~0.4, containing 20mg/L of acetosyringone) for 25 min. Then, the calli were transferred to the co-culture medium (containing 20 mg/L of acetosyringone) and incubated for 2-3 days in the dark at 28°C. After co-cultivation, the callus tissues were washed with sterile water and then transferred to selective medium with an appropriate concentration of hygromycin for two months (successively cultured one time in the middle time of this time frame). After selection, the vigorously growing transgenic calli were transferred to pre-differentiation medium for an incubation of about 20 days. Then, the pre-differentiated calli were transferred to the differentiation medium and incubated for differentiating and sprouting with a photoperiod of 14h per day. After 2-3 weeks, the resistant regenerating plantlets were transferred to the rooting medium. The well-grown regenerated plantlets were washed of excess agar and transplanted to soil in a greenhouse. Approximately 100 independent T0 rice transformants were generated for each construct. The events with high yield, big seeds, or high biomass traits, which can increase rice output, were chosen for breeding new varieties.

Example 5

Corn transformation

pCambial300-pZmTEI-Ps-iaaM, respectively, was prepared to infect the immature embryos of corn Hi-II of 8-10 days after fertilization (1.0—1.5 mm). The immature embryos were incubated with the Agrobacterium for 2-3 days at 22 °C on the co-culture medium (MS, 2 mg/L 2,4-D, 30 g/L sucrose, 8 g/L agar of sigma 7921, 40 mg/L acetylsyringone), and then were moved to callus induction medium (MS, 2 mg/L 2,4-D, 30 g/L sucrose, 2.5 g/L gelrite, 5 mg/L AgNO3, 200 mg/L timentin) for an incubation of 10—14 days at 28 °C in the dark. For selection of transformed cells, the calli were moved to selection medium (the same gradients with callus induction media) containing 50 mg/L hygromycin. After 2-3 weeks selection, all the tissues were transferred onto fresh selection medium for another 2-3 weeks. The surviving embryogenic tissues were moved to regeneration medium (MS, 30 g/L sucrose, 0.5 mg/L KT, 2.5 g/L gelrite, 200 mg/L timentin) and cultured for 10-14 days at 28 °C in the dark. Then they were transferred onto fresh regeneration media for another 10-14 days at 26 °C in the light. The fully developed plants were then moved to rooting medium (1/2 MS, 20 g/L sucrose, 2.5 g/L gelrite, 200 mg/L timentin) and cultured at 26 °C in the light until the roots grew well. The plantlets survived were moved to green house for growth to produce seeds.

**Example 6**

Evaluation of transgenic plants

Total of 55 independent transgenic corn events were obtained from transformation of pCambial300-pZmTEI-Ps-iaaM. In these transgenic events, IAA synthase from *Pseudomonas syringae* was under the control of TEl promoter of Zea mays. Four events were evaluated for their yield enhancement potential. The weight of the ears of the transgenic plants was 5%, 8%, 10%, and 11% more than the null segregants, respectively. The plant height was also about 5, 15, 24 and 28 cm taller than the null segregants.

A total of 40 independent transgenic corn events were obtained from transformation of pCambial300-pOsTEI-Ps-iaaM. In these transgenic events, IAA synthase from *Pseudomonas syringae* was under the control of TEl promoter of Oryza sativa. Events OA-3, OA-19, and OA-26 were evaluated for their yield enhancement
potential. The weight of the ears of the transgenic plants was 9%, 12%, and 8% more than the null segregants, respectively. The plant height was also about 5, 11 and 14 cm taller than the null segregants.

Total of 51 independent transgenic rice events were obtained from transformation of pCambia300-pOsTE1-Ps-iaaM. In these transgenic events, IAA synthase from *Pseudomonas syringae* was under the control of TE1 promoter of *Oryza sativa*. Among the 51 events, event OsA-29 was the only one that showed phenotype of yield enhancement. OsA-29 has significantly bigger seeds, average of 31 mg, compared to 26 mg of the non-transgenic control rice.

Example 7

Canola transformation

The methods for canola transformation is well established (Gopalan Selvaraj and Igor Kovalchuk, 2011, New Biotechnology 29: 144-155; Wang, 2006, Agrobacterium transformation Protocols Second Edition Volume 1. Humana Press). The method is as follows: seeds of winter canola or spring canola sterilized by 20% NaClO were germinated on MS medium (30 g/L sucrose and 6 g/L agar) for 8-day. The hypocotyls from the 8-d old seedlings were cut into about 0.5-1.0 cm pieces and pre-conditioned for 3 d on callus induction medium (MS, 1 mg/L 2,4-D) at 25 °C. A single colony of *Agrobacterium* stain LBA4404 harboring binary vector pCambia300-pAtTE1-Ag-iaaM, pCambia300-pAtTE1-Ps-iaaM, pCambia300- pAtML1-Ag-iaaM, pCambia300- pAtML1-Ps-iaaM, pCambia300-pBrTE1-Ag-iaaM and pCambia300- pBrTE1-Ps-iaaM separately was cultured in YEP liquid medium with antibiotics until the concentration of cell suspension reached to \( \text{OD}_{600} = 1.0 \). The *Agrobacterium* was then pelleted by centrifugation and resuspended in MS liquid medium (containing 10 mg/L acetylsyringone) to a final concentration of \( \text{OD}_{600} = 0.2 \). Then, the preconditioned hypocotyls were immersed in the prepared *Agrobacterium* cell suspension for 5 min, blotted on sterile filter paper and transferred onto callus induction medium (MS, 1 mg/L 2,4-D, 10 mg/L acetylsyringone) for cocultivation for 3d at 22 °C. After that, they were transferred to the selection medium (MS, 3 mg/L 6-BA, 5 mg/L AgNO\(_3\), 400 mg/L timentin, 50 mg/L hygromycin) and cultured for 15 days. The selected explants were
transferred to differentiation medium (MS, 5 mg/L BAP, 5 mg/L AgNO₃, 200 mg/L timentin, 10 mg/L hygromycin) until shoot regeneration. When the shoots were 5-10 mm in length, they were cut and transferred to shoot elongation medium (MS medium containing 0.05 mg/L BAP) Shoots of about 2 cm in length were transferred to the rooting MS medium for root induction. The rooted shoots were transplanted to soil in the greenhouse.

Example 7

Wheat transformation

There are different methods for wheat transformation described specifically in various research papers including, but not limited to Wang et al., 2002, Acta Genetica Sinica 29: 260-265; Cheng et al., 1997, Plant Physiol 115: 971-980; Supartana et al., 2006, Journal of Bioscience and Bioengineering, The Society for Biotechnology, Japan 102: 162-170. The following is a brief description of the wheat transformation method via Agrobacterium.

The ordinary cultivars of winter wheat (Triticum aestivum L.), BAU170 and BAU146, were used in transformation. Immature caryopses 12-15 days after anthesis were collected and sterilized using 0.1% HgCl for 10 min. After washing in sterile water for 3-4 times, the immature embryos were peeled from the seeds on a benchtop and placed on calli-inducing MS medium with scuteHums upside for incubation at 25°C in the dark for calli induction which were subcultured on the same medium for 20 days.

Single colonies of Agrobacterium strain LBA4404 harboring pCambial300-pOsTEI-Ag-iaaM, pCambial300-pOsTEI-Ps-iaaM, pCambial300-pZmTEI-Ag-iaaM, and pCambial300-pZmTEI-Ps-iaaM, respectively, were separately cultured in 5ml YEB liquid medium containing the selective antibiotics and shaken on an orbital shaker with 200 rpm at 28°C overnight. 0.5ml of above bacterial solution was added to 50ml YEB medium containing the same antibiotics. When the bacteria grew to OD₆₀₀ = 0.6, the cultures were centrifuged at 5000 rpm for 5min. The bacteria cells were collected and resuspended in MS liquid medium (MS with 3% sucrose, pH 5.4) after being washed by the medium twice. The bacteria concentration was adjusted to OD₆₀₀ = 0.1-1.0. Before infection, part of the peeled immature embryos or the calli derived from the embryos
were soaked into high osmotic MS medium containing 0.4 mol/L mannitol for pre-incubation for 12h or placed on the MS medium containing 200 mmol/L acetosyringone (AS) for an 3d pre-incubation. Then, the immature embryos or the calli were infected with the Agrobacterium in the cell suspension for 0.5-3.5 h. After the bacteria liquid being discarded, the explants were dried using sterile filter paper and transferred to co-culture medium (containing 200 mmol/L AS) for an incubation of 3 days at 26-28 °C in dark. Then, the explants were transferred to selection medium with containing 50 mg/L hygromycin. After being further selected on the selection medium containing 350 mg/L Cef and 50 mg/L hygromycin for 4-8 weeks at 26-28°C in the dark, the resistant calli were transferred to the differentiation medium (MS, 10 mg/L indoleacetic acid (IAA), 1.0 mg/L ZT, 3% sucrose, 0.7% agar, pH 5.8) containing 350mg/L Cef and 3-5 mg/L hygromycin and induced for differentiation in light. The plantlets derived by differentiation were transplanted to the rooting medium for root development.

Example 8

Soybean transformation

The genetically transformation method for soybean is well established in the art (Kan Wang, 2006, Agrobacterium transformation Protocols, Second Edition, Volume 1, Humana Press; Ma et al. (2008) Scientia Agricultura Sinica 41(3): 661-668). The detailed description of soybean transformation method is as following. The healthy, plump and mature soybeans were surface-sterilized with chlorine gas inside a bell jar under a fume hood. Seeds were kept in Petri dishes with chlorine gas produced by pouring 100mL of 4% sodium hypochlorite into a beaker and adding 5mL of 12N hydrochloric acid. The sterile soybeans were sowed into B5 medium and incubated at 25°C with a photoperiod of 18h light and 6h dark for pre-germination. When the cotyledon turned green and the first euphylla had not grown out completely, the seed husk, the root and the prophyll were removed. The remaining cotyledon with 3-5cm of hypocotyl was cut in the middle vertically. The resulted two pieces of explant materials with one cotyledon, half hypocotyl and half epicotyl of each were planted in the co-culture medium (1/10 B5 major and trace elements, 1/10 MS Fe salts, B5 vitamins, 3% sucrose, 1 mg/L BA, 200 μmol/L acetosyringone, pH 5.4) for an pre-incubation with the cotyledon and embryonic
tip upside.

Single colonies of Agrobacterium strain LBA4404 containing vector pCambial300-pGmTE 1-Ag-iaaM, pCambia 1300-pGmTE 1-Ps-iaaM, pCambia 1300-pGmFCA-Ag-iaaM and pCambia300-pGmFCA-Ps-iaaM, respectively, were separately cultured till the cell suspension reached to a concentration of OD₆₀₀°=0.8-1.0. After centrifugation for 10 min, the cells were collected and resuspended in the co-culture medium. The explants pre-incubated for 24h were transferred to the prepared Agrobacterium suspension for approximately 30 minute. The infected explants were plated adaxial or wounded side down on the filter paper overlaying co-culture medium and incubated at 25°C for 3 days.

Then, the explants were washed off the excess bacteria and transferred to shoot-inducing medium (B5 major and trace elements, MS Fe salts, 3% sucrose, 1.68 mg/L BAP, 400 mg/L timentin, 50mg/L hygromycin, 2.5g/L gelrite, pH 5.6) for a 14-day incubation at 25°C. After another 14-day incubation in the shoot-inducing medium containing 50mg/L hygromycin, the cotyledon and the dead tissue of the explants were cut off, and the explants were transferred to shoot-elongation medium (MS major and trace elements and Fe salts, B5 vitamins, 3% sucrose, 0.1 mg/L IAA, 0.5 mg/L GA3, 1mg/L ZR, 200 mg/L timentin, 2.5g/L gelrite, pH 5.6) for a 14-day incubation. Finally, the healthy shoots with at least 3 leaves were transferred to the rooting medium (1/2 B5, MS Fe salts, 2% sucrose, 1 mg/L IBA, pH 5.6) for root development.

The invention used many techniques in molecular biology, biochemistry and tissue culture. These techniques are available in the art. Detailed methods of the techniques can be referenced in Current Protocols in Molecular Biology (ed. by Ausubel, John Wiley and Sons Press) and Molecular Cloning: A Laboratory Manual, 3rd ED (ed. by J. Sambrook, Cold Spring Harbor Laboratory Press (2001)).

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.
Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

<table>
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<tr>
<th>SEQ ID NO:</th>
<th>description</th>
<th>Note</th>
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<td>1</td>
<td>Promoter of Oryza sativa TE1</td>
<td>DNA</td>
</tr>
<tr>
<td>2</td>
<td>Promoter of Zea mays TE1</td>
<td>DNA</td>
</tr>
<tr>
<td>3</td>
<td>Promoter of Glycine max TE1</td>
<td>DNA</td>
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<tr>
<td>4</td>
<td>Promoter of Glycine max TE2</td>
<td>DNA</td>
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<td>5</td>
<td>Promoter of Arabidopsis thaliana TEL1</td>
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<td>6</td>
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<td>7</td>
<td>Promoter of Arabidopsis thaliana AtML1</td>
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<td>9</td>
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<td>DNA</td>
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<td>12</td>
<td>Agrobacterium tumefaciens IAAM</td>
<td>Artificial synthesis cDNA</td>
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<tr>
<td>13</td>
<td>Oryza sativa flavin-containing monooxygenase-like (Os-YC)</td>
<td>Artificial synthesis cDNA (including the terminator)</td>
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<td>14</td>
<td>Pseudomonas syringae IAAM</td>
<td>Protein</td>
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<tr>
<td>19</td>
<td>Arabidopsis thaliana YUCCA family monooxygenase (YUC1)</td>
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THAT WHICH IS CLAIMED:

1. A method for increasing plant yield in a plant of interest, said method comprising transforming said plant with a DNA construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence wherein said auxin synthase coding sequence is selected from the group consisting of:
   i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
   ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO:11, 12, or 13;
   iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
   iv) a nucleotide sequence that encodes an amino acid sequence having at least 40% sequence identity to any one of SEQ ID NOs: 14-19, wherein said promoter is a promoter from a plant Mei2-like gene.

2. The method of claim 1, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO:1 - SEQ ID NO: 10.

3. The method claim 1 or 2, wherein said auxin synthase coding sequence is from a microorganism or from a plant.

4. The method of any one of claims 1-3, wherein plant is selected from the group consisting of rice, corn, cotton, wheat, barley, soybean, sunflower, canola, and sorghum.

5. An expression cassette comprising a DNA construct, said construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence, wherein said auxin synthase coding sequence is selected from the group consisting of:
   i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
   ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO:11, 12, or 13;
iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
iv) a nucleotide sequence that encodes an amino acid sequence having at least 40% sequence identity to any one of SEQ ID NOs: 14-19,
wherein said promoter is a promoter from a plant Mei2-like gene.

6. The expression cassette of claim 5, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO:1 - SEQ ID NO:10.

7. The expression cassette of claim 5 or 6, wherein said auxin synthase coding sequence is from a microorganism or from a plant.

8. A plant transformed with the expression cassette of any one of claims 5-7.

9. A transformed seed of the plant of claim 8.

10. A transformed plant that exhibits increased expression of auxin synthase in its shoot meristem and inflorescence as compared to a control plant, said plant having been transformed with a DNA construct comprising a promoter that drives expression in a plant shoot meristem and inflorescence operably linked to an auxin synthase coding sequence, wherein said auxin synthase coding sequence is selected from the group consisting of:
i) the sequence set forth in SEQ ID NO: 11, 12, or 13;
ii) a sequence having at least 60% sequence identity to the sequence set forth in SEQ ID NO:11, 12, or 13;
iii) a nucleotide sequence that encodes the amino acid sequence set forth in any one of SEQ ID NOs: 14-19; and,
iv) a nucleotide sequence that encodes an amino acid sequence having at least 40% sequence identity to any one of SEQ ID NOs: 14-19,
wherein said promoter is a promoter from a plant Mei2-like gene.
11. The transformed plant of claim 10, wherein said promoter comprises a sequence selected from the sequences set forth in SEQ ID NO:1 - SEQ ID NO:10.

12. The transformed plant of claim 10 or 11, wherein said auxin synthase coding sequence is from a microorganism or from a plant.

13. Transformed seed from the plant of any one of claims 10-12.

14. The transgenic plant of any one of claims 10-13, wherein said plant is selected from the group consisting of rice, corn, cotton, wheat, barley, soybean, sunflower, canola, and sorghum.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

See the extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC: C12N.A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Database: DWPI, SipoABS, CNABS, Cntxt, ISI WEB OF KNOWLEDGE(Biosis, medline, Embase), CNKI, Genbank;

Search terms: auxin synthase, tryptophan monoxygenase, iaa, mei2-like, tel, gene, promoter, plant, sequence search on the sequence of SEQ ID NO: 1-11, 14

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>A</td>
<td>WO20040000152A(BASF PLANT SCIENCE GMBH), 31 Dec.2003(31.12.2003), see the description, paragraphs 26-27 and 58, claims 14 and 16</td>
<td>1-14 (all partly)</td>
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<td>A</td>
<td>CN10217933A(PLASTID AS), 27 Jul.2011(27.07.2011), see the whole document</td>
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<td>Genbank accession no.P06617, 01 Feb.1991(01.02.1991), see the whole document</td>
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</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  
  "A" document defining the general state of the art which is not considered to be of particular relevance
  
  "E" earlier application or patent but published on or after the international filing date
  
  "L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)
  
  "O" document referring to an oral disclosure, use, exhibition or other means
  
  "P" document published prior to the international filing date but later than the priority date claimed

  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  
  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  
  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 Mar. 2013 (18.03.2013)

Date of mailing of the international search report

18 Apr. 2013 (18.04.2013)

Name and mailing address of the ISA/CN

The State Intellectual Property Office, the P.R.China

6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088

Facsimile No. 86-10-62019451

Form PCT/ISA/210 (second sheet) (July 2009)

Authorized officer

PAN, Junyu

Telephone No. (86-10)62411086
**INTERNATIONAL SEARCH REPORT**

<table>
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<tr>
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<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
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<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
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<td>1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:</td>
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<td>2. ☑ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
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<td>3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
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<tr>
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<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
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<td>1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
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<td>2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.</td>
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<td>3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
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<td>4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-14 (all partly) related to SEQ ID NO: 11 and 14.</td>
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**Remark on protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
## INTERNATIONAL SEARCH REPORT

**Information on patent family members**

<table>
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<th>Patent Documents referred in the Report</th>
<th>Publication Date</th>
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<td>AU2003238286A1</td>
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Form PCT/ISA /210 (patent family annex) (July 2009)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

C12N15/82(2006.01)i
C12N15/53(2006.01)i
C12N5/04 (2006.01)i
A01H4/00 (2006.01)i

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This Authority considers that there are 7 inventions covered by the claims 1-14 indicated as follows:

I: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a sequence set forth in SEQ ID NO: 11 (which encodes an amino acid sequence set forth in SEQ ID NO: 14);

II: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a sequence set forth in SEQ ID NO: 12 (which encodes an amino acid sequence set forth in SEQ ID NO: 15);

III: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a sequence set forth in SEQ ID NO: 13;

IV: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NO: 16;

V: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NO: 17;

VI: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NO: 18;

VII: claims 1-14 (all partly) directed to a method for increasing plant yield in a plant of interest, an expression cassette, a transformed plant, a transformed seed of the plant, which relate to a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NO: 19.

The sequences of the 7 inventions are different from each other, i.e. they do not process a common structure. Therefore, the 7 inventions relating to different sequences do not share a same or corresponding technical feature, obviously, they do not share a same or corresponding special technical feature that makes a contribution over the prior art. The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1.