

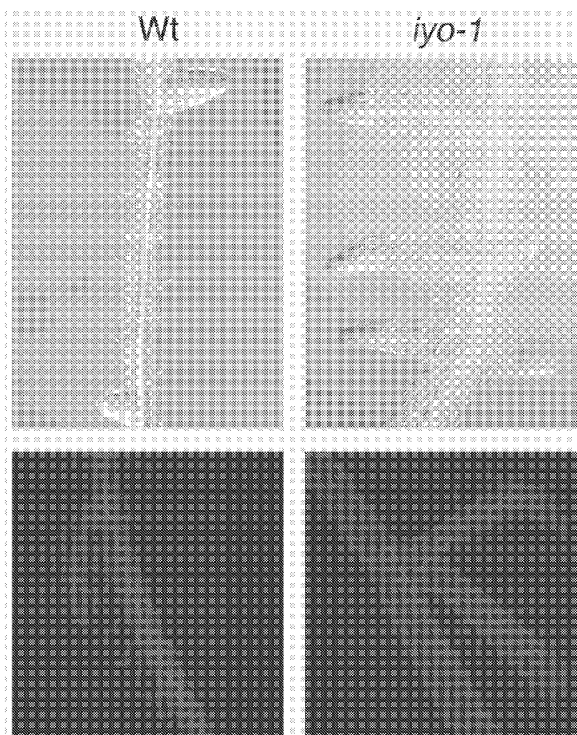


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(54) Title: PROCESS FOR MODIFYING THE ARCHITECTURE AND IMPROVING THE YIELD OF CROP PLANTS

FIG. 2



(57) Abstract: This invention identifies the plant MINIYO (IYO) gene and the AtRTR1 gene which are essential for the initiation of cell differentiation in all plant meristems and in embryogenesis. This invention relates methods for generating transgenic plants in which expression of the IYO and/or At RTR1 genes or their orthologous genes is modified to advancing or delaying the onset of differentiation in one or more meristems of the plant.



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**PROCESS FOR MODIFYING THE ARCHITECTURE AND IMPROVING THE YIELD
OF CROP PLANTS**

This invention lies within the technical field of agriculture.
5 The various aspects of the invention can also be applied to
the crop productivity for food and feed production as well as
biomass production for the energy sector, specifically the
development of transgenic plants for use in obtaining
biofuels, such as in the production of bioethanol.

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STATE OF THE ART

Plant architecture has a marked effect on the yield of plants.
This influence is exerted through photosynthetic potential,
15 the transfer of assimilated materials and nutrients to the
different organs of the plant, and finally manifests itself in
the potential growth and yield of the plant.

Cell differentiation is a fundamental process in all organisms
20 and, together with cell proliferation, dictates the size and
shape of the organisms (Ramirez-Parra *et al.*, *Int. J. Dev.*
Biol. 2005). Thus, modification of plant architecture and
yield depend at the most fundamental level on regulating cell
proliferation and differentiation. The molecular mechanisms
25 controlling the cell cycle in plants are well known (De
Veylder *et al.*, *Nat. Rev. Mo. Cell Biol.* 2007) and are largely
conserved relative to the mechanisms that control this cycle
in other eukaryotes. Many of the methods for modifying the
architecture of plants are based on controlling cell
30 proliferation, for example by altering the dormancy/activation
of buds (*TCP* genes) or altering the balance of proliferation
in meristems or organ primordia (*STM*, *WUS*, *AS1*, *SWP* genes).

On the other hand, the mechanisms responsible for initiating
35 cell differentiation are very poorly understood. In addition,
it is not known which genetic factors initiate cell
differentiation. In animals, it is assumed that the start of
differentiation involves a transcriptional re-ordering which

makes it possible to activate developmental programmes which are silenced in progenitor stem cells. There is evidence that silencing of these programmes is due to blocking the productive transcriptional elongation of developmental regulatory genes (Guenther *et al.*, Cell 2007; Stock *et al.*, Nature Cell Biol. 2007), but the factors which activate this elongation upon differentiation have not been identified.

An example of manipulating plant architecture and crop yield is disclosed in US 2009/0320163 which describes a method for obtaining transgenic plants whose plant architecture has been modified through alterations in the expression of PDR genes (Plant Developmental Regulators). These genes have similarities with phosphatidyl ethanolamide binding proteins (PEBPs), which act as inhibitors in the signalling cascades of MAP kinases, with the result that transgenic plants with a greater number of seeds, a better plant foliage architecture, stronger stems and a larger plant biomass in general are obtained.

Another specific example is transgenic *Arabidopsis* plants which have reduced levels of expression of *AtMago* mRNA (RNAi-*AtMago* plants). It is known that the *Mago Nashi* gene is involved in organisation of apical and root meristems, but not floral meristems, and also affects the formation of pollen and the development of seeds in *Arabidopsis* (Nam-Il *et al.* Plant Science 176 (2009) 461-469). RNAi-*AtMago* plants generally presented delayed vegetative growth, producing a larger number of leaves of smaller size, apical meristems with excessively vacuolated cells and large intercellular spaces giving rise to shorter and branched stems, smaller root meristems and shorter lateral roots with premature differentiation of root hairs. RNAi-*AtMago* plants also show reduced pollen production and germination, occasionally giving rise to non-viable seeds.

At the present time, there are many methods available for modifying root and above-ground architecture and improving crop yields, including modifying the size of meristems. These

methods are based on changing the expression of genes that have specific effects in each meristem, for example in shoot apical meristems through altering the expression of genes such as *STM*, *CLV3* or *WUS*, in root apical meristems through altering the expression of genes such as *PLT*, *SHY2* or *RGF1*, and in general modifying the synthesis, transport and signalling of auxins and cytokinins.

Nevertheless, to the best of our knowledge, in the state of the art, no gene has been identified which acts directly to switch on cell differentiation. The decision as to whether to differentiate or not is shared by cells of all the meristems throughout the plant as well as in the developing embryo, which means that identification of a conserved genetic switch controlling this fate decision makes it possible to control cell differentiation throughout the plant in a targeted way. This includes, for example, control during embryogenesis and in the apical, floral and vascular meristems. Modulation of this unique switch is thus a novel mechanism, directly applicable to all the meristems of the plant and in embryos, which makes it possible to activate, block or delay the initiation of differentiation in a highly specific way.

The present invention identifies this common switch which initiates cell differentiation. The invention is aimed at methods for generating transgenic plants with altered cell differentiation and at transgenic plants obtained through such methods. Plants with altered cell differentiation are desirable tools in agriculture as they can be used to increase yield and the present invention is aimed at addressing the need for more productive crop plants.

BRIEF DESCRIPTION OF THE INVENTION

The invention relates to methods for improving the root and above-ground architecture of plants to obtain better crop yields. According to the invention, the *MINIYO* and *RTR1* genes are good tools for genetic manipulation to control the timing

of the onset of differentiation in embryogenesis and in all of the meristems of the plant. According to the invention, *MINIYO* and *RTR1* nucleic acid sequences can therefore be used to regulate the size and number of plant embryos, meristems and the organs generated from them. *MINIYO* and *RTR1* interact and regulate the activity of RNA polymerase II (Pol II), and are jointly involved in the activation of transcriptional elongation and the expression of growth programmes which control the initiation of cell differentiation in the plant.

5 The transgenic plants according to the invention differ from the parent plants in that they have an increase or decrease in the expression and/or genetic activity of *MINIYO* and/or *RTR1*, including the *Arabidopsis thaliana AtMINIYO* and *AtRTR1* genes or their orthologues in other plant species. This gives rise to advance, delay or blocking of the initiation of differentiation in apical, floral and/or root meristems, thereby modifying the number and size of meristems and/or the number and size of the organs generated from them.

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20 Preferably, the transgenic plants according to the invention have partly or wholly reduced expression of the *MINIYO* and/or *RTR1* genes, including *AtMINIYO* and *AtRTR1* or their orthologues in other plant species, in such a way that there is an improvement in their plant architecture which leads to improved crop yields in comparison with the wild plants. For example, the transgenic plants have meristems with increased size (greater stem thickness), and they also have ectopic meristems giving rise to additional inflorescences, multiple flowers and/or a large number of side roots and seeds with double embryos.

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In a first aspect, the invention relates to an isolated nucleic acid sequence comprising a nucleotide sequence encoding for an amino acid sequence of SEQ ID NO: 5 or an orthologue thereof.

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In a second aspect, the invention relates to an isolated nucleic acid sequence comprising a nucleotide sequence encoding for amino acid sequence of SEQ ID NO: 11 or an orthologue thereof.

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In a further aspect, the invention relates to an expression vector comprising one or more of the isolated nucleic acid sequence(s) of the invention.

10 In another aspect, the invention relates to a transgenic plant wherein the activity of a MINIYO and/or RTR1 polypeptide is inactivated, repressed or down-regulated.

In an additional aspect, the invention relates to a transgenic
15 plant wherein the activity of a MINIYO and/or RTR1 polypeptide is increased or up-regulated.

In a further aspect, the invention relates to a use of a
MINIYO and/or RTR1 polypeptide to control the initiation of
20 cell differentiation in plant apical, root and/or floral meristems.

In an additional aspect, the invention relates to a use of a
MINIYO and/or RTR1 polypeptide to delay the initiation of cell
25 differentiation in plant apical, root and floral meristems.

In another aspect, the invention relates to a method for
delaying the onset of cell differentiation and increasing the
number of undifferentiated cells in a plant said method
30 comprising decreasing the activity of a MINIYO and/or RTR1
polypeptide.

In another aspect, the invention relates to a method for
increasing cell differentiation in a plant said method
35 comprising increasing the activity of a MINIYO and/or RTR1
polypeptide.

In further aspect, the invention relates to an isolated nucleic acid sequence comprising SEQ ID No. 48 or SEQ ID No. 49 and uses thereof to direct spatial and temporal expression of target genes.

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Also included are methods of producing transgenic plants with altered activity of a *MINIYO* and/or *RTR1* polypeptide and method of increasing yield by decreasing the activity of activity of a *MINIYO* and/or *RTR1* polypeptide.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

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The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature.

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The basis of this invention is the characterisation of the *AtMINIYO* (At4g38440) and *AtRTR1* (At5g26760) genes which comprise a highly conserved, common molecular switch that initiates differentiation in all plants. The acronym *IYO* is used herein to refer to the *AtMINIYO* gene.

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We have identified and characterised the mutant *miniyo-1* (*iy0-1*) which shows a delay in the initiation of cell differentiation (Figures 1 and 2). Through positional mapping, we have shown that phenotypes with delayed differentiation in

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iyo-1 plants are due to a point mutation in the *IYO* gene (At4g38440, Figure 3A-B). The *iyo-1* allele causes a change in amino acid 962 from a glycine to a glutamate residue. This disrupts an RGG RNA-binding motif which is strictly conserved in all *MINIYO* orthologues (Figures 3B and 18a). This mutation gives rise to a partial loss of activity in the *IYO* gene and results in delayed onset of differentiation in all the meristems of the plant (Figure 1). As a consequence, ectopic stem cells are generated (Figures 1A-E, 2) and these give rise to new shoot and root meristems, new floral meristems (double and triple flowers), adjacent groups of stomas (Figure 1F-I) and a larger number of lateral roots, including in positions in which they do not develop in wild-type plants (Figure 2). Total blocking of *IYO* activity causes embryogenesis to be arrested at early stages (Figure 3E-F).

In addition, the combination of the hypomorphic *iyo-1* allele with null alleles (*iyo-2* or *iyo-3*) gives rise to the formation of seeds with double embryos (Figures 1J and 3D) which mature and upon germinating give rise to plants with multiple meristem poles which generate rudimentary leaves (Figure 1K). Also in the case of the *iyo-1* allele there are cases of double embryos which develop in a similar way to wild-type embryos (Figure 1L).

Expression of the *IYO* gene is regulated at the transcriptional and post-transcriptional level to direct its activity specifically to the periphery of the meristems where differentiation begins (Figures 4 and 5). The promoter of the *IYO* gene is exclusively active in developing seeds, in meristems and in cells in the early stages of differentiation (Figures 4A-B, 5C-E). Moreover, specific signal sequences present in the *IYO* protein restrict nuclear accumulation of the protein exclusively to cells which are about to begin differentiation (Figures 4B-F, 5G-I). The protein is excluded from the nucleus in proliferating meristematic cells through active Exportin1-dependent export, as it is inhibited by treatment with leptomycin B (Figure 5j).

The overexpression of *IYO* or its fusions with proteins or peptides such as GFP, HA, FLAG under the control of the constitutive 35S promoter leads to premature differentiation in meristems, including in the rib meristem with the consequent shortening of internodes and the compaction of inflorescences (Figure 6B-C), and even to meristem consumption (Figure 7).

The *IYO* gene codes for a protein which interacts physically with RNA polymerase II (Pol II) and with elongation complexes (Figure 8A-B, G-I) and which activates transcriptional elongation (Figure 8C, E-F) and the expression of development programmes directing differentiation (Figures 8C-D, 6A).

Through the analysis of co-expressed genes, we identified the gene At5g26760 (*AtRTR1*) which has a Pearson correlation coefficient of $r = 0.625$ with *IYO*. At5g26760 codes for a protein which is highly conserved in all plant species, is homologous to the RTR1 protein from *Saccharomyces cerevisiae*, and also has homologues in animals. RTR1 interacts with Pol II and results have recently been published which indicate that it acts as a transition phosphatase for Pol II in yeast (Mosley *et al.*, 2009). This phosphatase is thought to be involved in the dephosphorylation of serine 5 in the C-terminal domain of the RPB1 (CTD) sub-unit, a modification preceding phosphorylation in serine 2 which is necessary for Pol II to enter into productive elongation. Bearing in mind the homology between At5g26760 and this phosphatase which acts in the transition between initiation and elongation in transcription and its co-expression with the *IYO* gene, which is an activator of transcriptional elongation, we postulate that At5g26760/*AtRTR1* is jointly involved with the *IYO* gene in the activation of transcriptional elongation and the initiation of cell differentiation in plants.

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As a first step in analysing the function of *AtRTR1*, we studied two mutant alleles in the SALK collection. The allele *atrtr1-1* (SALK_012339) has an insertion of T-DNA in the first

exon which gives rise to the total loss of function of *RTR1*. Homozygous *atrtr1-1* plants arrest their growth during early stages of embryogenesis (Figure 9). The arrested embryos have a phenotype which is very similar to strong alleles of *iy0*, comprising an almost total block on cell differentiation and the production of twin embryos from suspensor cells. The *atrtr1-2* allele (SALK_115762) has a T-DNA insertion in the third intron of the gene, which is spliced out but with low efficiency, resulting in very low accumulation of the full-length transcript. The *atrtr1-2* allele results in a partial loss of *AtRTR1* activity and phenocopies almost exactly the weak allele *iy0-1*. *atrtr1-2* plants have delayed onset of differentiation in the different meristems of the plant and therefore maintain ectopic stem cells that generate additional shoot, flower and root meristems (Figure 10A-C) and ectopic meristemoids (Figure 11). Ectopic embryos that germinate and give rise to cloned plants are also generated.

The phenotype of a double *iy0-latrtr1-2* mutant shows a clear interaction between the *IYO* and *AtRTR1* genes in control of the initiation of cell differentiation. These double mutants have a total block on differentiation that gives rise to growth in the form of a mass of undifferentiated cells (Figure 10D). In addition, we have demonstrated that the over-expression of *IYO* in a mutant *atrtr1-2* ecotype does not give rise to any phenotype, demonstrating that the *RTR1* gene is necessary for the function of *MINIYO*.

In order to study the expression of *RTR1*, we generated transgenic plants expressing the promoter of *RTR1* and the first three exons and introns of *RTR1* translationally fused to the UidA (GUS) reporter gene. This construct directs the activity of GUS specifically to meristems and differentiating tissues (Figure 10E), in a pattern which is very similar to that of plants which express GUS under the promoter of *IYO*, which is consistent with the high level of co-expression of the corresponding transcripts and is compatible with the fact that both genes function together and in a coordinated way.

Furthermore, we analysed the pattern of accumulation of AtRTR1 fused to the GFP reporter under the control of the constitutive CaMV 35S promoter . In the same way as IYO-GFP, the fusion product AtRTR1-GFP does not accumulate in the nuclei of undifferentiated cells (Figure 10F), being excluded through active export dependent on Exportin1 (Figures 10F and 12).

Although the bulk of AtRTR1 is present in the cytosol, we found high levels of nuclear fluorescence reconstitution when split YFP fused to IYO and AtRTR1 was expressed in *Nicotiana benthamiana* leaves (Figures 12-14). This confirms that IYO and AtRTR1 interact physically, and that they do so in the nucleus. Moreover, reconstituted YFP stabilizes complexes, and this leads to large accumulation of AtRTR1 in the nucleus, which suggest that complex formation serves to retain AtRTR1 in that compartment. To test this, we analyzed the subcellular distribution of AtRTR1 in the presence or absence of co-expressed IYO. These experiments confirmed that co-expressed IYO increases the accumulation of AtRTR1 in the nucleus.

These results demonstrate that MINIYO and RTR1 form a complex in the nucleus and that they have a common and shared function in combining to initiate cell differentiation by the activation of transcriptional elongation through the interaction and modification of Pol II.

We have shown that downregulation of *MINIYO* and/or *RTR1* genes or their proteins leads to a delayed onset of differentiation, increased meristem size/number and ectopic meristems. Thus, downregulation of *MINIYO* and/or *RTR1* can be useful in increasing plant yield. The term "yield" as described herein relates to yield-related traits. Specifically, these include an increase in biomass and/or seed yield. This can be achieved by increased growth. An increase in yield can be, for example, assessed by the harvest index, i.e. the ratio of seed yield to aboveground dry weight. Thus, according to the invention, yield comprises one or more of: increased seed

yield per plant, increased seed filling rate, increased number of filled seeds, increased harvest index, increased number of seed capsules/pods, increased seed size, increased growth or increased branching, for example inflorescences with more
5 branches. Preferably, yield comprises an increased number of seed capsules/pods and/or increased branching. Yield is increased relative to control plants. An increase in yield may be about 5, 10, 20, 30, 40, 50% or more compared to a control plant.

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In contrast, overexpression of *MINIYO* and/or *RTR1* genes can be used to eliminate branches of the inflorescence meristem in crops where this is useful.

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The invention is therefore based on the generation of plants having either increased or reduced activity of *MINIYO* and/or *RTR1* genes or their proteins, including *AtMINIYO* and *AtRTR1* or their orthologues in other plant species, to advance or delay the onset of differentiation in the meristems, at the required
20 time in each case. Thus, the activity of *MINIYO* and/or *RTR1*, including *AtMINIYO* and *AtRTR1* or their orthologues in other plant species, may be inactivated, repressed or downregulated. In another aspect, the activity of *MINIYO* and/or *RTR1*, including *AtMINIYO* and *AtRTR1* or their orthologues in other
25 plant species, is increased or up-regulated. Transgenic or mutant plants which express *MINIYO* and/or *RTR1* genes, including *AtMINIYO* and *AtRTR1* or their orthologues in other plant species, but where the function of the protein is partly lost, may be obtained according to the various aspects of the
30 invention.

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Alternatively, null mutants are obtained which are transformed with or carry attenuated or mutant versions of *MINIYO* or *RTR1* genes, such as the *iy0-1* alleles and *atrtr1-2* alleles or a
35 combination thereof, or other alleles which have mutated amino acids in regions which are highly conserved in *MINIYO* and *RTR1* proteins, including *AtMINIYO* and *AtRTR1* or their orthologues in other plant species (Figures 18 and 21). The expected

phenotypes are similar to those already observed in *Arabidopsis*, i.e.: larger meristems and directly generated organs (thicker stems), ectopic meristems which give rise to additional inflorescences, duplicated flowers, a larger number
5 of side roots and seeds with double embryos.

Throughout this disclosure, *MINIYO* and *RTR1* are used to refer to the genes homologous/proteins to the *Arabidopsis AtMINIYO* and *AtRTR1* genes/proteins respectively, as described herein.

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Specifically, a skilled person would therefore understand that the invention not only relates to isolated *AtMINIYO* and *AtRTR1* genes/proteins as defined in SEQ ID No. 1, 8, 5 and 11 and their uses in the various aspects of the invention, but that
15 the present invention relates to methods and uses of homologues and orthologues of the *AtMINIYO* or *AtRTR1* genes and their polypeptides in other plant species, including transgenic plants where expressing or activity of such an orthologous gene/protein is increased or decreased.

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Thus, in a first aspect, the invention relates to an isolated nucleic acid molecule or sequence comprising a nucleic acid molecule of SEQ ID No. 1 coding for the *AtMINIYO* protein of SEQ ID No. 5, or its orthologue in another plant species. In
25 another aspect, the invention relates to an isolated nucleic acid sequence or molecule comprising a nucleic acid of SEQ ID No. 8 coding for the *AtRTR1* protein of SEQ ID No. 11 or its orthologue in another plant species.

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As explained herein, said nucleic acid molecule(s) control(s) the initiation of cell differentiation in apical, root and floral meristems of the plant. Preferably, the nucleic acid molecule(s) is/are homologous to the corresponding nucleic acid molecules which code for the *AtMINIYO* proteins of SEQ ID
35 No. 5 or its orthologue in another plant species, and/or the *AtRTR1* protein of SEQ ID No. 11 or its orthologue in another plant species.

In a preferred embodiment of the invention, the nucleic acid molecule is characterised by interacting and/or modifying the RNA polymerase II (Pol II) involved in the activation of transcriptional elongation and the expression of developmental programmes which direct the initiation of cell differentiation in seeds and in all the apical, root and floral and other meristems of the plant. In a preferred embodiment, the protein AtMINIYO or its orthologue in other plant species, and the protein AtRTR1 or its orthologue in other plant species, interact and/or modify the RNA polymerase II (Pol II), and are jointly involved in the activation of transcriptional elongation and the expression of developmental programmes which direct the initiation of cell differentiation in seeds and in all the apical, root and floral and other meristems of the plant.

This invention also protects an isolated nucleic acid comprising a nucleotide sequence coding for an amino acid sequence of the protein IYO and/or the protein AtRTR1, or their orthologues in another plant species, which are at least 30% identical to the sequences coded by SEQ ID NO: 1 and/or SEQ ID NO: 8 to control the initiation of cell differentiation in seeds and in apical, root and floral and other meristems of a plant, and their uses, preferably their use to control the initiation of cell differentiation in seeds and in apical, root and floral meristems of a plant.

Accordingly, the invention relates to an isolated nucleic acid sequence comprising or consisting of SEQ No. 1 or 8 or a homologue, orthologue or functional variant thereof. In one embodiment, the isolated nucleic acid sequence comprises or consists of SEQ No. 1 or 8. In another embodiment, the isolated nucleic acid sequence comprises or consists of a nucleic acid sequence that encodes for an orthologue of the protein identified in SEQ No. 5 or 11.

As used herein, the words "nucleic acid", "nucleic acid sequence", "nucleic acid molecule", "nucleotide", or

"polynucleotide" are intended to include DNA molecules (e.g., cDNA- as is the case for SEQ ID NO: 1 and SEQ ID NO: 8- or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. The skilled person will understand that where the nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term "gene" or "gene sequence" is used broadly to refer to a DNA nucleic acid associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence, or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in combination with regulatory sequences. The sequences may also be synthetically made sequences. The nucleic acid may be wholly or partially synthetic, depending on design.

The term "functional part or functional variant" as used herein refers to a variant gene or polypeptide sequence or part of the gene or polypeptide sequence which retains the biological function of the full non-variant sequence, i.e. acts as a molecular switch to initiate cell differentiation. Variant degenerate sequences of the nucleotide sequences according to the invention whose product is a protein having the same function as the protein coded by each of the sequences SEQ ID NO: 5 and SEQ ID NO: 11 are thus included within the scope of the invention. The amino acid sequence may be coded by any nucleotide sequence which gives rise to any of the amino acid sequences according to the invention. Due to the fact that the genetic code is degenerate, the same amino acid may be coded for by different codons (triplets), and thus the same amino acid sequence may be coded for by different nucleotide sequences.

The homologue, orthologue or functional variant of SEQ ID No. 1 or 8 encodes a polypeptide that is 30%-99% identical to a sequence encoded by SEQ No. 1 or 8. For example, the polypeptide of the invention has, in increasing order of preference, at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% overall sequence identity to the amino acid represented by SEQ ID NO: 5 or 11, respectively, and/or represented by the AtMINIYO or AtRTR1 orthologues and paralogues shown herein.

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In one embodiment, the isolated nucleic acid of the invention encodes a polypeptide that is at least 30% identical to a sequence encoded by SEQ No. 5 or 11. In another embodiment, the degree of identity between the amino acid sequences encoded by SEQ ID NO: 1 or SEQ ID NO: 8 originating from *Arabidopsis thaliana* and an amino acid sequences from another plant, preferably a plant belonging to the superfamily *Viridiplantae*, is around 90% or 95%. Furthermore, all sequences whose transcription product is substantially identical to the amino acid sequences SEQ ID NO: 5 and SEQ ID NO: 11 according to this invention are included.

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The amino acid sequences which are at least 30% identical to those coded by SEQ ID NO: 1 and SEQ ID NO: 8 are homologous sequences from *A. thaliana* or other organisms in which the protein for which they code has an equivalent function to the protein coded by the said MINIYO and RTR1 genes of plant origin, for example from *Arabidopsis*. The homologous sequences in general relate to sequences from different species originating from a common ancestral sequence. Two types of homology are generally distinguished in sequence homology: orthology and paralogy. Orthologous sequences belong to species which have a common past. Paralogous sequences are

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those which are found in the same organism and originate from duplication of a given gene. In one embodiment, the invention relates to any homologous sequences, including both orthologous and paralogous, which are at least 30% identical to the amino acid sequences encoded by SEQ ID NO: 1 or SEQ ID NO: 8, without prejudice to whether other sequences with lower degrees of identity with MINIYO and RTR1 are also regarded as being an object of the invention.

10 The overall sequence identity is determined using a global alignment algorithm, for example the Needleman Wunsch algorithm in the program GAP (GCG Wisconsin Package, Accelrys), preferably with default parameters and preferably with sequences of mature proteins (i.e. without taking into
15 account secretion signals or transit peptides).

The orthologue may be selected from a MINIYO or RTR1 gene in any other plant, preferably a plant of superfamily *Viridiplantae*, in particular monocotyledonous and
20 dicotyledonous plants, including forage plants and vegetables for livestock, ornamental plants, crop plants for use in human or animal nutrition and plants for use as bioenergy. Specific plants from which the orthologue may be derived are listed elsewhere in this application as non-limiting examples of
25 transgenic plants. In one embodiment of the various aspects of the invention, the MINIYO gene is selected from one of the following plants:

Oryza sativa (SEQ ID No. 12 peptide sequence, SEQ ID No. 18
30 nucleic acid sequence), Zea mays (SEQ ID No. 13 peptide sequence, SEQ ID No. 19 nucleic acid sequence), Glycine max (SEQ ID No. 14 and 15 peptide sequences, SEQ ID No. 20, 21 nucleic acid sequences), Brachypodium distachyon (SEQ ID No. 16 peptide sequence), Sorghum bicolor (SEQ ID No. 17 peptide
35 sequence).

In one embodiment of the various aspects of the invention, the RTR1 gene is selected from one of the following plants:

Oryza sativa (SEQ ID No. 22 peptide sequence, SEQ ID No. 28 nucleic acid sequence), Zea mays (SEQ ID No. 23 peptide sequence, SEQ ID No. 29 nucleic acid sequence), Glycine max (SEQ ID No. 24, 25 peptide sequences, SEQ ID No. 30, 31 nucleic acid sequences), Brachypodium distachyon (SEQ ID No. 27 peptide sequence), Sorghum bicolor (SEQ ID No. 26 peptide sequence).

As shown in figures 16 to 21, genes encoding for MINIYO and RTR1 in plants and their resulting proteins are conserved and show a number of conserved domains.

For the MINIYO protein, one of these domains is a glycine rich domain comprising an RGG element. This domain is located at position 960-980 in Arabidopsis AtMINIYO. Mutating G962E results in a partial loss of function mutant. Therefore, orthologues of AtMINIYO are characterised by the presence of a conserved glycine rich domain as shown in Figure 3b for MINIYO proteins from different species. Thus, orthologues proteins comprise a sequence which has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the domain in Arabidopsis SEQ ID No. 32: RGGLAPGVGLGWGASGGGFWS. FIG. 18 (a).

In addition, as shown in figure 18, orthologues are characterised by the presence of one or more further conserved domains which show a high degree of sequence identity, preferably at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to the following domains in Arabidopsis:

- amino acids 209-255 SEQ ID No. 33
SDIDVENHAKLQTMSPDEIAEAQAELLDKMDPALLSILKKRGEAKLK. FIG 18 (b);

- amino acids 317-396 SEQ ID No. 34
RDLRFSFDGNVVEEDVSPAETGGKWSGVESAERDFLRTEGDPGAAGYTIKEAIALARSVI
PGQRCLALHLLASVLDKA. FIG 18 (c);

- amino acids 350-389 SEQ ID No. 35
ERDFLRTEGDPGAAGYTIKEAIALARSVIPGQRCLALHLL. FIG 18 (d);

- 18 -

- amino acids 417-437 SEQ ID No. 36 DWEAIWAYALGPEPELVLALR. FIG 18 (e).;

- amino acids 529-559 SEQ ID No. 37 TIQKDVVAGQDVAAGLVRMDILPRIYHLLLEE. FIG 18 (f);

5 - amino acids 529-597 SEQ ID No. 38 TIQKDVVAGQDVAAGLVRMDILPRIYHLLLEEPTAALEDSIISVTIAIARHSPKCTTAILKY PKFVQT. FIG 18 (g);

- amino acids 1136-1145 SEQ ID No. 39 EWAHQRMPLP. FIG 18 (h);
and/or

10 - amino acids SEQ ID No. 40 1144-1416
LPPHWFLSAISAVHSGKTSTGPPESTELLEVAKAGVFFLAGLESSSGFGSLPSPVSVPLVW
KFHALSTVLLVGMDDIEDKNTRNLYNYLQELYGQFLDEARLNHRDTELLRFKSDIHENYSTF
LEMVVEQYAAVSYGDVVYGRQVSVYLHQCVESVRLSAWTVLSNARVLELLPSLDKCLGEAD
GYLEPVEENEAVLEAYLKSWTGALDRAATRGVAYTLVVHFFSSLVFCNQAKDKVSLRNKI
15 VKTLVRDLRSKRHREGMMLDLLRYK. FIG 18 (i)

For the RTR1 protein, there is a conserved domain (DUF408) with a zinc-finger like motif located at the N-terminus of the protein that is found in all the orthologues from plants,
20 animals and fungi. This domain is located at position 45-98 in Arabidopsis AtRTR1. The zinc-finger-like-motif has been implicated in interaction with the RNA Polymerase II C-terminal domain (CTD) and the Integrator complex in humans and is required for CTD-phosphatase activity in yeast and humans
25 (Mosley et al., 2009; Egloff et al., 2011). Interestingly, this motif is also required for interaction of RTR1 with IYO. Substituting the putative zinc coordinating cysteine residues (C56A/C61A or C94A/C98A) in the full-length AtRTR1 protein for alanine abrogates interaction with IYO. Intriguingly, however,
30 both the truncated N-terminal and the C-terminal halves of RTR1 can interact with IYO, suggesting that although RTR1 binds at both ends of the protein to IYO, it requires an intact zinc-finger-like motif in the context of the full length protein for binding.

35

A consensus sequence for the zinc-finger like motif derived from sequences from multicellular eukaryotes is (the putative Zinc-coordinating cysteines are highlighted in bold):

D[IV]V[TDEV]ER[ASTF]I[AVIS][KND][LAV]CGY[TP][LRA]CXXXXLX₇-

₁₅[YF][RK]IS[LT][KSR][TAED][HKN][KR]VYD[IL][THEQ]EXXX[FY]CXXXXC

A blast search against the non-redundant protein sequence database at NCBI with the corresponding sequence from Arabidopsis

DVVTERAIAKL**CGYTLCQRFLPSDVSRRGKYRISLKDHKVYDLQETSKFCSAGC** SEQ ID No. 41 retrieved the RTR1 orthologues from plants, animals and fungi with a low E-value ($< 10^{-6}$).

Therefore, orthologues of AtRTR1 from plants animals and fungi are characterised by the presence of a conserved zinc-finger like motif as shown in Figure 21. Thus, orthologues proteins comprise a sequence which has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the domain in Arabidopsis SEQ ID No. 42: DVVTERAIAKL**CGYTLCQRFLPSDVSRRGKYRISLKDHKVYDLQETSKFCSAGC**. FIG. 21 (a).

In addition, orthologues to the AtRTR1 Arabidopsis protein are characterised by the presence of one or more further conserved domains as shown in figure 21, which show a high degree of sequence identity, preferably at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the following domains in Arabidopsis:

- amino acids 39-61 SEQ ID No. 43 SRSDYEDVVTERAIAKL**CGYTLC**. This domain includes catalytic cysteine C56 which is strictly conserved in all the orthologues. FIG. 21 (b).

- amino acids. 77-89 SEQ ID No. 44 ISLKDHKVYDLQE. FIG. 21 (c);

- amino acids. 429-435 SEQ ID No. 45 SVTWADQ. FIG. 21 (d);;

- amino acids 473-507 SEQ ID No. 46 AEALATALSQAAEAVSSGNSDASDATAKAGIILLP. FIG. 21 (e);

and/or

- amino acids 552-589 SEQ ID No. 47. SWFDGPPEGFNLTLSNFAVMWDSLFGWSSSSLAYIYG. FIG. 21 (f)

Thus, a skilled person would understand that MINIYO and RTR1 are highly conserved in plants and characterised by the presence of the conserved domains above. Accordingly, the

MINIYO and RTR1 proteins according to the invention can be defined and identified through the presence of these domains set out herein, in particular with reference to figures 18 and 21. A skilled person would therefore be able to identify orthologues to AtMINIYO and ATRTR1 by reference to these domains through routine methods.

Another aspect of the invention relates to an expression vector which comprises one or more isolated nucleic acid molecule(s) of the invention. The invention also relates to the use of an expression vector as described herein to control the initiation of cell differentiation in the apical, root floral and/or other meristems of a plant.

The term "vector" refers to a fragment of DNA which has the ability to replicate in a given host and, as the term indicates, it can act as a vehicle to multiply another DNA fragment which has been fused to it ("insert"). "Insert" refers to a fragment of DNA fused to the vector; in the case of this invention the vector may comprise any of the sequences described in accordance with the aspects of the invention which, when fused to the same, can replicate in a suitable host. The vectors may be plasmids, cosmids, bacteriophages or lentiviral vectors suitable for transforming or transfecting fungal or animal cells, without excluding other kinds of vectors which correspond to the definition of vector provided.

Expression of the said nucleic acid molecules may be under the control of a promoter sequence. The promoter used in the gene constructs of the vectors described above to express MINIYO or RTR1 may be an endogenous MINIYO or RTR1 promoter, for example the AtMINIYO or AtRTR1 promoter (SEQ Id No 48 and 49) or a MINIYO or RTR1 promoter from a AtMINIYO or ATRTR1 orthologue.

Alternatively, the promoter may regulate overexpression of the gene. Overexpression according to the invention means that the transgene is expressed at a level that is higher than expression of endogenous counterparts (MINIYO or RTR1) driven

by their endogenous promoters. For example, overexpression may be carried out using a strong promoter, such as the cauliflower mosaic virus promoter (CaMV35S), the rice actin promoter or the maize ubiquitin promoter or any promoter that gives enhanced expression.

Alternatively, an inducible expression system may be used, where expression is driven by a promoter induced by environmental stress conditions (for example the pepper pathogen-induced membrane protein gene CaPIMPI or promoters that comprise the dehydration-responsive element (DRE), the promoter of the sunflower HD-Zip protein gene *Hahb4* or *Hahb1*, which is inducible by water stress, high salt concentrations and ABA, or a chemically inducible promoter (such as steroid- or ethanol-inducible promoter system). Such promoters are described in the art. Other suitable promoters and inducible systems are also known to the skilled person.

As a skilled person will know, the expression vector may also comprise a selectable marker which facilitates the selection of transformants, such as a marker that confers resistance to antibiotics, such as kanamycin.

In any of the expression vectors described herein, wild type sequences that encode MINIYO or RTR1 polypeptides can be included, but in one embodiment, variant sequence or fragments may also be used, provided such sequences encode a polypeptide that has the same biological activity as the wild type sequence. Sequence variations in the wild type sequence include silent base changes that do not lead to a change in the encoded amino acid sequence and/or base changes that affect the amino acid sequence, but do not affect the biological activity of the polypeptide. Changes may be conservative amino acid substitutions, i.e. a substitution of one amino acid residue where the two residues are similar in properties. Thus, variant/mutant polypeptides encoded by such sequences retain the biological activity of the wild type polypeptide and act on cell differentiation.

In another embodiment, mutant sequence or fragments may also be used, which encode a polypeptide that has a different biological activity as the wild type sequence. These modifications are described below.

5

A sequence or vector described herein encoding for the MINIYO or RTR1 protein is introduced as a transgene into the plant. This can be carried out by various methods as known in the field of plant genetic engineering, for example using transformation with *Agrobacterium* or particle bombardment.

Another embodiment of the invention relates to a host cell which comprises the expression vector of the invention.

The term "cell" as understood in this invention relates to a prokaryotic or eukaryotic cell. The cell may be a bacterium capable of replicating a transformed foreign DNA such as for example any of the strains of the species *Escherichia coli*. Preferably cell refers to a eukaryotic fungal, plant or animal cell. Thus, in the case where the cell is a fungus, the term cell comprises at least an individual cell of a yeast, a mycelium of a filamentous fungus, or other fungal cell of any type, whether germinal (spore) or vegetative, differentiated or undifferentiated. In the case of an animal cell it may be any normal or tumour cell line, from any tissue or organ, adult or embryonal, multipotent (undifferentiated) or differentiated. Likewise a protoplast (a fungal cell without a cell wall) is also included in this definition.

The invention also includes a method for generating of transgenic plants which constitutively or conditionally express or over-express a nucleic acid of the invention, that is a nucleic acid that encodes for a plant MINIYO and/or RTR1 protein, throughout the plant or in specific meristems, to advance the onset of differentiation, reducing the size of the meristems or eliminating them, depending upon the level of over-expression obtained. It also includes the over-expression of mutated versions of MINIYO and/or RTR1 which are not

excluded from the nucleus in undifferentiated cells. In one aspect of the invention, mutated constructs of MINIYO and/or RTR1 that are retained in the cytosol or in the nucleus are expressed under constitutive, inducible, tissue-specific or developmental-stage-specific promoters, to modify specifically cell proliferation or cell differentiation rates in different meristems and during embryogenesis. These constructs are described below.

The invention also includes a method for generating transgenic plants in which a nucleic acid of the invention that encodes for a plant MINIYO and/or RTR1 protein is expressed throughout the plant or in specific meristems, to delay the onset of differentiation. Such nucleic acids include mutated constructs of MINIYO and/or RTR1 as described herein.

These methods include introducing an nucleic acid of the invention into said plant by means of recombinant DNA technology and expressing said transgene in the plant.

In another aspect, the invention relates to a transgenic plant wherein the activity of a MINIYO polypeptide as described herein is inactivated, repressed or down-regulated. As described above, said MINIYO protein is at least 30% identical to the sequences coded by SEQ ID NO:1. In one embodiment, the MINIYO protein comprises or consists of SEQ ID No. 5. Thus, in another aspect, the invention relates to a transgenic plant wherein the activity of a RTR1 polypeptide as described herein is inactivated, repressed or down-regulated. As described above, said RTR1 protein is at least 30% identical to the sequences coded by SEQ ID NO:8. In one embodiment, the RTR1 protein comprises or consists of SEQ ID No. 11.

In one embodiment, the transgenic plant may be characterised in that activity of both a MINIYO and RTR1 polypeptide as described herein is inactivated, repressed or down-regulated.

In another embodiment, RNA-mediated gene suppression or RNA silencing may be used to achieve silencing of the MINIYO or

RTR1 gene. "Gene silencing" is a term generally used to refer to suppression of expression of a gene via sequence-specific interactions that are mediated by RNA molecules. The degree of reduction may be so as to totally abolish production of the encoded gene product, but more usually the abolition of expression is partial, with some degree of expression remaining. The term should not therefore be taken to require complete "silencing" of expression.

10 Transgenes may be used to suppress endogenous plant genes. This was discovered originally when chalcone synthase transgenes in petunia caused suppression of the endogenous chalcone synthase genes and indicated by easily visible pigmentation changes. Subsequently it has been described how many, if not all plant genes can be "silenced" by transgenes. Gene silencing requires sequence similarity between the transgene and the gene that becomes silenced. This sequence homology may involve promoter regions or coding regions of the silenced target gene. When coding regions are involved, the transgene able to cause gene silencing may have been constructed with a promoter that would transcribe either the sense or the antisense orientation of the coding sequence RNA. It is likely that the various examples of gene silencing involve different mechanisms that are not well understood. In different examples there may be transcriptional or post transcriptional gene silencing and both may be used according to the methods of the invention.

RNA-mediated gene suppression or RNA silencing according to the methods of the invention includes co-suppression wherein over-expression of the MINIYO or RTR1 gene sense RNA or mRNA leads to a reduction in the level of expression of the genes concerned. RNAs of the transgene and homologous endogenous gene are co-ordinately suppressed.

35

Other techniques used in the methods of the invention include antisense RNA to reduce transcript levels of the endogenous MINIYO and/or RTR1 gene in a plant. In this method, RNA

silencing does not affect the transcription of a gene locus, but only causes sequence-specific degradation of target mRNAs. An "antisense" nucleic acid sequence comprises a nucleotide sequence that is complementary to a "sense" nucleic acid sequence encoding a MINIYO and/or RTR1 protein, or a part of a MINIYO and/or RTR1 protein, i.e. complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA transcript sequence. The antisense nucleic acid sequence is preferably complementary to the endogenous MINIYO and/or RTR1 gene to be silenced. The complementarity may be located in the "coding region" and/or in the "non-coding region" of a gene. The term "coding region" refers to a region of the nucleotide sequence comprising codons that are translated into amino acid residues. The term "non-coding region" refers to 5' and 3' sequences that flank the coding region that are transcribed but not translated into amino acids (also referred to as 5' and 3' untranslated regions).

The length of a suitable antisense oligonucleotide sequence is known in the art and may start from about 50, 45, 40, 35, 30, 25, 20, 15 or 10 nucleotides in length or less. An antisense nucleic acid sequence according to the invention may be constructed using chemical synthesis and enzymatic ligation reactions using methods known in the art.

Antisense nucleic acid sequences may be introduced into a plant by transformation or direct injection at a specific tissue site. Alternatively, antisense nucleic acid sequences can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense nucleic acid sequences can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid sequence to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid sequences can also be delivered to cells using vectors.

RNA interference (RNAi) is another post-transcriptional gene-silencing phenomenon which may be used according to the methods of the invention. This is induced by double-stranded RNA in which mRNA that is homologous to the dsRNA is specifically degraded.

Thus, a plant may be transformed to introduce a RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA or cosuppression molecule that has been designed to target the expression of the MINIYO and/or RTR1 gene and selectively decreases or inhibits the expression of the gene or stability of its transcript. Preferably, the RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA or cosuppression molecule used in the methods of the invention comprises a fragment of at least 17 nt, preferably 22 to 26 nt and can be designed on the basis of the information shown in SEQ ID No. 1 and/or 8. Guidelines for designing effective siRNAs are known to the skilled person.

siNA molecules may be double stranded. In one embodiment, double stranded siNA molecules comprise blunt ends. In another embodiment, double stranded siNA molecules comprise overhanging nucleotides (e.g., 1-5 nucleotide overhangs, preferably 2 nucleotide overhangs). In some embodiments, the siRNA is a short hairpin RNA (shRNA); and the two strands of the siRNA molecule may be connected by a linker region (e.g., a nucleotide linker or a non-nucleotide linker). The siNAs of the invention may contain one or more modified nucleotides and/or non-phosphodiester linkages. Chemical modifications well known in the art are capable of increasing stability, availability, and/or cell uptake of the siNA. The skilled person will be aware of other types of chemical modification which may be incorporated into RNA molecules. In one embodiment, recombinant DNA constructs as described in US 6635805, incorporated herein by reference, may be used.

35

Silencing of the MINIYO and/or RTR1 gene may also be achieved using virus-induced gene silencing.

For example, the transgenic plant having reduced activity of the *MINIYO* and/or *RTR1* polypeptides may be characterised in that in comparison with the wild phenotype plant said plant has a reduction of between 50% and 100% in the expression of a gene encoding for an amino acid sequence of the *MINIYO* and/or *RTR1* protein as described herein.

10 In one embodiment, the endogenous *MINIYO* or *RTR1* gene carries a functional mutation.

In another embodiment, the transgenic plant expresses a transgene said transgene comprising a modified *MINIYO* or *RTR1* nucleic acid sequence when compared to a wild type sequence.

15 For example, said modification/functional mutation of the *MINIYO* nucleic acid sequence results in a polypeptide comprising a substitution of the second conserved G in the RGG motif (SEQ ID No. 32).

20 With reference to the *Arabidopsis* sequence, this is G962E. The numbering of the amino acid residues as used in this disclosure is based on the numbering of the *Arabidopsis AtMINIYO*. Because the *MINIYO* or *RTR1* amino acid sequence in other species may comprise fewer or more amino acids, the position of the second conserved G in the RGG motif residue may not be 962, but may be between position 947 to 1067 (Fig. 16). The positions of the targeted residues according to the invention in some exemplified plant species are shown in figure 3B.

30 In another embodiment, the modification is a substitution or deletion of one or more residues within the nuclear localisation signals present in the *MINIYO* and/or *RTR1* protein. In another embodiment, it is an insertion.

35 *MINIYO* and *RTR1* are required for cell proliferation in meristematic cells, where they accumulate primarily in the cytosol, albeit shuttling through the nucleus, as evidenced by

the fast nuclear accumulation when export is blocked with leptomycin B. Moreover, in the meristem periphery, MINIYO accumulates in the nucleus and together with RTR1 switches on cell differentiation. The shuttling between the cytosol and the nucleus implies that MINIYO and RTR1 have domains responsible for nuclear import and for nuclear export. Those signals can be identified through a blind genetic search, testing the localization of mutant versions of the proteins (i.e: a deletion series), or by a directed bioinformatic search for nuclear localization signals (NLS) and nuclear export signals (NES) in the protein sequences. Those skilled in the art will be aware that by mutating the domains required for nuclear import or nuclear export, it is possible to generate MINIYO and RTR1 constructs such that the encoded proteins are retained in the cytosol or in the nucleus, respectively. In this way, the two activities of MINIYO and RTR1 (promoting cell proliferation and promoting cell differentiation) may be uncoupled. Constructs that cause retention of MINIYO and RTR1 in the cytosol may specifically promote proliferation without affecting the timing of differentiation, whereas the constructs that cause MINIYO and RTR1 to be retained in the nucleus will specifically promote cell differentiation.

The predicted NLS in AtMINIYO are located at aa. 250-262 (GEAKLKKRKHSVQ, SEQ ID No. 50) and at aa. 1397-1420 (RDLSRKRHREGMMLDLLRYKKGSA, SEQ ID No. 51).

Thus, the invention relates to a nucleic acid construct comprising a MINIYO nucleic acid sequence which encodes for a polypeptide that has a mutation in one of both of the NLS of the resulting MINIYO polypeptide. The mutation may be a substitution or deletion of one or more residues in the NLS, preferably all residues. In one embodiment, residues aa. 250-262 and/or aa. 1397-1420 in AtMINIYO or corresponding residues in orthologues are deleted. Said construct may be introduced and expressed in a transgenic plant according to the methods

of the invention to exclude the MINIYO polypeptide from the cell nucleus and thus block cell differentiation and stimulate cell proliferation. Using inducible promoters, the nucleic acid may be included in an expression vector as described
5 herein so that the timing of the expression can be specifically determined.

In one embodiment of the invention, an MINIYO protein impaired in nuclear import is expressed under the control of an embryo
10 specific promoter (such as the Arabidopsis cruciferin promoter, the Brassica napus Napin A promoter, the rice glutelin promoter, the maize 19 Kda zein promoter, the wheat SPA promoter or the pea legumin promoter) or an endosperm-specific promoter (such as the wheat gliadin promoter, the
15 rice prolamin promoter, or the maize END promoter) to increase cell proliferation, seed size and yield in a seed crop.

In another embodiment of the invention, an MINIYO protein impaired in nuclear import is expressed under the control of a shoot meristem promoter (such as KNOX gene promoters from
20 Brassica, rice or maize) to increase cell proliferation, meristem size, meristem number, production of aerial organs and crop yield (leaves, flowers).

In one embodiment of the invention, an MINIYO protein impaired in nuclear import is expressed under the control of axillary
25 bud specific promoter (BRC1 promoter from Arabidopsis, TB1 promoter from maize, OSTB1 promoter from rice, ATC085 promoter from tobacco, SlBRC1a and SlBRC1b promoters from tomato) to increase branching and yield.

In one embodiment of the invention, an MINIYO protein impaired
30 in nuclear import is expressed under the control of a root meristem specific promoter (RCH1 promoter, the brassica G1-3b promoter) to increase cell proliferation, root growth, nutrient uptake and plant yield.

In one embodiment of the invention, an MINIYO protein impaired
35 in nuclear import is expressed under the control of the IYO

promoter that is active in embryos and in plant meristems, to increase seed size, meristem size, plant growth and improve yields in target crops.

The predicted NLS in AtRTR1 is located at aa. 340-368.
5 LKGDLQTLDGKNTLSGSSSGSNTKGSKTK, SEQ ID No.52.

Thus, the invention relates to a nucleic acid construct comprising a RTR1 nucleic acid sequence which encodes for a polypeptide that has a mutation in the NLS of the resulting
10 RTR1 polypeptide. The mutation may be a substitution or deletion of one or more residues in the NLS, preferably all residues. Said construct may be introduced and expressed in a transgenic plant according to the methods of the invention to
15 exclude the RTR1 polypeptide from the cell nucleus and thus block cell differentiation and stimulate cell proliferation. Using inducible promoters, the nucleic acid may be included in an expression vector as described herein so that the timing of the expression can be specifically determined.

20 In one embodiment of the invention, an RTR1 protein impaired in nuclear import is expressed under the control of an embryo specific promoter (such as the Arabidopsis cruciferin promoter, the Brassica napus Napin A promoter, the rice glutelin promoter, the maize 19 Kda zein promoter, the wheat
25 SPA promoter or the pea legumin promoter) or an endosperm-specific promoter (such as the wheat gliadin promoter, the rice prolamin promoter, or the maize END promoter) to increase cell proliferation, seed size and yield in a seed crop.

In another embodiment of the invention, an RTR1 protein
30 impaired in nuclear import is expressed under the control of a shoot meristem promoter (such as KNOX gene promoters from Brassica, rice or maize) to increase cell proliferation, meristem size, meristem number, production of aerial organs and crop yield (leaves, flowers).

35 In one embodiment of the invention, an RTR1 protein impaired in nuclear import is expressed under the control of a root

meristem specific promoter (RCH1 promoter, the brassica G1-3b promoter) to increase cell proliferation, root growth, nutrient uptake and plant yield.

5 In one embodiment of the invention, an RTR1 protein impaired in nuclear import is expressed under the control of the MINIYO promoter, active in embryos and in plant meristems, to increase seed size, meristem size, plant growth and improve yields in target crops.

10 Also within the scope of the invention are transgenic plants wherein both the MINIYO protein and the RTR1 are impaired in nuclear import. Combinations of the manipulations of the NLS in MINIYO protein and the RTR1 as set out above can be used to achieve this.

15 The activity of RTR1 may also be decreased by manipulating the interaction between MINIYO and RTR1 proteins. This can be achieved by manipulating certain residues in the MINIYO and/or RTR1 polypeptide sequences. For example, substituting the putative zinc coordinating cysteine residues for alanines (C56A/C61A or C94A/C98A in the Arabidopsis sequence) in the full-length AtRTR1 protein abrogates the interaction with
20 MINIYO.

Another aspect of the invention refers to a transgenic plant wherein the activity of a MINIYO polypeptide is increased or up-regulated. Another aspect of the invention refers to a
25 transgenic plant wherein the activity of a RTR1 polypeptide is increased or up-regulated.

In one embodiment, the transgenic plant is characterised in that the activity of both a MINIYO and a RTR1 polypeptides is
30 increased or up-regulated in the same plant.

For example, said plant overexpresses a nucleic acid encoding for a MINIYO protein that is at least 30% identical to the sequences coded by SEQ ID NO:1. In another embodiment, said
35 plant overexpresses a nucleic acid encoding for a RTR1 protein

that is at least 30% identical to the sequences coded by SEQ ID NO:8.

In another embodiment, said plant expresses a transgene said
5 transgene comprising a modified *MINIYO* and a *RTR1* nucleic acid sequence when compared to a wild type sequence.

For example, said modification is a substitution or deletion
of one or more residues within the nuclear export signal
10 present in the *MINIYO* or *RTR1* protein.

Preferably, over-expression will be between 2 and 100 times the expression of the endogenous mRNA.

15 One way of increasing the activity of *MINIYO* or *RTR1* is to retain the protein in the nucleus.

The predicted NES in At*MINIYO* is located at 432-440. LVLALRMAL
SEQ ID No. 53.

20

Thus, the invention relates to a nucleic acid construct comprising a *MINIYO* nucleic acid sequence which encodes for a polypeptide that has a mutation in the NES of the resulting *MINIYO* polypeptide. The mutation may be a substitution or
25 deletion of one or more, preferably all residues of the NES. In one embodiment, residues 432-440 in At*MINIYO* or corresponding residues in orthologues are deleted. Said construct may be introduced and expressed in a transgenic plant according to the methods of the invention to retain the
30 *MINIYO* polypeptide in the cell nucleus and stimulate cell differentiation. Using inducible promoters, the nucleic acid may be included in a an expression vector as described herein so that the timing of the expression of the mutated nucleic acid can be specifically determined.

35

In one embodiment of the invention, a *MINIYO* protein impaired in nuclear export is expressed under the control of axillary bud specific promoter (BRC1 promoter from *Arabidopsis*, TB1

promoter from maize, OSTB1 promoter from rice, ATC085 promoter from tobacco, SlBRC1a and SlBRC1b promoters in tomato) to reduce branching and increase yield. This is particularly important for forestry applications, for instance for growing
5 closely packed trees used for pulp production in the paper or biofuel industry.

In one embodiment of the invention, a MINIYO protein impaired in nuclear export is expressed under the control of an inflorescence meristem specific promoter (such as the LFY
10 promoter) to terminate the inflorescence meristem in crops that are cultivated for their vegetative organs and in which flowering reduces the harvest (lettuce, spinach, sugar beet, potato, and others).

The predicted NES in RTR1 is located at 340-349 (LKGDQLQTLDG,
15 SEQ ID No.54).

Thus, the invention relates to a nucleic acid construct comprising a RTR1 nucleic acid sequence which encodes for a polypeptide that has a mutation in the NES of the resulting
20 RTR1 polypeptide. The mutation may be a substitution or deletion of one or more, preferably all residues of the NES. In one embodiment, residues 432-440 are deleted. Said construct may be introduced and expressed in a transgenic plant according to the methods of the invention to retain the
25 RTR1 polypeptide in the cell nucleus and stimulate cell differentiation. Using inducible promoters, the nucleic acid may be included in an expression vector as described herein so that the timing of the expression of the mutated nucleic acid can be specifically determined.

30 Also within the scope of the invention are transgenic plants wherein both the MINIYO protein and the RTR1 are impaired in nuclear import. Combinations of the manipulations of the NLS in MINIYO protein and the RTR1 as set out above can be used to
35 achieve this.

In one aspect, the invention relates to transgenic plants wherein both, MINIYO and RTR1 have been manipulated. As shown in the examples, MINIYO and RTR1 are jointly responsible for the control of cell differentiation, supporting a close functional interaction. Differentiation in the *iy0-latrtr1-2* double mutants was almost completely blocked and the plants eventually developed as a friable callus of undifferentiated cells. This phenotype is much stronger than the sum of the phenotypes of the single mutants. Thus, transgenic plants according to the invention may have reduced or increased activity for both, MINIYO and RTR1 by manipulating activity of MINIYO and RTR1 as explained herein.

For the purposes of the invention, "transgenic", "transgene" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

- (a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or
- (b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or
- (c) a) and b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length

of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette - for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above - becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815 incorporated by reference.

A transgenic plant for the purposes of the invention is thus understood as meaning, as above, that the nucleic acids used in the method of the invention are not at their natural locus in the genome of said plant, it being possible for the nucleic acids to be expressed homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acids according to the different embodiments of the invention are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place. Preferred transgenic plants are mentioned herein.

Transgenic plants according to the invention display altered cell differentiation and proliferation compared to a control plant. A control plant according to the invention is a plant that is not modified in the same way as the transgenic plant of the invention with respect to MINIYO and/or RTR1 expression or polypeptide activity. In one embodiment, this plant is a wild type plant. In another embodiment, this plant is a parent plant that may comprise additional modifications through

expression of other transgene of interest that modify desired pathways, for example stress resistance.

The *MINIYO* or *RTR1* genes according to the different aspects of the invention may be an exogenous gene, such as *Arabidopsis AtMINIYO* or *AtRTR1*, overexpressed in a different plant species. Alternatively, the *MINIYO* or *RTR1* may be an endogenous plant gene, i.e. a gene that is endogenous to the plant in which it is introduced via recombinant methods and (over)-expressed.

In a preferred embodiment of the invention, the transgenic plant is characterised in that it is selected from the group comprising: plants for particular use in the methods according to the invention include all the plants belonging to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including forage plants and vegetables for livestock, ornamental plants, crop plants for use in human or animal nutrition, plants for use as bioenergy, trees, and bushes selected from the list comprising: *Acer spp.*, *Actinidia spp.*, *Abelmoschus spp.*, *Agropyron spp.*, *Allium spp.*, *Amaranthus spp.*, *Ananas comosus*, *Annona spp.*, *Apium graveolens*, *Arabidopsis thaliana*, *Arachis spp.*, *Artocarpus spp.*, *Asparagus officinalis*, *Avena sativa*, *Averrhoa carambola*, *Benincasa hispida*, *Bertholletia excelsea*, *Beta vulgaris*, *Brachypodium spp.*, *Brassica spp.*, *Cadaba farinosa*, *Camellia sinensis*, *Camelina spp.*, *Canna indica*, *Capsicum spp.*, *Carex elata*, *Carica papaya*, *Carissa macrocarpa*, *Carya spp.*, *Carthamus tinctorius*, *Castanea spp.*, *Cichorium endivia*, *Cinnamomum spp.*, *Citrullus lanatus*, *Citrus spp.*, *Cocos spp.*, *Coffea spp.*, *Colocasia esculenta*, *Cola spp.*, *Coriandrum sativum*, *Corylus spp.*, *Crataegusspp.*, *Crocus sativus*, *Cucurbita spp.*, *Cucumis spp.*, *Cynara spp.*, *Daucus carota*, *Desmodium spp.*, *Dimocarpus longan*, *Dioscorea spp.*, *Diospyros spp.*, *Echinochloa spp.*, *Eleusine coracana*, *Eriobotrya japonica*, *Eucalyptus spp.*, *Eugenia uniflora*, *Fagopyrum spp.*, *Fagus spp.*, *Ficus carica*, *Fortunella spp.*, *Fragaria spp.*, *Ginkgo biloba*, *Glycine spp.*, *Gossypium hirsutum*, *Helianthus*

spp., *Hemerocallis fulva*, *Hibiscus* spp., *Hordeum* spp., *Ipomoea*
batatas, *Juglans* spp., *Jatropha* spp., *Lactuca sativa*, *Lathyrus*
spp., *Lens culinaris*, *Linum usitatissimum*, *Litchi chinensis*,
Lotus spp., *Luffa acutangula*, *Lupinus* spp., *Luzula*
5 *sylvatica*, *Macrotyloma* spp., *Malus* spp., *Malpighia emarginata*,
Mammea americana, *Mangifera indica*, *Manihot* spp., *Manilkara*
zapota, *Medicago sativa*, *Melilotus* spp., *Mentha* spp.,
Momordica spp., *Morus nigra*, *Musa* spp., *Nicotiana* spp., *Olea*
spp., *Opuntia* spp., *Ornithopus* spp., *Oryza* spp., *Panicum*
10 *miliaceum*, *Passiflora edulis*, *Pastinaca sativa*, *Persea* spp.,
Petroselinum crispum, *Phaseolus* spp., *Phoenix* spp., *Physalis*
spp., *Pistacia vera*, *Pisum* spp., *Poa* spp., *Populus* spp.,
Prosopis spp., *Prunus* spp., *Psidium* spp., *Punica granatum*,
Pyrus communis, *Quercus* spp., *Raphanus sativus*, *Rheum*
15 *rhabarbarum*, *Ribes* spp., *Ricinus communis*, *Saccharum* spp.,
Sambucus spp., *Secale cereale*, *Sesamum* spp., *Sinapis* sp.,
Solanum spp., *Sorghum bicolor*, *Spinacia* spp., *Syzygium* spp.,
Tamarindus indica, *Theobroma cacao*, *Trifolium* spp.,
Triticosecale rimpau, *Triticum* spp., *Tropaeolum minus*,
20 *Tropaeolum majus*, *Vaccinium* spp., *Vicia* spp., *Vigna* spp.,
Viola odorata, *Vitis* spp., *Zea mays*, *Zizania palustris*,
Ziziphus spp., among others.

According to a preferred embodiment of the invention, the crop
25 plant is a plant such as tomato, potato, pepper, fruiting
plants of the prunus and citrus genuses, *Jatropha curcas*,
soya, sunflower, rape, alfalfa, canola, cotton, brassica
genuses or tobacco. Even more preferably, the plant is a
monocotyledonous one such as sugar cane, and even more
30 preferably a cereal such as rice, maize, wheat, rye, barley,
millet, sorghum or oats. Most preferred plants are maize,
rice, wheat, sorghum, canola and cotton.

Another preferred embodiment of the invention relates to a
35 product obtained from the transgenic plant as described above,
the said product being selected from seeds, stones, leaves,
flowers, roots, flour and fruit. In a more preferred
embodiment the said product is a transgenic seed. In one

embodiment the products produced by said methods of the invention are plant products such as, but not limited to, a foodstuff, feedstuff, a food supplement, feed supplement, fiber, cosmetic or pharmaceutical. Foodstuffs are regarded as compositions used for nutrition or for supplementing nutrition. Animal feedstuffs and animal feed supplements, in particular, are regarded as foodstuffs. In another embodiment the inventive methods for the production are used to make agricultural products such as, but not limited to, plant extracts, proteins, amino acids, carbohydrates, fats, oils, polymers, vitamins, and the like. It is possible that a plant product consists of one or more agricultural products to a large extent.

The invention also relates to a method for generating a transgenic plant with altered cell differentiation and cell proliferation comprising altering the activity of a gene encoding a *MINIYO* polypeptide. This may be achieved by expressing a *MINIYO* transgene in a plant so that activity is altered. In another aspect, the invention relates to a method for generating a transgenic plant with altered cell differentiation and cell proliferation comprising altering the activity of a gene encoding a *RTR1* polypeptide as defined herein. In another aspect, plants are generated where the activity of both, *MINIYO* and *RTR1*, is manipulated. As explained elsewhere, alteration of the activity of *MINIYO* and/or *RTR1* means that the activity may be increased or decreased. This may be achieved by manipulating the NLS/NES sequences and introducing constructs that express *MINIYO* and/or *RTR1* proteins modified in this way as explained herein. Other ways of manipulating the activity of *MINIYO* and *RTR1*, such as gene silencing or the generation of partial loss of function mutants, are also set out herein.

In another aspect, the invention relates to a plant obtained/obtainable by said methods.

In another aspect, the invention relates to a method for altering localisation of MINIYO and/or RTR1 in a plant by manipulating the NLS/NES sequences of MINIYO and/or RTR1 as described herein. In this way, MINIYO and/or RTR1 may be retained or excluded from the nucleus in one or more meristems. The NLS/NES sequences can be manipulated to achieve this as set out herein and transgenes carrying such manipulations can be introduced and expressed in a plant.

10 In another aspect, the invention also relates to a method for improving the architecture and yield of plants through genetic changes to the MINIYO (SEQ ID No 1), *AtRTR1* (SEQ ID No 8) genes or their orthologues in other plants. The expression of improving architecture refers to the non-exclusive list of altering the size/number of one or more meristems, altering the number of side branches, altering inflorescence, altering thickness of the stems, modify thickness of the stems and increasing plant yield.

20 In a preferred embodiment of the method for improving the architecture and yield of plants, the method is used to alter the size of one or more meristems, including increasing or decreasing the activity of the MINIYO and RTR1 genes.

25 In a preferred embodiment of the method, it is used to increase the size of the meristems by delaying the onset of differentiation and consequently increasing the number of undifferentiated cells brought about through the loss of function of MINIYO and/or RTR1. Preferably, to obtain ectopic shoot apical meristems through delaying the onset of differentiation and the consequent increase in the number of undifferentiated cells caused by the loss of function of MINIYO and/or RTR1.

35 In another preferred embodiment of the improving the architecture and yield of plants, the method is to obtain ectopic floral meristems through delaying the onset of differentiation and the consequent increase in the number of

undifferentiated cells caused by the loss of function of MINIYO and/or RTR1.

5 In another preferred embodiment of the method, it is used to obtain ectopic root meristems through delaying the onset of differentiation and the consequent increase in the number of undifferentiated cells caused by the loss of function of MINIYO and/or RTR1.

10 In another preferred embodiment, the method is to obtain ectopic embryos through delaying the onset of differentiation in the suspensor cells caused through the loss of function of MINIYO and/or RTR1.

15 In a preferred embodiment, the method is used to reduce or eliminate meristems through delaying the onset of differentiation caused by the increased activity of MINIYO and/or RTR1. Preferably, to reduce the number of side branches in crops through increasing the activity of MINIYO and/or
20 RTR1, specifically in axillary buds.

In another preferred embodiment, the method is used to compact inflorescence through increasing the activity of MINIYO and/or RTR1 in reproductive meristems.

25

In another preferred embodiment, the thickness of the stems of herbaceous plants is increased.

30 In another preferred embodiment, secondary growth in shrubs is modified.

In another more aspect, the invention includes a method to increase plant yield by decreasing or downregulating the activity of MINIYO and/or RTR1 in a transgenic plant. This may
35 be achieved as described elsewhere, including through manipulating of the NLS sequences, creating mutant proteins that lead to partial loss of function or gene silencing.

Another preferred embodiment of the invention relates to the development of transgenic plants for use in obtaining biofuels, such as in the production of bioethanol.

5 The invention also relates to the use of a polypeptide having at least 30% sequence identity to a polypeptide encoded by SEQ ID NO. 1 or 8 in altering cell differentiation, cell proliferation, meristem formation/growth and/or increasing crop yield.

10

In another aspect, the invention relates to manipulating the interaction between *MINIYO* and *RTR1* proteins,. This can be achieved by manipulating certain residues in the *MINIYO* and/or *RTR1* polypeptide sequences. For example, substituting the putative zinc coordinating cysteine residues for alanines (C56A/C61A or C94A/C98A in the *Arabidopsis* sequence) in the full-length *AtRTR1* protein abrogates the interaction with *MINIYO*.

15 In another embodiment, the invention relates to an isolated nucleic acid sequence comprising or consisting of SEQ ID No. 48 (*AtMINIYO* promoter). In another embodiment, the invention relates to an isolated nucleic acid sequence comprising or consisting of SEQ ID No. 49 (*RTR1* promoter).

20 Such promoter sequences may be fused to any gene of interest to direct spatial and temporal expression of the target gene. The invention also relates to the use of these promoter sequences in directing expression at sites of active cell proliferation and differentiation (for example shoot apical meristem (SAM), in leaf and flower primordia, in unfertilized ovules and in developing embryos, but not in mature organs).

25 The invention also relates to methods for screening for loss of function mutants of *MINIYO* and/or *RTR1* in plants. These methods comprise generating a mutant population by using mutagens known in the art. Specifically included are modifications of the endogenous locus by mutagenesis,

35

including chemical mutagenesis, leading to a deletion, insertion or substitution in the endogenous locus. The mutagen may be fast neutron irradiation or a chemical mutagen, for example selected from the following non-limiting list: ethyl methanesulfonate (EMS), methylmethane sulfonate (MMS), N-ethyl-N-nitrosourea (ENU), triethylmelamine (1'EM), N-methyl-N-nitrosourea (MNU), procarbazine, chlorambucil, cyclophosphamide, diethyl sulfate, acrylamide monomer, melphalan, nitrogen mustard, vincristine, dimethylnitrosamine, N-methyl-N'-nitro-Nitrosoguanidine (MNNG), nitrosoguanidine, 2-aminopurine, 7,12 dimethyl-benz(a)anthracene (DMBA), ethylene oxide, hexamethylphosphoramide, bisulfan, diepoxyalkanes (diepoxyoctane (DEO), diepoxybutane (BEB), and the like), 2-methoxy-6-chloro-9 [3-(ethyl-2-chloroethyl)aminopropylamino]acridine dihydrochloride (ICR-170) or formaldehyde.

In one embodiment, the method used to create and analyse mutations is targeting induced local lesions in genomes (TLLING).

A skilled person will know that different approaches can be used to generate such mutants. In one embodiment, insertional mutagenesis is used. In this embodiment, as discussed in the examples, T-DNA may be used as an insertional mutagen which disrupts *MINIYO* or *RTR1* gene expression. These plants thus do not carry a transgene to alter expression of the endogenous locus, but the endogenous locus is modified by mutagenesis. The methods also involve analyzing cell proliferation/differentiation compared to control wild type plants. If cell proliferation/differentiation is delayed, then this may be due to a mutation in *MINIYO* and/or *RTR1*.

In one embodiment, methods that solely rely on essentially biologically processes are specifically disclaimed.

Through the description and the claims the word "comprises" and its variants is not intended to exclude other technical

features, additives, components or steps. To those skilled in the art other objects, advantages and characteristics of the invention will be apparent partly from the description and partly from the practice of the invention. The following 5 figures and examples are provided by way of illustration, and are not intended to restrict the invention.

The disclosure of all references cited is incorporated.

10 DESCRIPTION OF THE FIGURES

Figure 1. The mutant *iy0-1* has delayed differentiation in the shoot (A) and root (B) apical meristems, in the procambium (C) and in the protoderm (D). (E) The expression of stem cell 15 markers in *iy0-1* plants extends beyond its normal meristematic niche. The boxes show the pattern of expression of the markers in wild plants (Wt). *iy0-1* plants develop ectopic shoot apical meristems (F), multiple flowers from a single bud (G), ectopic root apical meristems (H) and additional stomas (I). (J) *iy0-1/iy0-2* plants develop twin (double) embryos. (K) The double 20 embryos are viable and germinate, developing into plants with a very low degree of differentiation. (L) *iy0-1* plants also occasionally develop double embryos which develop normally.

25 **Figure 2.** The *iy0-1* mutant has a larger number of lateral roots, which develop ectopically in positions in which they do not appear in wild type plants (Wt): two lateral roots developing opposite one another or even emerging from the same point.

30

Figure 3. (A) Diagram of the positional map of the mutant *iy0-1*. The number of recombinant chromosomes in a total of 1500 genotyped mutant plants is indicated. (B) Alignment of the *MINIYO* sequence and its orthologues in plants. At: *Arabidopsis thaliana*; Mt: *Medicago truncatula*; Vv: *Vitis vinifera*; Os: 35 *Oryza sativa*; Pp: *Physcomitrella patens*. The G/E mutation of the *iy0-1* allele in the motif united to RNA RGG and conserved glycines is indicated. (C) The hypomorphic allele *iy0-1* does

not affect accumulation of the *IYO* transcript. (D) Phenotype of the null allele *iyo-2*. (E) Phenotype of *iyo-1/iyo-2* and *iyo-1/iyo-3* embryos. (F) Phenotype of *iyo-1/iyo-2* plants.

5 **Figure 4.** Expression of the β -glucuronidase (GUS) gene (A) and an *IYO*-GFP fusion (B) under the control of the *IYO* promoter in roots. (C) Detail of a root epidermis showing nuclear accumulation of *IYO*-GFP in cells about to begin differentiation. (D-F) Expression in shoot and root apices of
10 *IYO*-GFP and RPB10-GFP (RPB-GFP) fusions under the control of the 35S promoter. Arrowheads mark nuclear accumulation of *IYO*-GFP in cells about to begin differentiation at the transition zone of the root.

15 **Figure 5.** (A) Northern-blot with a specific *IYO* probe hybridised against samples of total RNA from different tissues and organs. Hypoc+SAM: Hypocotyl and shoot apical meristem of 7-day plants. Cotyledons: cotyledons of 7-day plants. Rosette leaves, cauline leaves, stems, inflorescence apices and
20 flowers were from 30-day plants. (B) *In situ* hybridisation of inflorescence apices of 22-day plants with an antisense *IYO* probe. The box shows hybridisation with an *IYO* sense probe. (C-E) Pattern of expression of the β -glucuronidase (GUS) gene under the control of the *IYO* promoter. (F) Complementation of
25 the *iyo-1* mutant phenotypes with the 35S::*IYO*-GFP construct. (G-H) Pattern of expression and subcellular accumulation of *IYO*-GFP under the control of the 35S promoter. (I) Pattern of expression and subcellular accumulation of GFP-*IYO* under the control of the 35S promoter. (J) Pattern of subcellular
30 accumulation of GFP-*IYO* under the control of the *IYO* promoter in root apices of plants treated with 0.9 μ M leptomycin B (+LMB) or with an equivalent quantity of the solvent used (-LMB) for 1 or 24 hours.

35 **Figure 6.** (A) The majority of the 380 genes most significantly induced ($p \leq 0.00015957$) in the inflorescence apices of wild type plants relative to those of *iyo-1* plants (Wt/*iyo-1*) are over-expressed in inflorescence apices of 35S::*IYO*-GFP plants

relative to those of wild-type plants (*IYO-GFPoe/Wt*). (B) Histological sections of the apices of wild-type (Wt) and *IYO-GFP* over-expressing plants (*IYO-GFPoe*) 14 and 21 days after sowing. The arrowheads indicate prematurely differentiated cells. (C) Compaction of inflorescence in plants which over-express *IYO-GFP* (*IYO-GFPoe*).

Figure 7. (A) Termination of the primary shoot apical meristem in plants which over-express *IYO-HA* under the 35S promoter (*IYO-HAoe*). (B) Termination of the primary root apical meristem in plants over-expressing *IYO-HA* under the 35S promoter (*IYO-HAoe*). The boxes show the meristem of wild type plants (Wt) at equivalent stages. (C) Premature differentiation of xylem vessels in lateral root primordia (arrows) and root hairs in epidermal cells prior to their elongation (asterisks).

Figure 8. (A) *IYO* interacts *in vitro* with the Rpb3 sub-unit from RNA Polymerase II as shown by the specific pull-down of Rpb3 together with *IYO*- (B) *IYO* interacts *in vivo* with the Rpb3 and Rpb10 sub-units from RNA Polymerase II as shown by bimolecular reconstitution of YFP in the nucleus. (C) *IYO* is necessary for the activity of RNA Polymerase II in differentiating organs. Left-hand panel: Levels of the Rpb1 sub-unit phosphorylated in Serine 2 of the CTD (Ser2P) in leaf primordia (LP) and in mature leaves (ML) from wild-type (Wt) and *iyo-1* mutant plants (*iyo*). Right-hand panel: Levels of the mRNA of *RPB1* in leaf primordia (LP) or mature leaves (ML) from wild-type (Wt) and *iyo-1* mutant plants (*iyo*) (D) Analysis of the relative expression in flowers versus undifferentiated tissues (calluses or cell cultures) of the 380 genes most significantly induced ($p \leq 0.00015957$) and the 380 most significantly repressed ($p \leq 0.00066388$) in floral meristems (and associated floral primordia) from wild-type plants relative to those from *iyo-1* plants (Wt/*iyo*). (E) Constitutive ubiquitination of Rpb1 in *iyo-1* plants. Extracts (Inputs) from wild (W) or *iyo-1* (i) plants treated (+) or not (-) with MG132 were immunoprecipitated (IP) with antibodies against Rpb1 and

analysed by Western blot with antibodies against Rpb1 or against ubiquitin (Ubq). (F) The over-expression of *IYO* increases resistance to the transcriptional elongation inhibitor 6-azauracil. Plants grown for 11 days in the presence of increasing concentrations of 6-azauracil (in μM) are shown. (G) *IYO* interacts *in vitro* with the histone acetyltransferase ELO3. (H) *IYO* interacts genetically with components of the Elongator complex to activate cell differentiation.

10

Figure 9. Development of *atrtr1-1* (*rtr1-1*) embryos compared to wild type embryos (Wt) at the corresponding stage in the panels above.

15 **Figure 10.** (A) Formation of ectopic shoot apical meristems in *atrtr1-2* plants causes fasciation and thickening of the stem. (B) Ectopic floral meristems give rise to duplicated flowers (and fruits) in *atrtr1-2* plants. (C) Duplicated root apical meristems in *atrtr1-2* plants. (D) Genetic interaction between
20 *AtRTR1* and *IYO*. The double *iy0-1atrtr1-2* mutants grow as an undifferentiated callus. (E) Expression of the UidA (GUS) gene under the control of the *AtRTR1* promoter in apical root meristems, lateral root primordia (arrow) and pericycle. (F)
25 The fusion protein AtRTR1-GFP is excluded from the nuclei in undifferentiated cells of the meristem (arrows).

Figure 11. (A) *atrtr1-2* plants (*rtr1-2*) develop ectopic meristemoids which give rise to clusters of stomas (arrowheads). (B) Cotyledons from *iy0-1* and *atrtr1-2* plants
30 maintain cells which express the stem cell marker pTMM::TMM-GFP (arrowheads) at later stages than cotyledons from wild type plants (Wt).

Figure 12. (A) The AtRTR1-GFP protein is excluded from the
35 nucleus in untreated plants (arrowheads). (B) Treatments with leptomycin B, an inhibitor of Exportin1, causes nuclear accumulation of AtRTR1-GFP (arrows).

Figure 13. *IYO* interacts *in vivo* with the AtRTR1 as shown by bimolecular reconstitution of YFP in the nucleus in two different combinations of fusions of AtRTR1 and IYO with nYFP and cYFP.

5

Figure 14. AtRTR1-YFP accumulates in the nucleus when co-expressed with IYO-HA (AtRTR1-YFP+IYO-HA) but not when expressed on its own (AtRTR1-YFP).

10 **Figure 15.** The *atrtr1-2* and *iyo-1* mutants have almost identical changes in the transcriptome relative to wild type plants. The majority of the 380 genes most significantly induced ($p \leq 0.00015957$) or repressed genes ($p \leq 0.00066388$) in the inflorescence apices of wild-type plants relative to those of
15 *iyo-1* plants (Wt/*iyo-1*) are similarly affected (induced or repressed respectively) in inflorescence apices of wild type plants relative to those of *atrtr1-2* plants (Wt/*art-2*).

Figure 16. Alignment of protein sequences coded by orthologous
20 genes of *MINIYO* in a representative set of plants using the Clustal W program. The ordered sequences, from top to bottom, are those of: *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brachypodium distachyon*, *Citrus clementina*, *Citrus sinensis*, *Manihot sculenta*, *Oryza sativa*, *Populus trichocarpa*, *Prunus*
25 *persica*, *Ricinus communis*, *Setaria italica*, *Sorghum bicolor*, *Vitis vinifera*, *Zea mays*, *Physcomitrella patens*, *Carica papaya*, *Glycine max*, *Medicago truncatula* and *Eucalyptus grandis*.

30 **Figure 17.** Phylogenetic tree constructed from the alignment of the polypeptide sequence of the orthologues of *MINIYO* using the Clustal W programme.

Figure 18. Examples of highly-conserved domains in the *MINIYO*
35 proteins of plants. The sequences, in order from top to bottom, are those of: *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brachypodium distachyon*, *Citrus clementina*, *Citrus sinensis*, *Manihot sculenta*, *Oryza sativa*, *Populus trichocarpa*,

Prunus persica, *Ricinus communis*, *Setaria italica*, *Sorghum bicolor*, *Vitis vinifera*, *Zea mays*, *Carica papaya*, *Glycine max*, *Medicago truncatula*, *Eucalyptus grandis* and *Physcomitrella patens*. The amino acids of the MINIYO protein of *Arabidopsis thaliana* corresponding to the domains shown are: (a) aa. 960-980. This domain contains the RGG motif mutated into the hypomorphic *iy0-1* allele of *Arabidopsis* which is conserved in all the plant orthologues; (b) aa. 209-255; (c) aa. 317-396; (d) aa. 350-389.; (e) aa. 417-437; (f) aa. 529-559; (g) aa. 529-597; (h) aa. 1136-1145; (i) aa. 1144-1416.

Figure 19. Alignment of protein sequences coded by orthologous genes of *AtRTR1* in a representative set of plants using the Clustal W programme. The sequences in order from top to bottom are those of: *Arabidopsis thaliana*, *Carica papaya*, *Cucumis sativa*, *Eucalyptus grandis*, *Glycine max*, *Mimulus guttatus*, *Manihot esculenta*, *Populus trichocarpa*, *Prunus persica*, *Ricinus communis*, *Vitis vinifera*, *Zea mays*, *Sorghum bicolor*, *Setaria italica*, *Oryza sativa*, *Brachypodium distachyon* and *Picea glauca*.

Figure 20. Phylogenetic tree constructed from the alignment of the polypeptide sequences of the orthologues of *AtRTR1* using the Clustal W programme.

Figure 21. Examples of highly-conserved domains in the RTR1 proteins of plants. The sequences, in order from top to bottom, are those of: *Arabidopsis thaliana*, *Carica papaya*, *Cucumis sativa*, *Eucalyptus grandis*, *Glycine max*, *Mimulus guttatus*, *Manihot esculenta*, *Populus trichocarpa*, *Prunus persica*, *Ricinus communis*, *Vitis vinifera*, *Zea mays*, *Sorghum bicolor*, *Setaria italica*, *Oryza sativa*, *Brachypodium distachyon* and *Picea glauca*. The amino acids of the *AtRTR1* protein of *Arabidopsis thaliana* corresponding to the domains shown are: (a) aa. 39-61. This domain includes catalytic cysteine C56 which is strictly conserved in all the orthologues; (b) aa. 39-61; (bc) aa. 77-89; (d) aa. 429-435; (e) aa. 473-507; (f) 552-589.

Figure 22. Seed yield test of a line co-suppressed in *AtRTR1*. Seed yield was measured in wild type *Arabidopsis* plants (Col-0 plants) and in plants from a *35S::AtRTR1-GFP* line displaying co-suppression of the *AtRTR1-GFP* transgene and of the endogenous *AtRTR1* gene (*si-art* plants). Six independent experiments were carried out. The plants were germinated in sterile MS media supplemented with 1% sucrose and transplanted to soil in individual pots after 1 week (10-16 plants per genotype in each experiment). Seeds were collected from individual plants after they were fully dried and the total seed weight measured. For comparing the results of the different experiments, we calculated the relative yield of each plant as the ratio of the yield of that plant relative to the average yield of control plants (Col-0) in that particular experiment. (A) Average relative seed yield of Col-0 and *si-art* plants in all six experiments combined. A 12% increase in the average seed yield of *si-art* plants is observed, with a p-value of 0,006 in an unpaired two tailed t-test. (B) Average relative seed yield of Col-0 and *si-art* plants in each of the six independent experiments. In experiments 3 and 6 the yield of *si-art* plants is increased 32% and 27% over that of the control plants (p-value=0,006). The error bars represent standard deviation.

Figure 23. Phenotype of *35S::IYO-GFP* tomato plants. Shown are untransformed tomato plants of the variety Moneymaker (TMM) and three independent transgenic plants of the same variety expressing a *35S::IYO-GFP* construct (Lines 1-3). Line 1 displays determinate growth of the primary shoot. The arrow marks the terminated primary shoot. Lines 2 and 3 display reduced apical dominance resulting in a shorter stature and increased branching. Arrowheads mark premature outgrowth of lateral buds.

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EXAMPLES

The following specific examples provided in this patent document serve to illustrate the nature of this invention. These examples are included purely for illustrative purposes and should not be interpreted as limiting the invention claimed here. Therefore the examples described below illustrate the invention without limiting the scope of its application.

EXAMPLE 1

1. Identification of the *miniyo-1* (*iy0-1*) mutant. The *iy0-1* mutant is identified in a population mutagenised by ethylmethane sulphonate in the Landsberg erecta ecotype which brought about a change of amino acid (G962E) in the coded protein.

2. Positional map of the *iy0-1* mutant to provide evidence of point mutations in the *IYO* gene. The mutant was crossed with accession Col-0 and it was proved that the *iy0-1* phenotype is inherited in a recessive way. F2 plants with a mutant phenotype were used to clone the mutation through positional mapping. The analysis of more than 1500 mutant plants made it possible to establish the position of the mutation to 6 genes in chromosome 4. Sequencing of the 6 genes showed that there was a single mutation in the At4g38440 gene in *iy0-1* mutants. This was a point mutation (G to A).

3. Generation of ectopic stem cells in an *iy0-1* mutant. It was proved by microscope studies and by analysing the expression of markers that one of the phenotypes of the *iy0-1* mutant was the formation of ectopic stem cells in all meristems of the plant.

4. Total blocking of *IYO* activity in *iy0-2* and *iy0-3* mutants. Mutants were obtained that had a T-DNA insertion in the At4g38440 gene and it was proved that they cause blocking of endosperm development and early arrest of embryogenesis. *iy0-2* has T-DNA insertion at nucleic acid position 234 (Salk

099873). *iy0-2* has T-DNA insertion at nucleic acid position 1357 (Salk 0692_g12).

5. Combination of the hypomorphic allele *iy0-1* with the null alleles (*iy0-2* or *iy0-3*). The allele combination *iy0-1/iy0-2* or *iy0-1/iy0-3* caused almost total blocking of differentiation of the embryo and the formation of ectopic embryos from the suspensor. The embryos were viable and after germination the plants grew as a callus-like structure with multiple meristematic poles and producing only rudimentary leaves.

6. Double embryos in an *iy0-1* mutant. Although with low penetrance, we observed that the *iy0-1* mutant can also form double embryos from the suspensor, which apparently developed normally.

7. Transcriptional and post-transcriptional regulation of the expression of *IYO*.

7.1. Monitoring of activity of the *IYO* promoter.

Through a Northern type analysis, *in situ* hybridisation and studies of the promoter we have demonstrated that the *IYO* gene is expressed specifically during embryogenesis in meristem cells and in cells adjacent to the meristem which are initiating differentiation.

7.2. Nuclear accumulation of *IYO* protein

Confocal microscopic studies of functional fusions of *IYO* protein with GFP which complement the *iy0-1* and *iy0-2* mutants have shown that *IYO* is excluded from the nucleus in undifferentiated cells in a manner which is dependent on Exportin1, while it accumulates in the nucleus in cells which are initiating differentiation.

8. Over-expression of *IYO* or its fusions with proteins or peptides such as GFP, HA, FLAG under the control of the 35S promoter.

Col-0 *Arabidopsis* plants were transformed with constructs which expressed fusions of the epitopes GFP, 3XHA or FLAG at the C-terminal of *IYO* under the control of the 35S promoter.

The lines which accumulated larger quantities of the transgene transcript and the transgenic protein had gain of function phenotypes, from premature differentiation in the shoot apical meristem and compaction of inflorescence but no apparent

effect on the root meristem to termination of the shoot and root apical meristems in lines with the maximum levels of accumulation.

9. Physical interactions between IYO protein and RNA
5 Polymerase II (Pol II) and with elongation complexes. Through pull-down studies with proteins synthesised in bacteria and *in vitro*, studies of bimolecular complementation of YFP, measurements of accumulation of different phosphorylated and ubiquitinated forms of the RPB1 sub-unit and analysis of
10 genetic interactions we have proved that IYO interacts with Pol II and with the elongator complex and is required for maintaining the global levels of elongating Pol II in differentiating tissues.

15 **EXAMPLE 2**

1. Identification of the gene At5g26760 (*atrtr1*). Through the analysis of co-expression we discovered the At5g26760 gene which is highly co-expressed with *IYO* and codes for a protein
20 homologous to RTR1, a yeast protein which functions as a transition phosphatase that dephosphorylates Ser5 in the CTD from the RPB1 sub-unit of Pol II to promote the shift from initiation to productive elongation.

2. Analysis of *AtRTR1* function in the mutant alleles *atrtr1-1*
25 (SALK_012339, T-DNA insertion at nucleic between nucleic acid 414 and 415) and *atrtr1-2* (SALK_115762, T-DNA insertion at nucleic between nucleic acid 864 and 865). We obtained homozygous mutants of *atrtr1-1*, showing by Nomarski microscopy that it caused blocking of differentiation in the embryos, the
30 formation of ectopic embryos from the suspensor and early embryo arrest. The homozygous mutant *atrtr1-2* has phenotypes very similar to the *iyo-1* mutant: enlarged shoot apical meristems, thicker stems, formation of ectopic shoot, floral and root meristems, delayed differentiation of the protodermis
35 and the formation of clusters of stomas.

3. Generation of ectopic stem cells in *atrtr1-2* mutants. Microscopic studies and analysis of marker expression reveal

that ectopic stem cells form in all meristems in the *atrtr1-2* mutants.

4. Investigation of gene interaction between *IYO* and *AtRTR1*. The double mutant *iyo-1atrtr1-2* was obtained and it was shown that cell differentiation was completely blocked, the plants growing as calluses of undifferentiated cells, demonstrating clear gene interaction.

5. Investigation into the over-expression of *IYO* in an *atrtr1-2* mutant context. Heterozygous plants from *IYO-3XHA* over-expressing lines displaying termination of shoot and root meristems were crossed with homozygous *atrtr1-2* plants. In the F₂, homozygous plants for the *IYO-3XHA* construct were recovered that were homozygous for *atrtr1-2* or were wild type for the *AtRTR1* locus. It was proved that while *IYO-3XHA* over-expression in wild type plants produced shoot and root meristem termination, in the *atrtr1-2* background it did not cause any phenotype, demonstrating that *AtRTR1* activity is required for *IYO* function.

6. Investigation into the expression of *AtRTR1* in transgenic plants which express an *AtRTR1* promoter construct and the first three exons and introns of *AtRTR1* translationally fused to the *Uida* (*GUS*) gene. Histological studies of *GUS* activity in transgenic plants expressing the *Uida* gene under the control of the *AtRTR1* promoter show that this is specifically located in embryos, meristem cells and cells adjacent to the meristem which are beginning to differentiate.

7. Investigation into the accumulation of *RTR1* fused to GFP under the control of the constitutive 35S promoter and the *AtRTR1* promoter. Confocal microscopy studies of plants expressing stable functional fusions of *AtRTR1* protein to GFP which complement *atrtr1-1* mutants have demonstrated that *AtRTR1* is excluded from the nucleus in undifferentiated cells in a form dependent on Exportin1.

Example 3 Bioinformatics analysis

To search for candidate nuclear localization signals (NLS), the Arabidopsis *IYO* and *AtRTR1* protein sequences were

submitted to the cNLS mapper server (<http://nls-mapper.iab.keio.ac.jp>). This tool searches for classical NLS (cNLS) recognized by the nuclear receptor importin α . cNLSs detected with this tool have been validated in a number of yeast proteins (Kosugi et al., PNAS 2009). The server predicted in IYO a monopartite NLS (aa. 250-262) and a bipartite NLS (aa. 1397-1420), both with a score of 10, the maximum possible. When a GUS-GFP reporter protein was fused with NLSs having a score between 8-10 they accumulated in the nucleus (Kosugi et al., PNAS 2009, JBC 2009). In the case of AtRTR1, the server predicted a bipartite NLS with a long linker and a score of 5.2 in the middle of the protein (aa. 340-368). GUS-GFP proteins fused with NLSs with a score 3-7 were localized in the cytosol and the nucleus (Kosugi et al., PNAS 2009, JBC 2009). Moreover, when bi-partite NLSs have long linkers they are functional normally in unstructured regions, which frequently coincide with the N- and C-terminal ends of the proteins. Thus, for IYO, two high confidence cNLSs were predicted, while for AtRTR1, no clear cNLSs were detected. It is possible that AtRTR1 is imported through an importin α - independent pathway, as is the case for the majority of nuclear proteins. In this regard, we have shown that nuclear IYO leads to AtRTR1 accumulation in the nucleus, which could be by mediating its import.

To search for candidate nuclear export signals (NES), the Arabidopsis IYO and AtRTR1 protein sequences were submitted to the NetNES server (<http://www.cbs.dtu.dk/services/NetNES>). This tool searches for leucine-rich NES, and predictions have been validated (for example for BRCA1, Thompson et al., JBC 2005). The server predicted a NES in IYO (aa. 432-440) and in AtRTR1 (aa 340-349). Consistent with these predictions, inhibition of the NES receptor CRM1 with leptomycin B, leads to nuclear accumulation of IYO and AtRTR1 in Arabidopsis and Nicotiana cells, suggesting that their nuclear export is NES-dependent. Moreover the truncated C-terminal half of AtRTR1 (aa 305-735) that contains the predicted NES is exported from the nucleus.

To test for altered localization, mutated versions of the proteins are fused to GFP and their subcellular distribution by transient expression in *Nicotiana benthamiana* leaves is analysed (where wild type IYO accumulates primarily in the nucleus and AtRTR1 in the cytosol). Analysis is also carried out in *Arabidopsis* cell cultures untreated (where both proteins are localized in the cytosol) or treated with leptomycin B (where both proteins are localized in the nucleus). In the case of IYO constructs are designed lacking each predicted NLSs and one combining mutations in both NLSs, as it has been shown in other proteins with multiple NLSs that only after mutating all of them is their nuclear import blocked (i.e. Zhang et al., PNAS 2000; Yeung et al., J. Cell Biochem. 2008).

15 Homology Analysis

Alignment by clustalW of the whole polypeptide sequence shows that the % identity between IYO orthologues from embryophytes (*A thaliana*, *A lyrata*, *Brachypodium*, *Carica*, *citrus*, *Eucalyptus*, *Manihot*, *Medicago*, *Oryza*, *Physcomitrella*, *Populus*, *Prunus*, *Ricinus*, *Selaginella*, *Sorghum*, *Vitis*, *Zea*) is higher than 30%. If only angiosperm sequences are aligned the overall identity is higher than 39%.

Alignment by clustalW of the whole polypeptide sequence shows that the % identity between IYO orthologues from embryophytes (*A thaliana*, *A lyrata*, *Brachypodium*, *Carica*, *citrus*, *Eucalyptus*, *Manihot*, *Medicago*, *Oryza*, *Physcomitrella*, *Populus*, *Prunus*, *Ricinus*, *Picea*, *Sorghum*, *Vitis*, *Zea*) is higher than 25%. If only spermatophyte sequences are aligned the overall identity is higher than 32%.

An Interpro scan of the polypeptide sequence of IYO reveals two conserved domains IPR013929 (PF08620) and IPR013930 (PF08621) in the N-terminus of the protein (aa 209-255 and 317-396, respectively), which are found in orthologues from plants, animals and fungi. Moreover, blast searches reveal two other domains highly conserved in IYO orthologues from

multicellular eukaryotes (aa 529-597 and 1144-1416). In addition, IYO has a glycine rich domain with an RGG motif (aa 960-980) that is strictly conserved in orthologues from plants. Glycine rich domains and RGG boxes have been linked to nucleic acid binding (Gendra et al., Plant Journal 2004).
 5 Moreover, the *iyo-1* allele is a missense mutation that changes the motif from RGG to RGE and reduces the transcriptional activity of the protein. This indicates that this domain contacts the DNA or the nascent transcript to facilitate
 10 transcription. This domain is not clearly identifiable in the animal orthologues of IYO, but alignment of their sequences reveals a high number of conserved glycines in this region.

AtRTR1 contains a conserved domain (DUF408) with a zinc-finger like motif located at the N-terminus of the protein that is
 15 found in all the orthologues from plants, animals and fungi. A consensus sequence for that motif derived from sequences from multicellular eukaryotes is (in bold the putative Zinc-coordinating
 cysteines):
 D[IV]V[TDEV]ER[ASTF]I[AVIS][KND][LAV]**CGY**[TP][LRA]**CXXXXLX**₇-
 20 ₁₅[YF][RK]IS[LT][KSR][TAED][HKN][KR]VYD[IL][THEQ]EXXX[FY]**CXXXC**

A blast search against the non-redundant protein sequence database at NCBI with the corresponding sequence from Arabidopsis
 DVVTERAIAKL**CGYTL****CQRFL**PSDVSRRGKYRISLKDHKVYDLQETSKF**CSAGC**
 25 retrieved the AtRTR1 orthologues from plants and animals and fungi with a low E-value ($< 10^{-6}$).

The zinc-finger-like-motif has been implicated in interaction with the RNA Polymerase II C-terminal domain (CTD) and the Integrator complex in humans and is required for CTD-phosphatase activity in yeast and humans (Mosley et al., 2009; Egloff et al., 2011). Interestingly, this motif is also
 30 required for interaction of AtRTR1 with IYO. Substituting for alanine the putative zinc coordinating cysteine residues (C56A/C61A or C94A/C98A) in the full-length AtRTR1 protein
 35 abrogates interaction with IYO. Intriguingly, however, both the truncated N-terminal and the C-terminal halves of AtRTR1

can interact with IYO, suggesting that although AtRTR1 binds at both ends of the protein to IYO, it requires an intact zinc-finger-like motif in the context of the full length protein.

5 **EXAMPLE 4**

We analyzed the expression pattern of an *AtRTR1* promoter construct driving the GUS reporter gene (*pART::GUS*). This same promoter driving an *AtRTR1* cDNA fully complements *atrtr1* mutant phenotypes, indicating that it reproduces the activity
10 of the endogenous gene. In roots, *pART::GUS* was strongly expressed in root apical meristem (RAM) and in transition cells, in the pericycle layer and in lateral root primordia. In the aerial part of the plant, *pAtRTR1::GUS* was expressed in the shoot apical meristem (SAM), in leaf and flower primordia,
15 in unfertilized ovules and in developing embryos, but not in mature organs. These results suggest that *AtRTR1* is exclusively expressed at sites of active cell proliferation and differentiation, in a pattern highly similar to that of *IYO*.

20

To determine the subcellular distribution of AtRTR1 we analyzed a translational fusion to GFP. Under the control of the constitutive 35S promoter (*35S::AtRTR1-GFP*) we only obtained transgenic lines expressing low levels of the tagged
25 protein that complemented partially the *atrtr1-1* null mutation (i.e: *atrtr1-1* plants transgenic for this construct were viable but resembled the hypomorphic *atrtr1-2* plants). These results suggest that expressing high levels of AtRTR1 protein in a constitutive manner may be deleterious for plant
30 development. We then transformed plants with AtRTR1-GFP driven by its own promoter (*pAtRTR1::AtRTR1-GFP*). The resulting lines had higher levels of expression and complemented fully the *atrtr1-1* null mutation. This indicates that *pAtRTR1::AtRTR1-GFP* reproduces the activity of the endogenous gene and can be
35 used as a proxy for localization of ART. *pAtRTR1::AtRTR1-GFP* fluorescence in the root was restricted to the tip, consistent

with the pattern of expression found in *pAtRTR1-GUS* lines. Importantly, the fluorescence was found in the cytosol and strongly excluded from the nucleus (Figure 6A-B). Similarly, in *Nicotiana benthamiana* leaf cells transiently expressing a
5 *35S::ATRTR1-GFP* construct, fluorescence was confined to the cytosol. In yeast and mammalian cells, the orthologues of ATRTR1, RTR1 and RPAP2, are also localized primarily in the cytosol, but they redistribute partially to the nucleus upon inhibition of the XPO1 nuclear export receptor with leptomycin
10 B (LMB), which also inhibits the Arabidopsis receptor (Kudo et al., 1999, Haasen et al., 1999). After treating Arabidopsis with LMB, nuclear accumulation of ATRTR1-GFP was observed in cells of the root transition zone and in *Nicotiana benthamiana* leaf epidermal cells. These results suggest that, at least in
15 differentiating and mature cells, AtRTR1 is imported into the nucleus, although the higher rate of nuclear export leads to its steady state accumulation in the cytosol. The remarkable conservation in nuclear-cytoplasmic shuttling of RTR1 homologues in all eukaryotic lineages indicates that it
20 constitutes an important regulatory mechanism for this family of phosphatases.

We tested for the *in vivo* interaction between IYO and AtRTR1 through a bimolecular fluorescence complementation assay in
25 epidermal cells from *Nicotiana benthamiana* leaves. YFP complementation was observed with different combinations of split YFP fused at the N- or C-terminus of the respective proteins and not in any of the multiple negative controls tested. Interestingly, the reconstituted fluorescence was
30 localized in the nucleus, suggesting that these proteins interact specifically in this compartment, possibly to regulate transcription. Moreover, we found that both the DUF408-containing N-terminal half and the C-terminal half of AtRTR1 interact with IYO, suggesting that the two proteins
35 bind through at least two sites. Unexpectedly, substituting the putative zinc coordinating cysteine residues for alanines (C56A/C61A or C94A/C98A) in the full-length AtRTR1 protein abrogates the interaction with IYO, suggesting that although

AtRTR1 binds at both ends of the protein to IYO, it requires an intact zinc-finger-like motif for binding in the context of the full length protein.

5 Considering that AtRTR1-GFP expressed in *Nicotiana benthamiana* cells is found exclusively in the cytosol, it was surprising to find AtRTR1 strongly interacting with IYO in the nucleus. We reasoned that when bound to IYO, AtRTR1 is retained in the nucleus. To test this we expressed AtRTR1-GFP together with IYO-HA or an empty vector. Importantly, co-expression with
10 IYO-HA led to nuclear AtRTR1-GFP accumulation in *Nicotiana* cells, confirming that IYO retains AtRTR1 in the nucleus. The levels of nuclear fluorescence were much lower than in the split YFP assays, where the IYO-AtRTR1 complex is stabilized through the irreversible reconstitution of YFP. This suggest
15 that the IYO-AtRTR1 association is very transient, explaining why nuclear AtRTR1 accumulation cannot detected in transition cells of the meristem, even though IYO is present in the nucleus of those cells.

20 To test for genetic interaction between *IYO* and *ATRTR1*, we combined the *atrtr1-2* with the *iy0-1* hypomorphic mutations. Differentiation in the *iy0-1atrtr1-2* double mutants was almost completely blocked and the plants eventually developed as a friable callus of undifferentiated cells. This phenotype is
25 much stronger than the sum of the phenotypes of the single mutants, and indicates a strong genetic interaction of IYO and AtRTR1 in the control of cell differentiation, supporting a close functional interaction. Transcriptome analysis of *iy0-1* mutants supports that IYO functions as a global
30 transcriptional regulator of developmental programs. In inflorescence meristems, IYO was required for proper expression of flower development programs, including activating the expression of the homeotic flower organ identity genes, which are the master regulators of
35 organogenesis in those meristems. We performed a similar analysis in the *atrtr1-2* mutant and we found a very high overlap (>80%) in the up-regulated and down-regulated genes in

iyo-1 and *atrtr1-2* inflorescences meristems relative to wild type. These results indicate that IYO and AtRTR1 regulate as a complex transcription of developmental programs. Consistent with their functioning together, we found that a functional AtRTR1 gene is required for IYO activity in cell differentiation. Over expression of IYO-HA provokes premature differentiation and termination of the root and shoot apical meristems. Importantly, in an *atrtr1-2* background or in *AtRTR1* co-suppressed line, these effects of IYO-HA over expression are eliminated, demonstrating that IYO requires AtRTR1 for its activity.

EXAMPLE 5 YIELD ANALYSIS IN ARABIDOPSIS

We have measured seed yield in a line co-suppressed in *AtRTR1*, which was chosen because it had a weak loss of function phenotype. This line was characterized by a few extra shoot meristems, but otherwise normal development.

Seed yield test

During the generation of lines transgenic for a *35S::AtRTR1-GFP* construct, we isolated a line (*si-art* line) showing co-suppression of the transgene and of the endogenous *AtRTR1* gene. The phenotype of *si-art* plants is weaker than that of the hypomorphic *atrtr1-2* allele, forming some ectopic shoot apical meristems (SAMs) that give rise to split primary shoots but otherwise developing very similarly to wild type plants. To test if the formation of ectopic SAMs affects yield, we measured seed production in *si-art* plants and in the corresponding wild type background (Col-0). We carried out six independent experiments (experiments 1-3 in the greenhouse, 4-6 in a growth chamber) and harvested seeds after the plants were fully dried. In each experiment, we measured the seed yield (in weight) of individual plants, and then calculated their yield relative to the average yield of control plants (Col-0) in that particular experiment. Combining in this way the data from all six experiments, we found a 12% increase in

the average seed yield of *si-art* plants, with a p-value of 0,006 in an unpaired two tailed t-test. The results are shown in Figure 22. If the individual experiments are analyzed separately, we find significant differences (p-value<0,05) between the average yields in experiments 3 and 6, in which the yield of *si-art* plants is increased 32% and 27%, respectively over that of the control plants (p-value=0,006).

EXAMPLE 6 EXPERIMENTS IN TOMATO

10

Tomato plants of the cultivar Moneymaker were transformed by co-cultivation with *Agrobacterium tumefaciens* with a sequence coding for the Arabidopsis IYO protein fused to GFP under the control of the 35S promoter. This construct fully complements the phenotypes of weak (*iy0-1*) and null (*iy0-2*) alleles in Arabidopsis. Plants were regenerated from independent transformed calli and transplanted to soil. We analyzed roots from those lines in the confocal microscope and observed accumulation of GFP fluorescence in nuclei of differentiated cells, demonstrating that they are transgenic for the construct and that subcellular localization of the IYO protein in tomato is the same as in Arabidopsis. The development of the transgenic lines reveals that the Arabidopsis protein is functional in tomato and that its overexpression provokes premature onset of cell differentiation as it does in Arabidopsis. Some of the transgenic lines (e.g. Line 1, Fig 23) show a determinate growth pattern, in contrast to untransformed Moneymaker plants that are indeterminate (Figure 23). In other lines (e.g. Lines 2 and 3, Fig 23) the branching pattern is altered (Figure 23). The transgenic lines are fertile and produces fruits with viable seeds.

Sequence listing

SEQ ID NO:1 MINIYO cDNA Arabidopsis thaliana

35

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ATGGAGCAAAGTAGCGGGAGAGTCAATCCGGAACAGCCGAACAACGTCTTGGCGAGCCTTGTCTGGGAGCATCGTGGA  
GAAAGGAATATCGGAGAATAAGCCTCCAAGCAAGCCGCTTCCCCAAGGCCCTCCCTTCTTTCTTCCCGTCTGCTCGT  
CATCGTTCTCACGGACCCCATTTGGCTCCTGTGGAAGCAGCATAGCACAACTAAGGATTACAATGACGATCAGGAAG
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AAGAAGAAGCAGAAGAACGTTTCATGAATGCAGACTCCATTGCTGCTTTTGCTAAACCGCTTCAAAGAAAAAGAGAAGAAA
 GACATGGACCTCGGGAGGTGAAAGATATGGTCTCTGGGGATGATCCTGCATCCACACATGTCCTCAGCAATCAAGG
 AAACCTTAAGATCATTGAAACGAGACCGCCCTATGTTGCTTCAGCCGATGCGGCCACTACATCCAGCAACACTTTACTGG
 CTGCCAGGGCATCAGACCAGAGAGAGTTTGTCTGATAAAGCACCGTTTATTAATAAATTTGGGAACCAAGGAAAGGGTT
 5 CCTTTAAACGCTTCTCCTCCCCTAGCTGTTTGAATGGACTTGGGACTCGACACGCGTCTTCGTCTCTTGAAGTGATAT
 TGATGTTGAGAACCATGCAAAGTTGCAGACAATGTCACCCGACGAGATTGCTGAGGCTCAGGCTGAGTTATTGGACAAG
 ATGGATCCTGCACTACTCTCCATTTGAAGAAACGAGGTGAGGCAAATTAAGAAGCGAAAGCATTCTGTGCAGGGGG
 TTTCCATCACCGATGAAACAGCAAAGAATCAAGAAGTCAAGGCTGATTTTGTACTCCTAAAGTGATGGCAATACCGAAA
 GAAAAAAGTGTGGTGCAAAGCCAGGGATAGCCCAAGGATTCGTGTGGGATGCATGGACTGAGAGGGTTGAGGCAGCC
 10 AGAGACTTGAGATTTTCTTTGACGGGAATGTTGTTGAGGAAGATGTTGTCTCGCCAGCTGAAACTGGTGGAAAGTGGT
 CTGGTGTGAATCTGCTGCCGAACGTGATTTCTTGAGAACCGAGGGGGATCCTGGGGCCGAGGTTACACTATCAAAG
 AAGCTATTGCTCTTGACGAAGTGTGATTTCCCGGGCAGAGATGTCTTGCTTTGCATCTGCTTGCATCTGTACTCGACAAA
 GCTTTGAACAACTTTGTCAAAGCAGAATAGGCTACGCAAGGGAAGAAAAAGATAAATCCACTGACTGGGAAGCCATCT
 GGGCTTATGCCCTTGACCGGAACCTGAGCTTGTCTTAGCATTGAGGATGGCTCTTGATGACAACCATGCCTCTGTTGT
 15 TATAGCATGTGTAAGTGTGATTCACTGAGTGTCTACTGAGCTGTTCTTAAACGAGAATTTCTTAATATTCTGGAGAACATGGG
 ACCACACGGGAAAGATATCTTACGGCCTCGGTGTTGAGGATAAGCCGGAATTTGATCTTGGCTTCCCTCGTGGTTGC
 TACTGGAAGTACAGCGCTAAACCCCTCCAATATTGTTGCGTTCCGTGAAGAAATCTGGATGACGGGACAGAAGATCGG
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 ATCACCTTCTGGAGACAGAACCAACAGCAGCGCTTGAGGACAGCATAATCTCTGTTACTATTGCGATAGCAAGGCATTCT
 20 CCAAAATGCACAACCTGCAATCTTGAAGTATCCCAAATTTGTGCAAACAATTGTGAAAAGATTCCAATTGAACAAAAGAATG
 GACGTTCTTTCTCTCAGATCAACTCTGTCCGCTCTTAAAGGTGTTGGCCCGGATGATCAAAGTACTTGCATGGAATT
 TGTGAAAGTGGGACTTTCAATGCGGTACATGGCATTGTTTCAGTTCACCTCATCTCTTACTCATGGGTGAAGCTAG
 GGAAGCAGAACTGCAAGCTTTCATCTACCTTGTGTTGAACAGCTCCGTTTTGGAAGGTCTGTATCCATAGTGGCTG
 TTGCGTATCTCGCTCCAGAGCTATTCCAGCTCTGTGTCTGTGGTTGAGTTGTCCATCATTGAAAAGCTCAGGGAGA
 25 AAAATCTCATCAGCGAGTTTACTTCTGTGTCAAACGAGGCCTACCTGGTCTTGGGCTTTTGGCAGACACTTCCCTAAT
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55 SEQ ID NO: 2 nucleic acid sequence mutant *iy0-1* Arabidopsis thaliana

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SEQ ID NO: 3 Arabidopsis thaliana nucleic acid sequence mutant
iy0-2

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SEQ ID NO: 6 *Arabidopsis thaliana*, protein allele *iyo-1*

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SEQ ID NO: 8 *Arabidopsis thaliana*, nucleic acid sequence
25 AtRTR1.

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35 SEQ ID NO: 11 *Arabidopsis thaliana*, Protein sequence AtRTR1

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 10 GVFHQAMWHWYRKAYTLEDWIRSGKEHCKLTSALMVEQLRFWRTCISYGFICITHFTDFFPILCLWLS
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 15 PGVGFGWGAYGGGFWSLNFLLAQLDLSHFVLELMKILSTGPEGLVTVNKSVNPIVQEGNNVTDVAITS
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 20 IEQFAAVSYGDALFGRQVAIYLHRSVEPTIRLAAWNALSNAVLELLPPLDKCVGDVQGYLEPLEDDE
 GILES YAKSWTSGALDKAFQRDAMSFTVARHHLSGFVFQCSGSGKVRNKLKSLIRCYGQKRHHEDML
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SEQ ID No. 13 MINIYO Zea mays, GRMZM2G156818_T01

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 30 TLDGDILGFQSSHEQQDGKKMPSESVAERDFLRTEGDPAAVGYTINEAVALTRSMVPGQRVLLALQLLA
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 40 LLAQLDSQLVVELIKCFSSVQGSPIILDEGVKLDNVTNTVVTASNWISSTLGLSLIAGPGQIYMLEKV
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 45 QVAIYLHRKAEPVRLAAWNALS SAYVLELLPPLDNCIGNAPGYLEPLEDDEKILES YAKSWTSGVLD
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SEQ ID No: 14 MINIYO Glycine max, Glyma01g08040.1

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15

SEQ ID No: 15 MINIYO Glyma02g13360.1

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 20 SVNGHAQSPQDAKHLHTEDGIAQTVIVPPSKEKLDDEKISTKTSTTASSSAWNAWSNRVEAVRELRF S
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 ICEDRTGHMTKIENKVDKSV DWEAVWAFALGPEPELVLSLRICLDDNHNSVVLACAKVVQCVLSYDAN
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 25 DIVVAGQDFTVGLVRMGILPRLRYLLETDP TTALEECIISVLI AIAARHSPTCANAVLKCERLVQTIAN
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 SATPLLVVYAAVTHMLFRVLERMTWGD TIETEGHVPWLPEFVPKIGLEVIKYWFLGFSASF GAKCGRD
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 SDTSSVTTPCCNSIMIEWAHQKLPVHFYLSPISTIFH SKRAGTKIVDDVLDHDP SNLLEVAKCGLFF
 VLGVEAMSIFHGTDIPSPVQVSLTWKLSL SVNFLVGM EILEQDWSRDIFEALQDLYGELL DNARLN
 35 QSKEVISDDKKHLEFLRFQTEIHESYSTFLEELVEQFSAVS YGDVIFGRQVSLYLHRCVETSIRLAAW
 NTLNSRVLLELLPPLEKCFSGAEGYLEPAEDNEAILEAYTNLWVSDALDRAAIRGSVAYTLVVHHLSS
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40

SEQ ID No: 16 MINIYO Brachypodium distachyon, BD1G37370

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 50 AEEEGDEEHTIQDDVVVSGQDAAGLIRMGILPRICSLLEMDPPP ILEDYLVSTLVALARHSPQSADA
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 5 WSLKCLLAQLDSQLVLELIKIFSAVPEVLVTPSKGVNSDNVTNPVAKASGRISPVLGVSLIAGPGQIT
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 10 QHLDMLCHKYYRSHSVKNDEVVGSVTTVEEAKAISLEILGFKEKIHGSYTTTFVESVIDQFAAVSYGD
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SEQ ID No: 17 MINIYO Sorghum bicolor, Sb10g022700

15 MDAPTKRRHQPGGAHPTRRKVVEEPFHPAPPTPAAAAAASASPARLVGAIVEKGFSAAPSSAPRP
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40 SEQ ID No: 18 MINIYO Oryza sativa ssp. japonica , >OS06G37640,
 Gene

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40 SEQ ID No: 24 RTR1 *Glycine max*, Glyma02g34860.1

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SEQ ID No: 27 RTR1 Brachypodium distachyon Bradi2g38650

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SEq ID No. 48 MINIYO promoter

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 GACCCGTAAGCCAATCCAATCCTCTAGTGGTGCTTTTTAGGTTCATCTTCCCTTTGTTGCCTTCT
 TCTAGATTTAAGCACCTTCTACTGTTGTTTAGTACTTGGGACTCCACAATTTTTACCCTGCCTGAC
 30 CTTGTAATTCAGCTTTCTGAGACATCTAATTTTTGTTTCTCATGTTTGATTTTGTAGCATTGGCTC

SEQ ID No. 49 AtRTR1 promoter

TGAATCATTCTCAAAAAGAAAATGGGAAAAATGTCATTCAAATAATCAGTTTACCATTTTCGTTGGT
 35 TTTAAACATAAATTTTGGACCTGGTGATTTAAATCCTCAATATTATGTTGACTTTCAGTTTAAACACA
 AATTTTCATTGATTAAGAGACATCGTTAGAAATCCCTAGATCACATACCCTTTATCCCAAACCGAA
 AACCGATTTTGGATTCCCTCTTCTTCTTCTCGAATTCGAAGTAATCTCTTGTCTGGAGGTTGACTGATG
 GCGTAAAAAAGAAGAATTTGTATCTCAATTAGTTTAGTTTACAAGAACTCGTGATTAATTTGAAAGT
 CAAAATAAAAATGAGAATTTAAATTACCAAATCAAGAGTTTTTCATATTTAAATGGTAAACTGATGAC
 40 ATTTTCCCTGTTGAACAATATTGGCCATAATGTAACCCAATTAATCGGCCAATTACGTGAACCGCC
 TTTACCTGGCTTAAGGAATAAGTAAGGACCATTCATGATCTCATCACTTTTAGCTTTCTGGCTTCTC
 TGCTTAAGCTCTCTCGAGTCTGCCTCAAGTGTTTTTGGGGGAAATGATTTTCGTTGAGAAAAACCTA
 AATTCCGAACCTGAAGCAATTTTTCAATTTTCGTTTGCAGAAAAATGGCAAAGGATAATGAAGCAATCG
 CCATTAACGATGCGGTTTACAAGCTTCAAGCTCTATATGCTCGAAAATACCACTGATCAGAACCAGCTC
 45 TTCGCGGCGAGGAAGTTAATGTCTCGATCAGATTACGAAGATGTCGTCACTGAACGAGCAATCGCTAA
 GCTCTGTGGTTATACTCTTTGCCAGAGATTTCTCCCTTCCGATGTTCTAGAAGAGGGAAGTATCGGAT
 TTCGTTGAAGGACCATAAGGTTTACGATTTACAGGAGACGAGCAAGTTTTGCTCCGCTGGTTGTTTAA
 TTGATAGCAAACGTTTTTCGGGGAGTTTGCAAGAGGCTCGTACATTGGAGTTTGAATCGGTGAAGTTG
 AATGAGATTTTGGATTTGTTTGGTGATTCTTTGGAAGTGAAAGGTTCTTTGGATGTGAATAAGGATTT
 50 GGATTTGCTAAGCTTATGATTAAGGAGAATTTGGAGTTAGAGGTGAAGAATTGCTTTTAGAGAAGT
 GGATGGGTCCTTCTAATGCTGTTGAAGGTTATGTTCCCTTTTATCGAAGCAAATCAAGTAATGGTAAG
 TTCGATGATGAACCTATGGTGTGAGCAAAAAATTCAGTTAAACAAATGTTTTATCGATGTGAATAAAT
 AAGTTTGGTTTTGGCAGATTCCAAGGCTACTACTCAAAGTAATCAAGAGAAGCATGAGATGGATTTCA
 CTAGCACAGTAATTATGCCTGATGTTAATAGTGTTCAAAGCTTCCACCGCAAACCAAGCAAGCTTCT

ACTGTTGTGGAATCTGTTGATGGCAAAGGGAAAACAGTTCTGAAAGAGCAAACCTGTAGTTCCTCCCAC
CAAAAAAGTTTCGAGTAAGCATTAAAGGAGTTTTTAAGAGTAATAGGCCTTATGACCAACATATCTCTA
AGAAATGTAGCTGTATATGTTATTTAGTCTTGCTTAAAGGTATTTGGATGGTATCATGAATGTTTTGA
5 TTTATTCGTCGGAGAGACAGATCTTTTGGTGGTTATTAGGCCATTTCTACTGATGGGTGAAGCAATAA
ATGTCGTTGTCCTTGCTCTCTGTTTATCTGAGTCTTAATGAATCTAATGTGTGTCTGCAGGATTTTCG
TCGTGAGAAAGAAAAGGAGAAGAAGACTTTCGGGGTTGATGGGATGGGTTGTGCCAGGAAAAAACTA
CAGTTCTCCCCAGAAAAATATTGAGTAAGCACTTAGGAAGCTGTGAAGATAGTTAGGCCTTACTTTCA
AGATATCTCTTAAAATAATCTGTATATGTTACGTTTTTTTCATTTTGCTGTATTCATTTGGTATCTCG
AATGAGATTCTTTATTCCTTGGGTCTCTAAGTTGTTCTAATGATTGTTAGGCAGTTTTTGTGCCTGTG
10 TGA CTCTGTTTATCTGTCTAACATATGCAGGTTTTTGTAATGAAATAGAGAAGGATT

CLAIMS

1. An isolated nucleic acid sequence comprising a nucleotide sequence encoding for an amino acid sequence of SEQ ID NO: 5
5 or an orthologue thereof.
2. An isolated nucleic acid sequence according to claim 1 wherein said orthologue is at least 30% identical to SEQ ID NO: 5.
10
3. An isolated nucleic acid sequence according to claim 1 or 2 wherein said nucleic acid sequence is SEQ ID No. 1.
4. An isolated nucleic acid sequence comprising a nucleotide sequence encoding for amino acid sequence of SEQ ID NO: 11 or
15 an orthologue thereof.
5. An isolated nucleic acid sequence according to claim 4 wherein said orthologue is at least 30% identical to SEQ ID
20 NO: 11.
6. An isolated nucleic acid sequence according to claim 4 or 5 wherein said nucleic acid sequence is SEQ ID No. 8.
- 25 7. An expression vector comprising the isolated nucleic acid sequence as defined in any of claims 1 to 6 characterised in that expression of the nucleic acid sequence is under the control of a promoter sequence.
- 30 8. A host cell which comprises the expression vector defined in claim 7.
9. A transgenic plant wherein the activity of a MINIYO and/or RTR1 polypeptide is inactivated, repressed or down-regulated.
35
10. A transgenic plant wherein the expression of a gene encoding a MINIYO and/or RTR1 polypeptide is inactivated, repressed or down-regulated.

11. A transgenic plant according to any of claims 9 or 10 wherein said MINIYO protein is at least 30% identical to the sequences coded by SEQ ID NO:1.

5

12. A transgenic plant according to claim 11 wherein said MINIYO protein comprises SEQ ID No. 5.

10

13. A transgenic plant according to any of claims 9 to 12 wherein said RTR1 protein is at least 30% identical to the sequences coded by SEQ ID NO:8.

14. A transgenic plant according to claim 13 wherein said RTR1 protein comprises SEQ ID No. 11.

15

15. A transgenic plant according to any of claims 9 to 14 characterised in that in comparison with the wild phenotype plant said plant has a reduction of between 50% and 100% in the expression of an amino acid sequence of the MINIYO and/or RTR1 protein.

20

16. A transgenic plant according to any of claims 9 to 15 wherein the endogenous *MINIYO* and/or RTR1 gene carries a functional mutation.

25

17. A transgenic plant according to any of claims 9 to 16 wherein said plant expresses a transgene said transgene comprising a modified *MINIYO* and/or or RTR1 nucleic acid sequence when compared to a wild type sequence.

30

18. A transgenic plant according to claim 17 wherein said modification in the *MINIYO* nucleic acid results in a polypeptide that has a substitution of the second conserved G in the RGG motif.

35

19. A transgenic plant according to claim 17 wherein said modification is a substitution or deletion of one or more

residues within one or more of the nuclear localisation signals present in the *MINIYO* and/or *RTR1* protein.

20. A transgenic plant wherein the activity of a *MINIYO* and/or *RTR1* polypeptide is increased or up-regulated.

21. A transgenic plant wherein the expression of a gene encoding a *MINIYO* and/or *RTR1* polypeptide is increased or up-regulated.

10

22. A transgenic plant according to claim 20 or 21 wherein said plant overexpresses a nucleic acid encoding for a *MINIYO* protein that is at least 30% identical to the sequences coded by SEQ ID NO:1.

15

23. A transgenic plant according to claim 22 wherein said *MINIYO* protein comprises SEQ ID No. 5.

24. A transgenic plant according to any of claims 20 to 23 wherein said *RTR1* protein is at least 30% identical to the sequences coded by SEQ ID NO:8.

20

25. A transgenic plant according to claim 24 wherein said *RTR1* protein comprises SEQ ID No. 11.

25

26. A transgenic plant according to any of claims 20 to 25 wherein said plant expresses a transgene said transgene comprising a modified *MINIYO* and/or *RTR1* nucleic acid sequence when compared to a wild type sequence.

30

27. A transgenic plant according to claim 26 wherein said modification is a substitution or deletion of one or more residues within one or more nuclear export signal present in the *MINIYO* and/or *RTR1* protein.

35

28. A transgenic plant according to any of claims 9 to 27, characterised in that the plant belongs to the superfamily *Viridiplantae*.

29. A transgenic plant according to claim 28, characterised in that the plant is a crop plant.

5 30. A product obtained from the transgenic plant defined in any of claims 9 to 29 wherein said product is selected from seed, stem, leaf, flower, root, flour and fruit.

10 31. Use of an isolated nucleic acid sequence comprising a nucleotide sequence coding for an amino acid sequence which is at least 30% identical to the sequences coded by SEQ ID NO: 1 to control the initiation of cell differentiation in plant apical, root and/or floral meristems.

15 32. A use according to claim 31 wherein said sequences is a modified *MINIYO* nucleic acid sequence when compared to a wild type sequence.

20 33. Use of an isolated nucleic acid sequence comprising a nucleotide sequence coding for an amino acid sequence which is at least 30% identical to the sequences coded by SEQ ID NO: 8 to control the initiation of cell differentiation in plant apical, root and/or floral meristems.

25 34. A use according to claim 33 wherein said sequence is a modified *RTR1* nucleic acid sequence when compared to a wild type sequence.

30 35. A method for altering plant architecture by increasing or decreasing activity of the *MINIYO* and/or *RTR1* protein.

35 36. A method for delaying the onset of cell differentiation and increasing the number of undifferentiated cells in a plant said method comprising decreasing the activity of a *MINIYO* protein which is at least 30% identical to the sequences encoded by SEQ ID NO: 1 and/or 8.

37. A method for increasing cell differentiation in a plant said method comprising increasing the activity of a MINIYO protein which is at least 30% identical to the sequences encoded by SEQ ID NO: 1 and/or 8.

5

38. A method for increasing yield of a plant by increasing or decreasing activity of the MINIYO and/or RTR1 protein.

39. An isolated nucleic acid sequence comprising SEQ ID No. 48.

10

40. An expression construct comprising a nucleic acid sequence according to claim 37 operably linked to a gene sequence to direct expression of the target gene in meristems and in cells in the early stages of differentiation.

15

41. An isolated nucleic acid sequence comprising a nucleotide sequence encoding for an amino acid sequence of SEQ ID NO: SEQ ID No. 49.

20

42. An expression construct comprising a nucleic acid sequence according to claim 39 operably linked to a gene sequence to direct expression of the target gene sites of cell differentiation and proliferation.

25

43. Use of an isolated nucleic acid sequence as define in claims 39 or 41 or of a vector as defined in claims 42 in directing spatial and temporal expression of a target gene.

FIG. 1

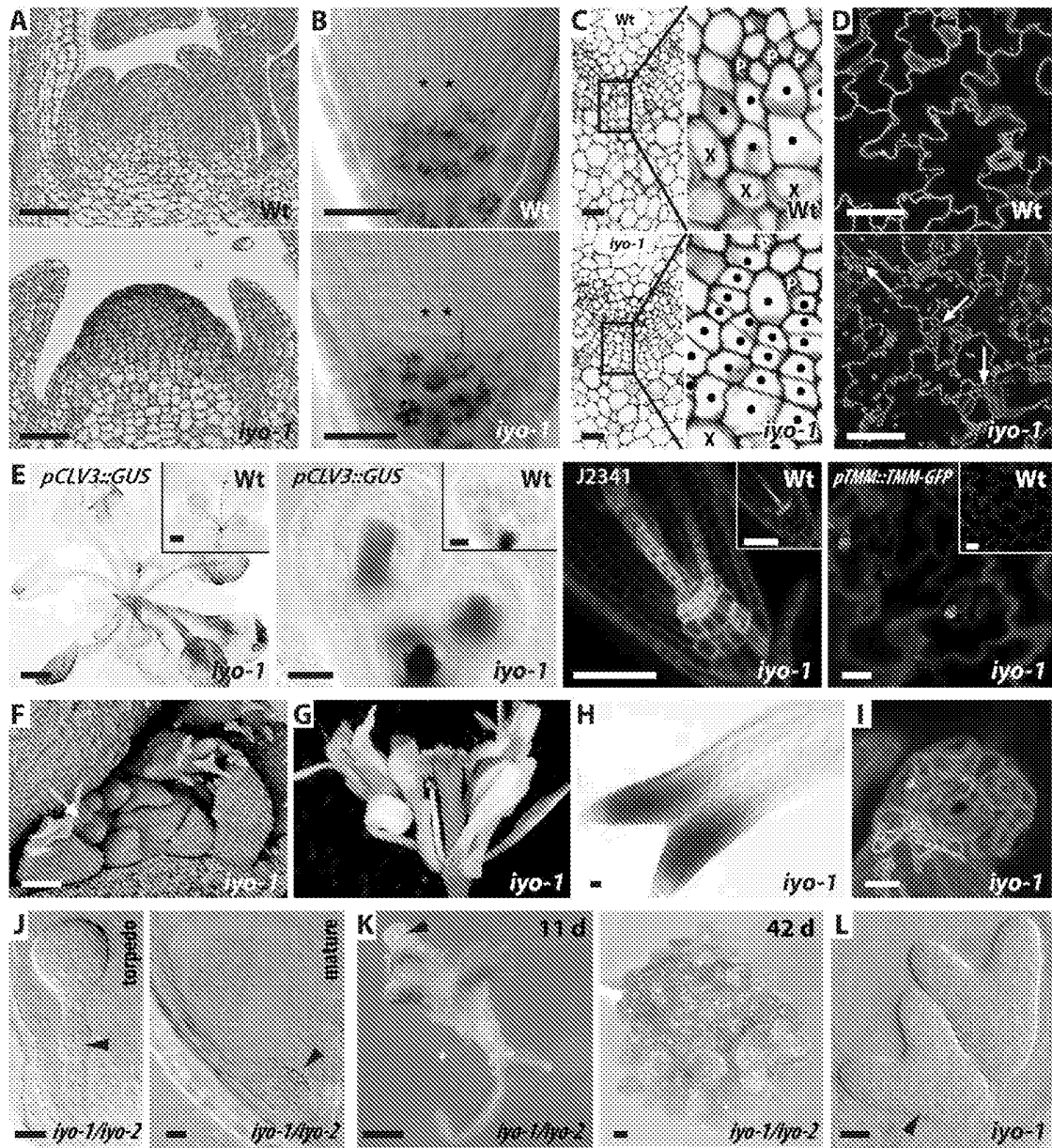
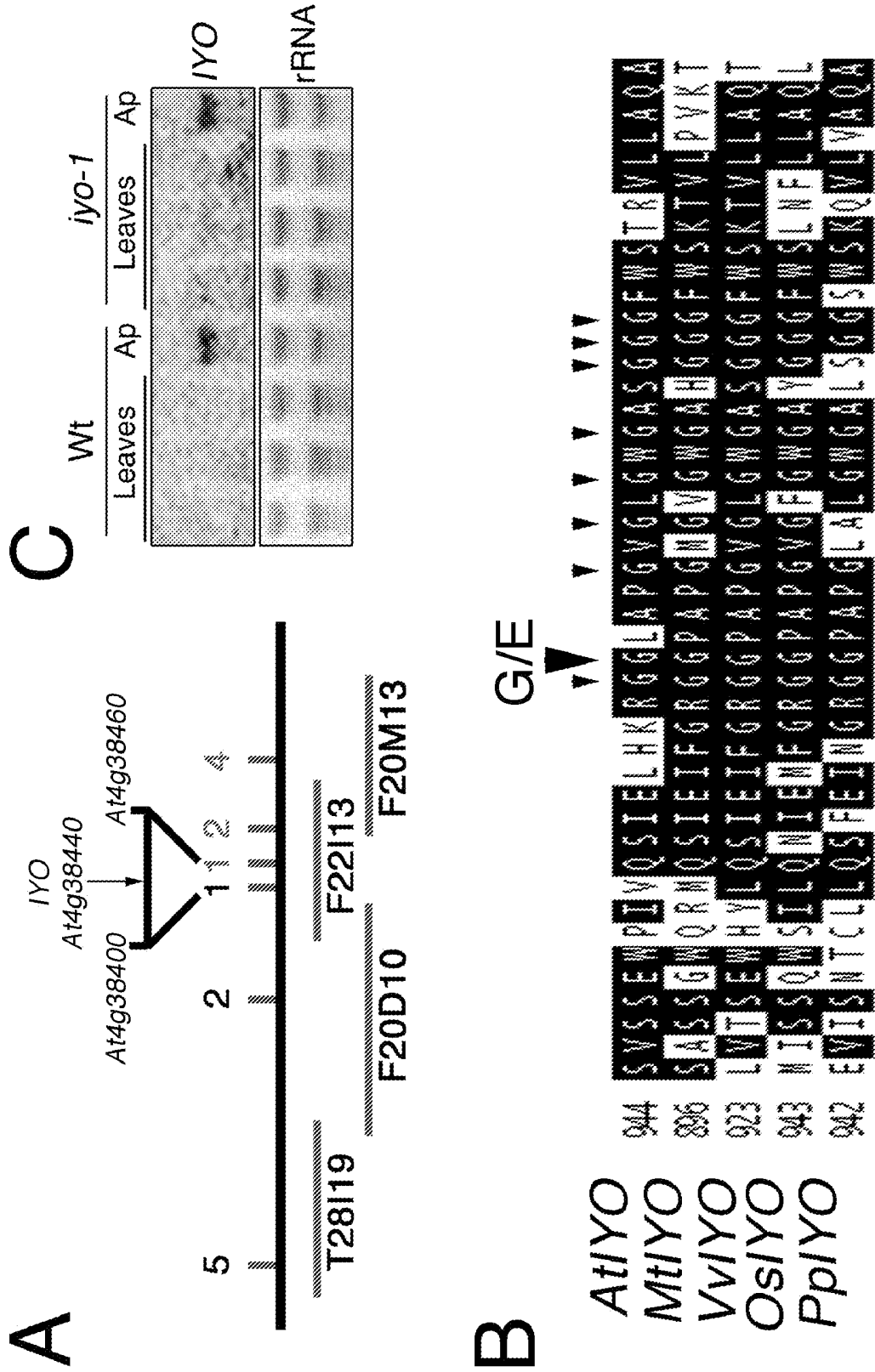


FIG. 2



FIG. 3



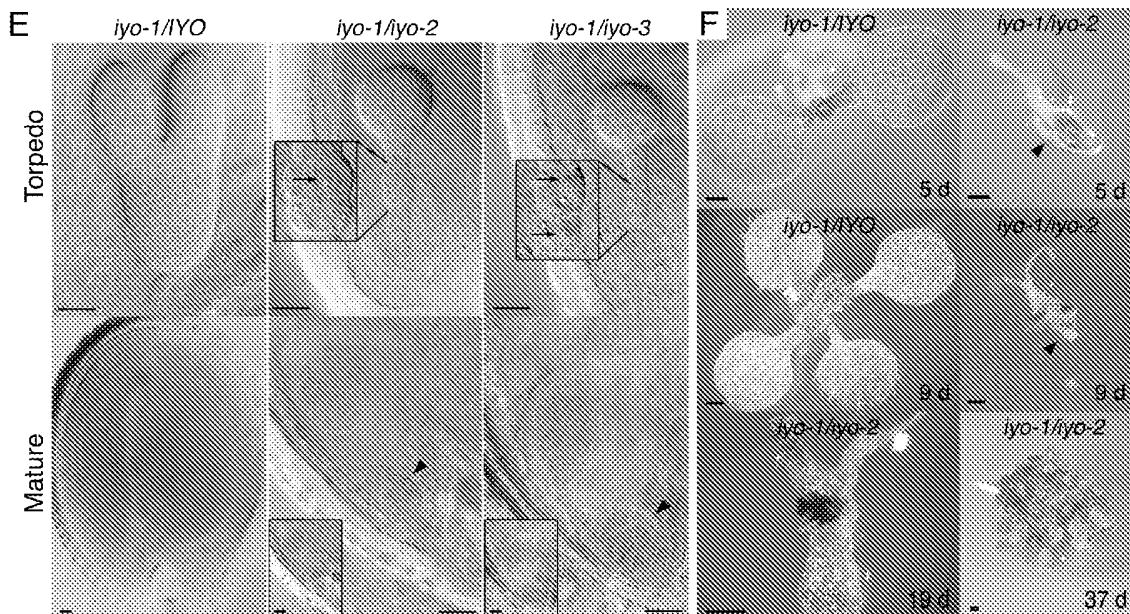
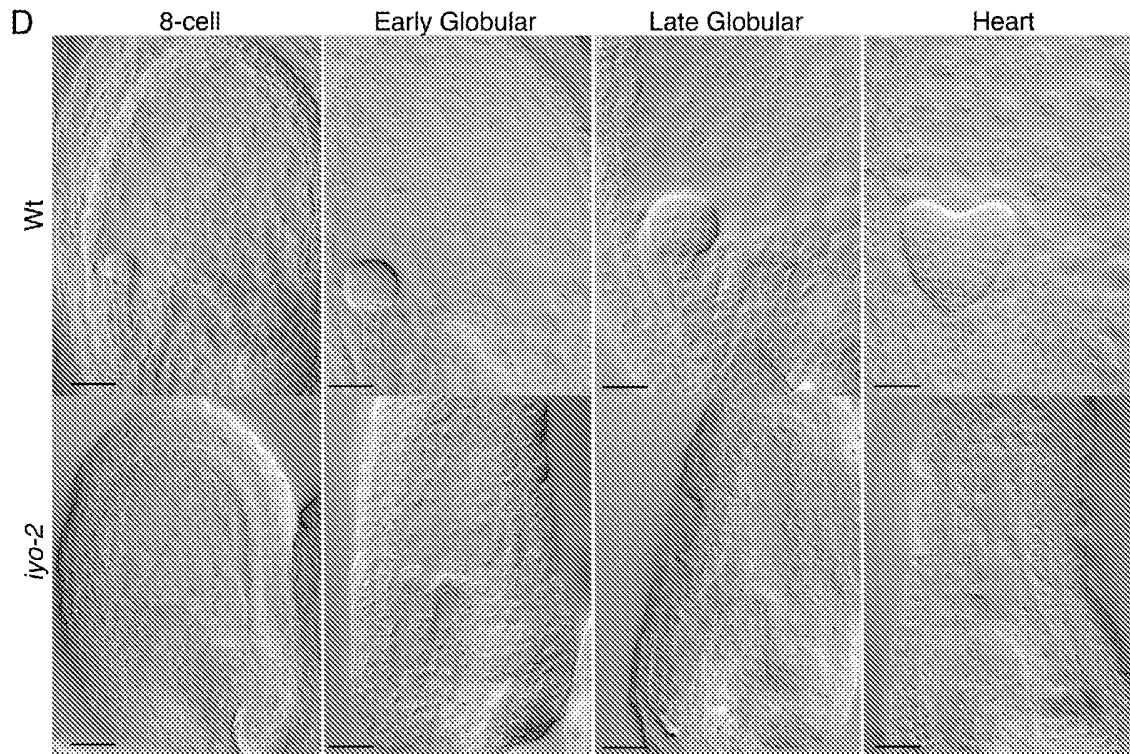


Fig 4

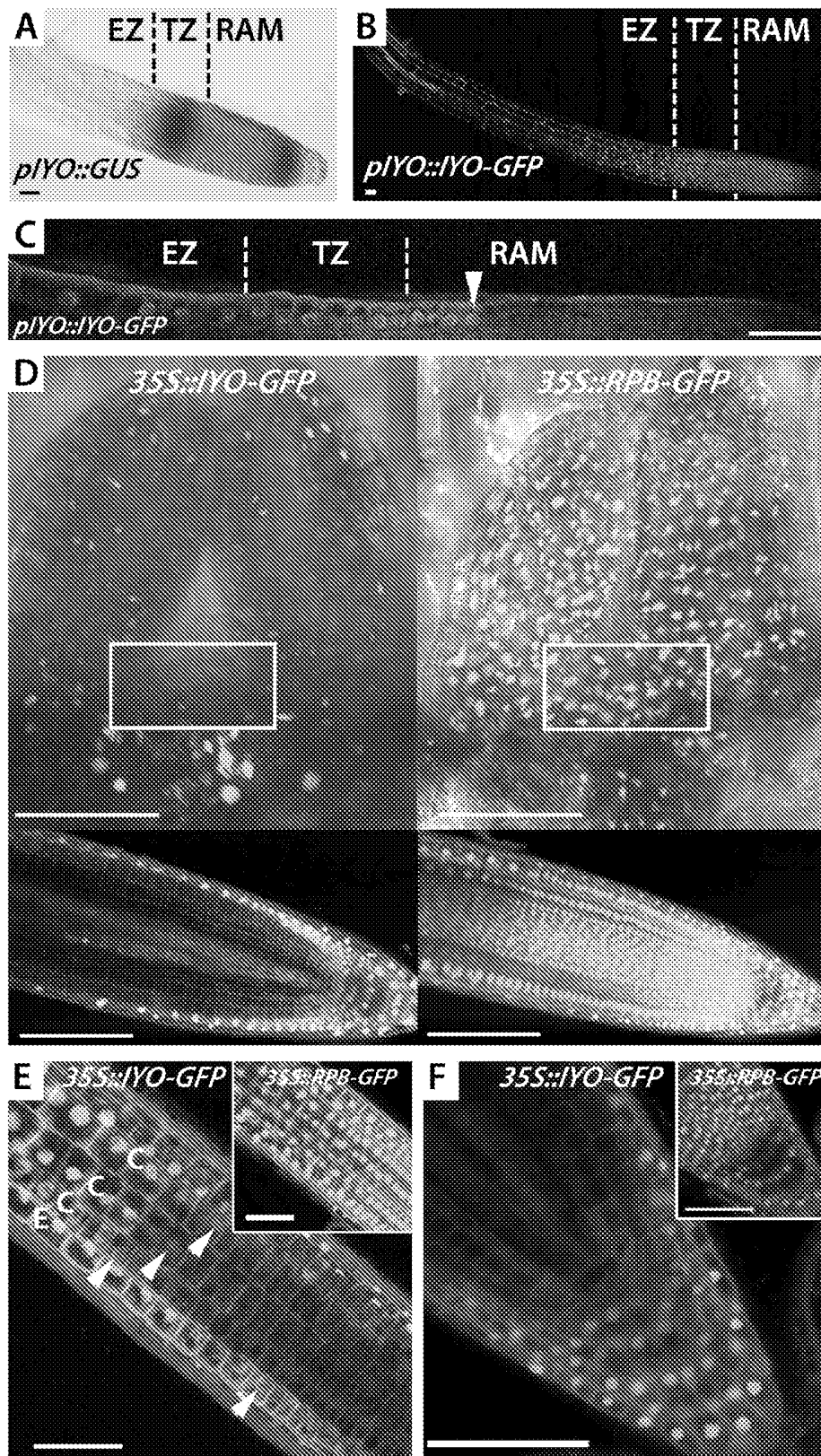


FIG. 5

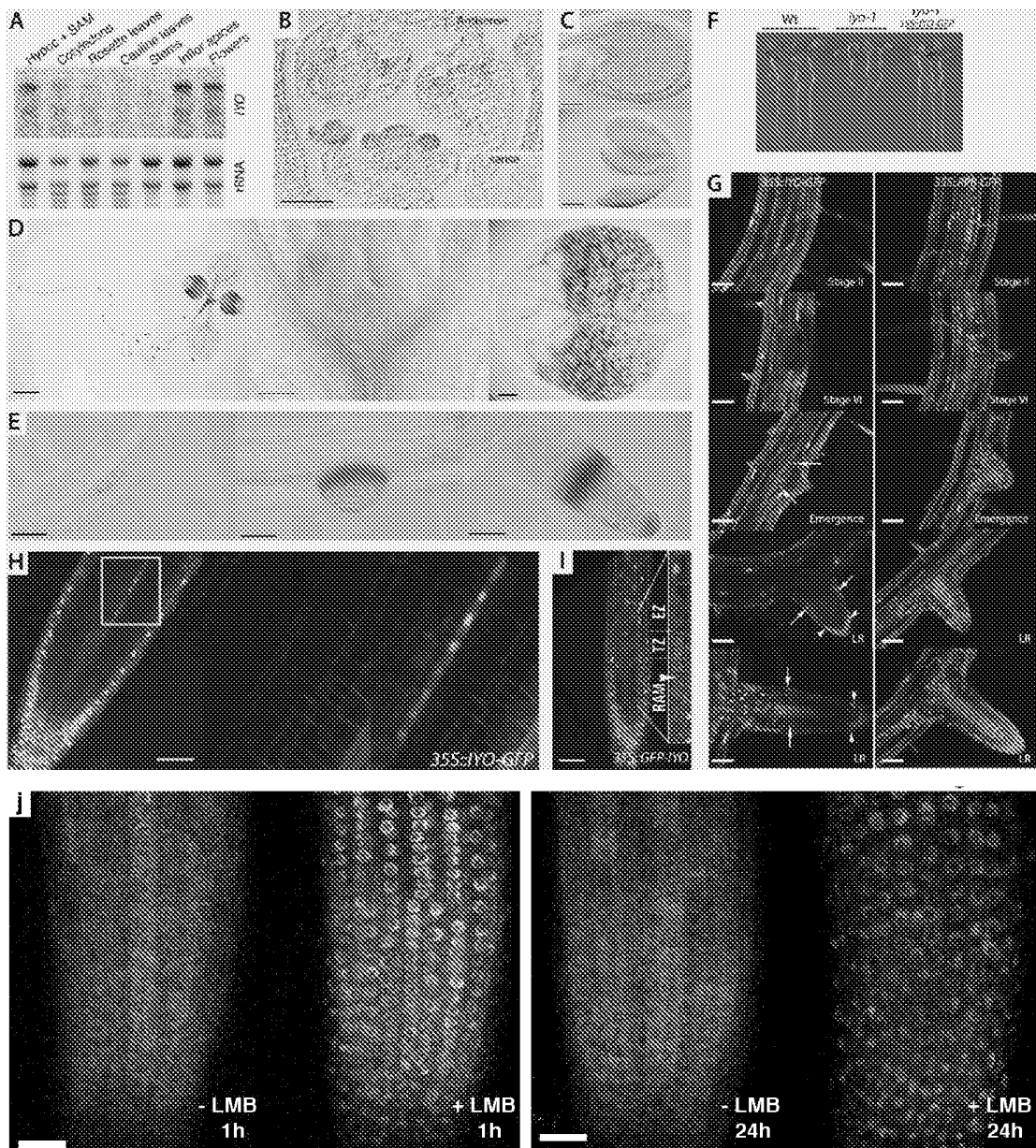


FIG. 6

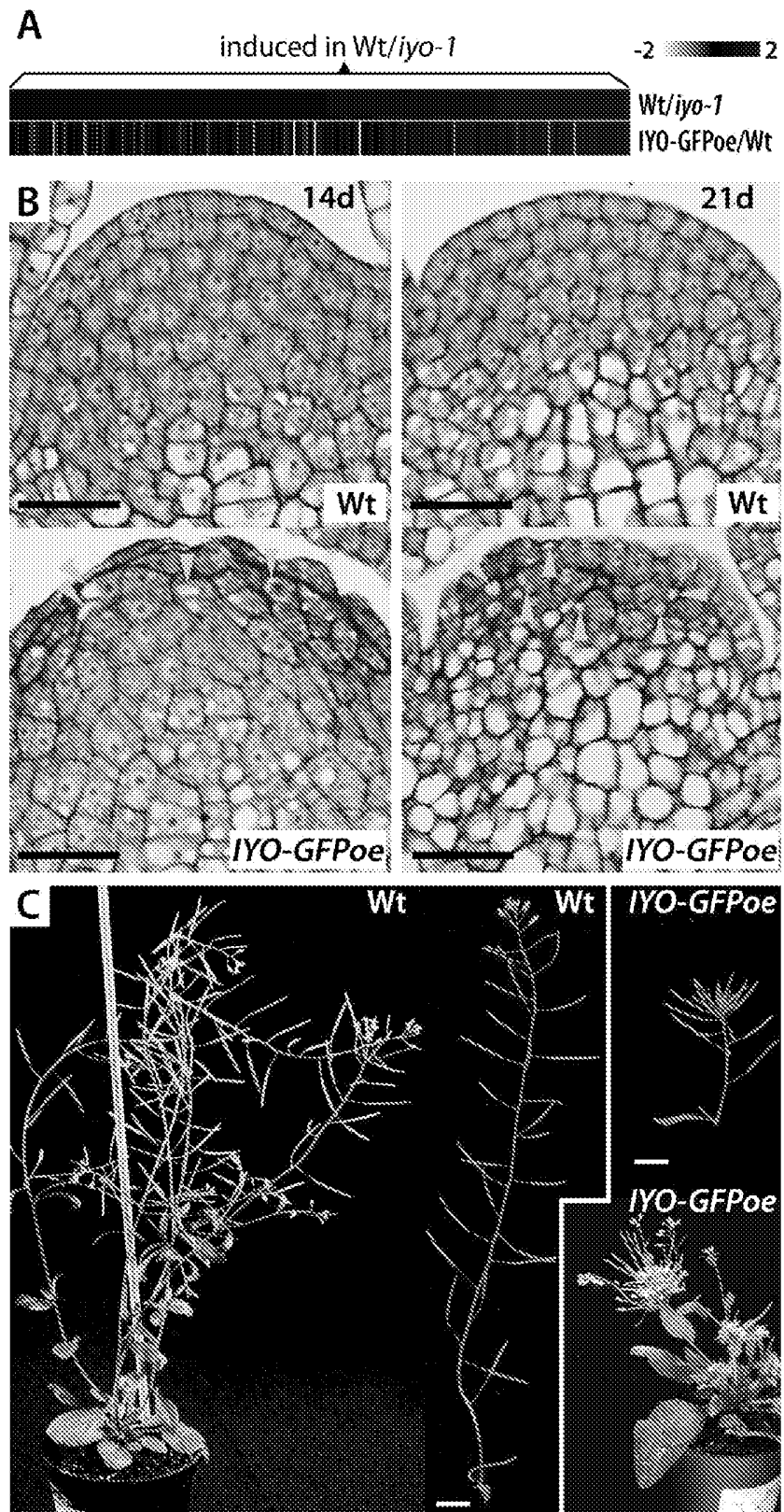


FIG. 7

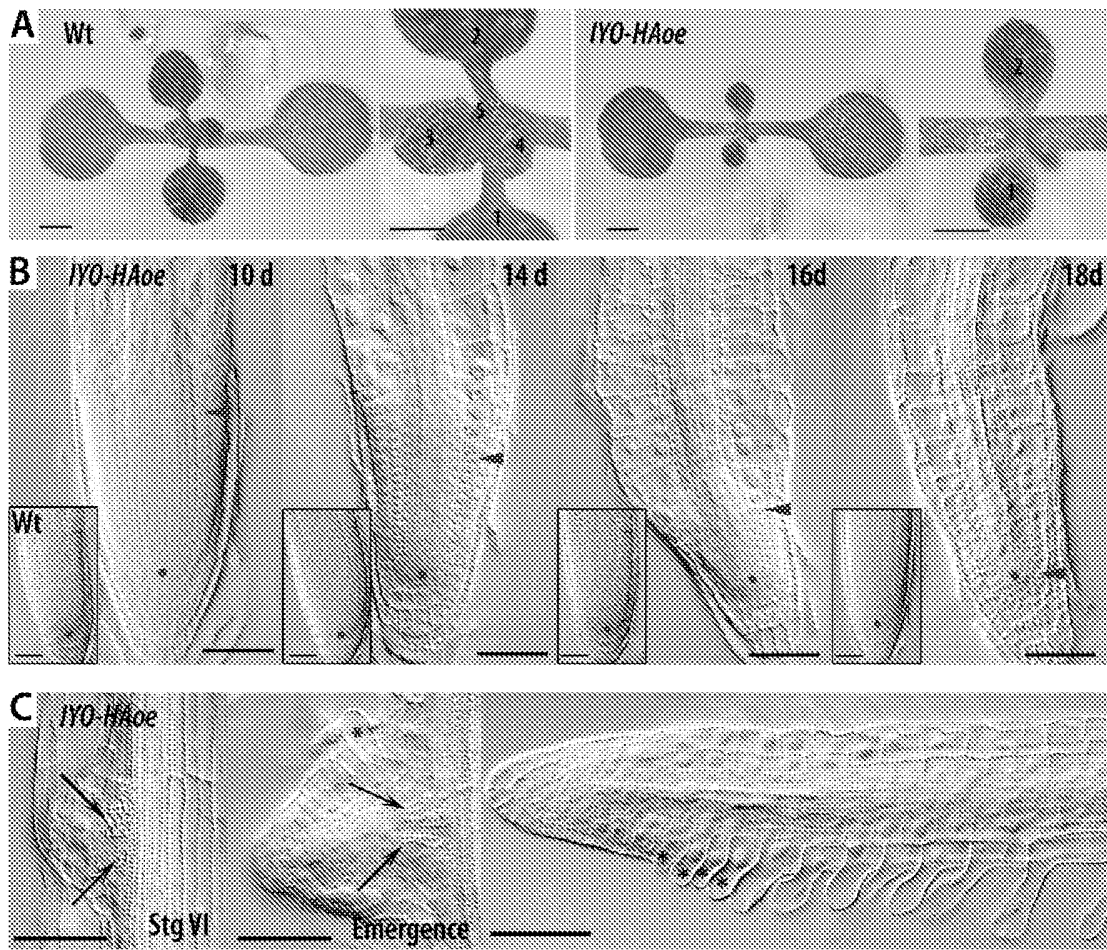


FIG. 8

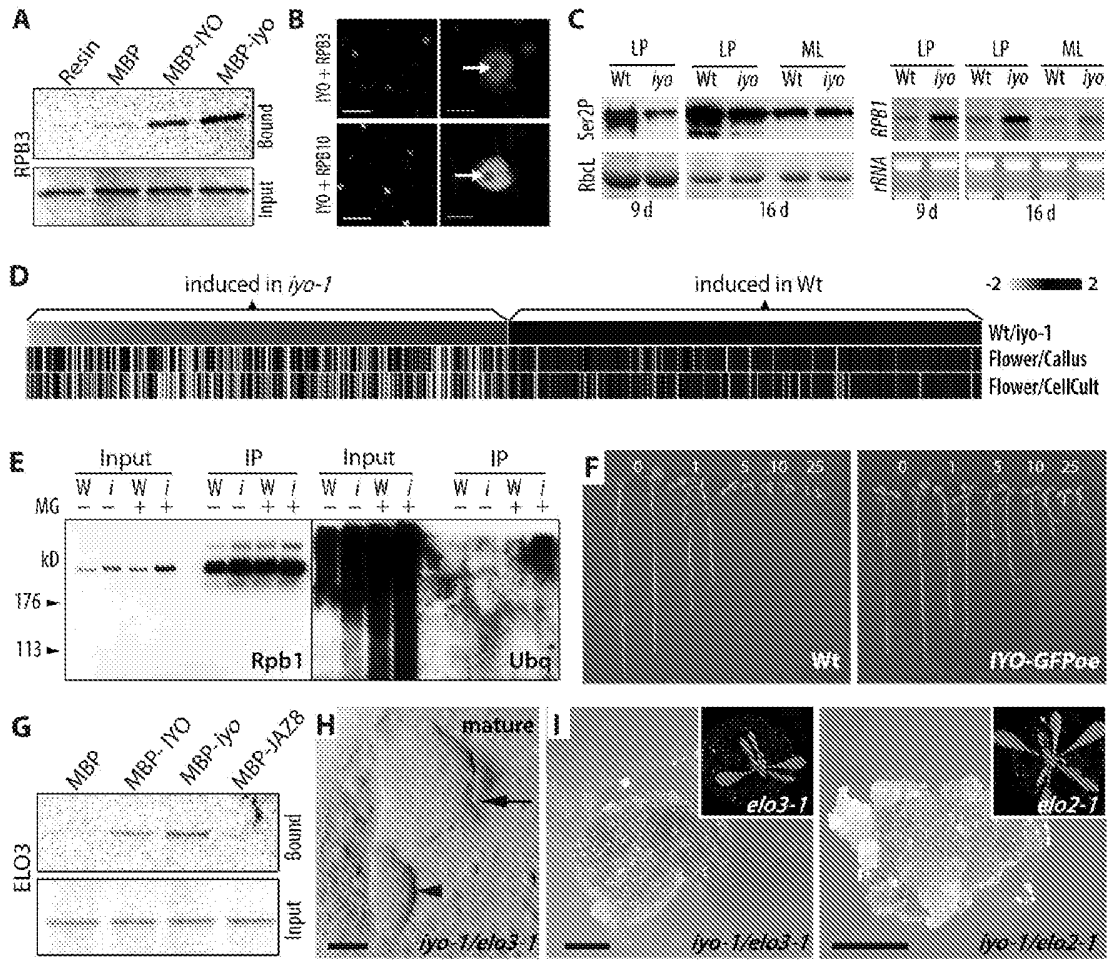


FIG. 9



FIG. 10

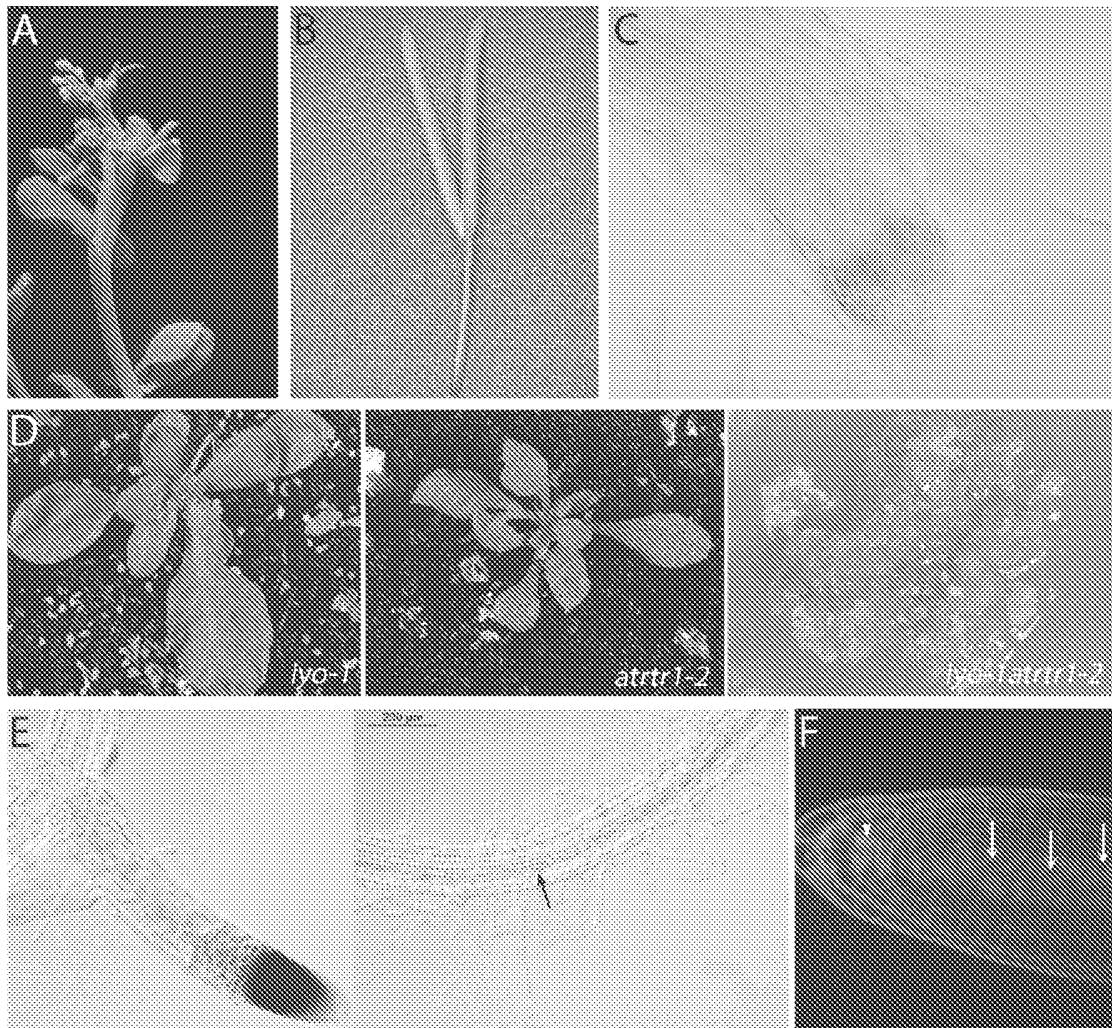


FIG. 11

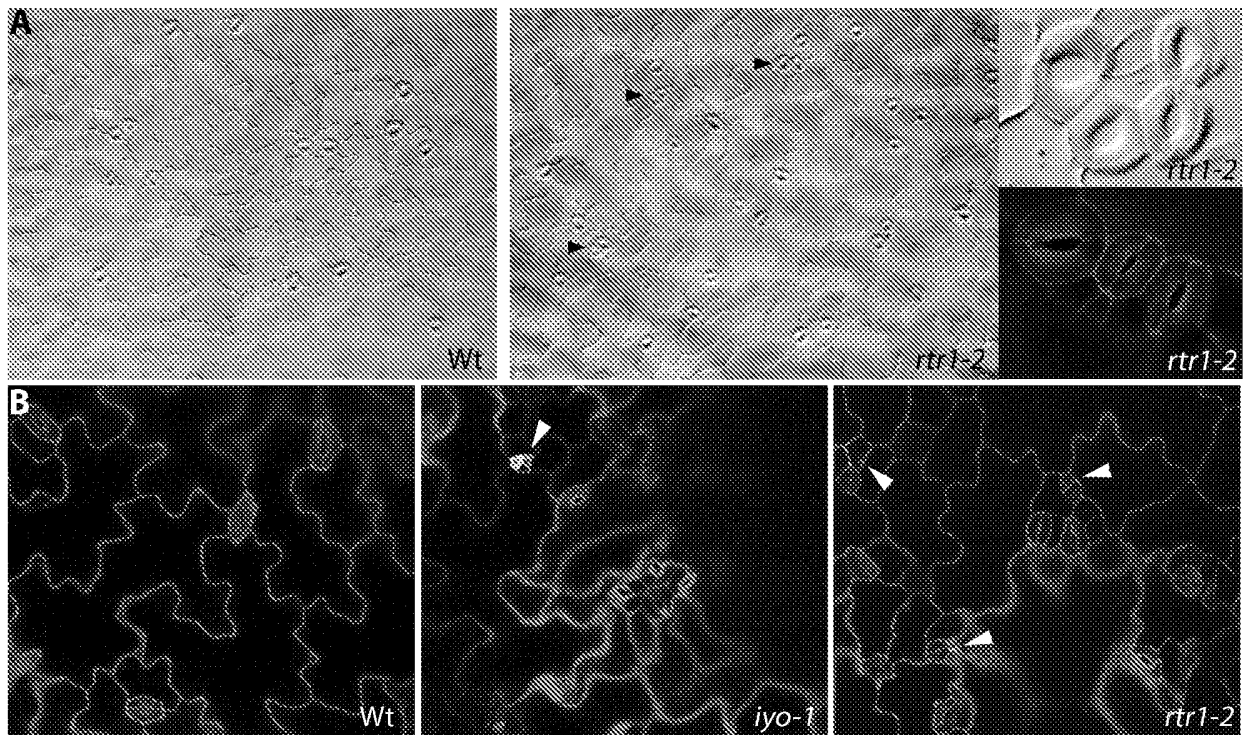


FIG. 12

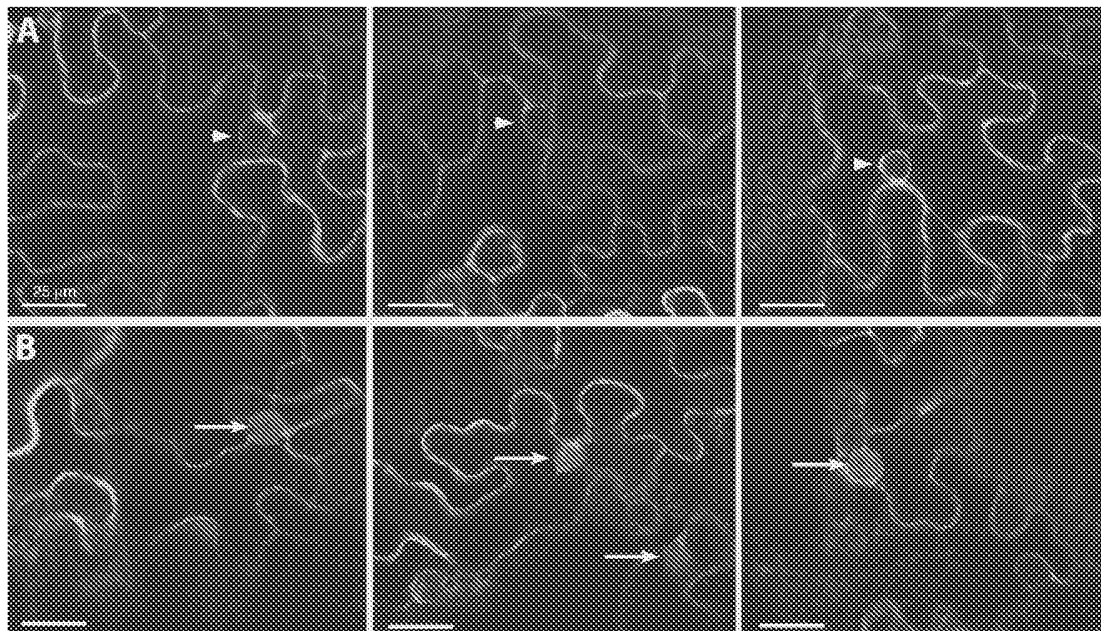


FIG. 13

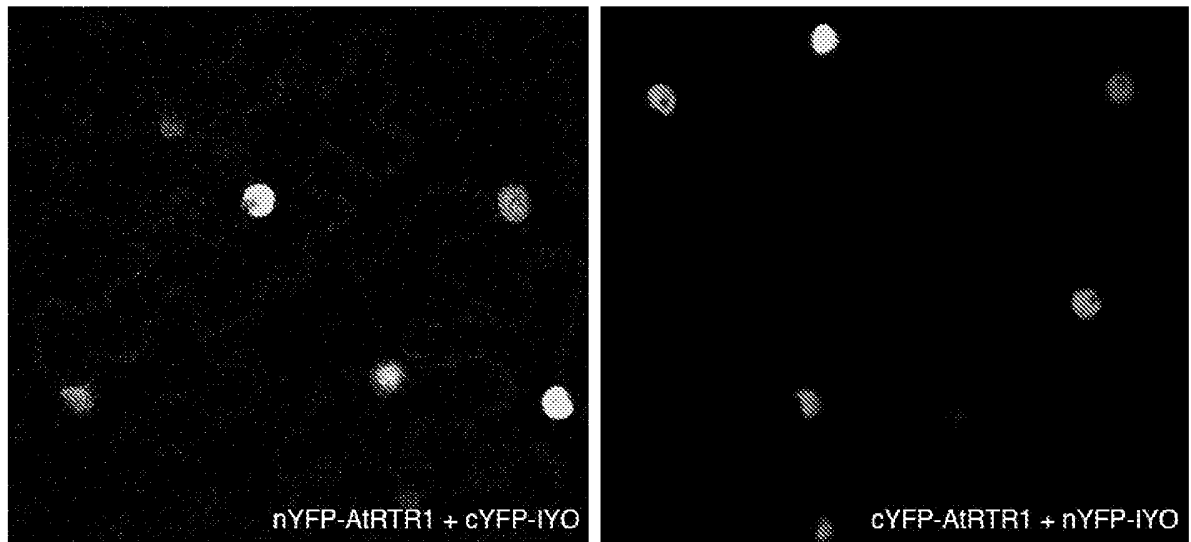


FIG. 14

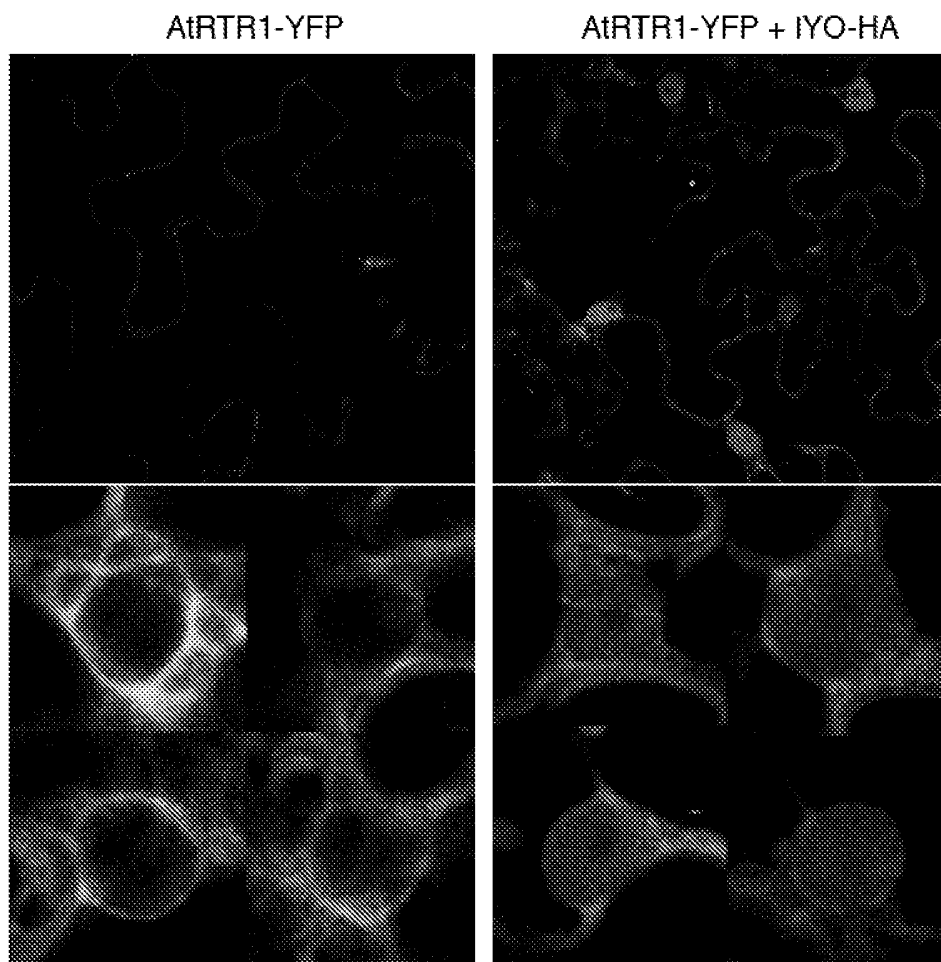
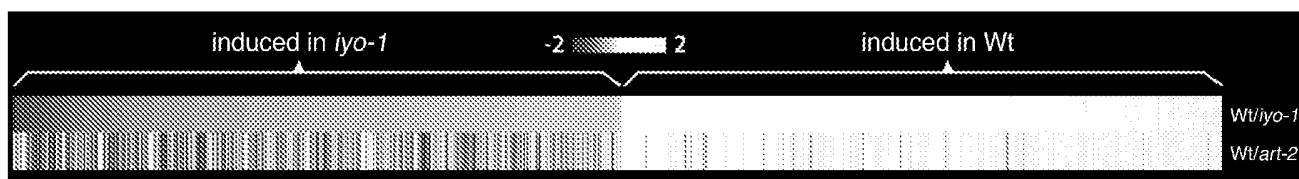
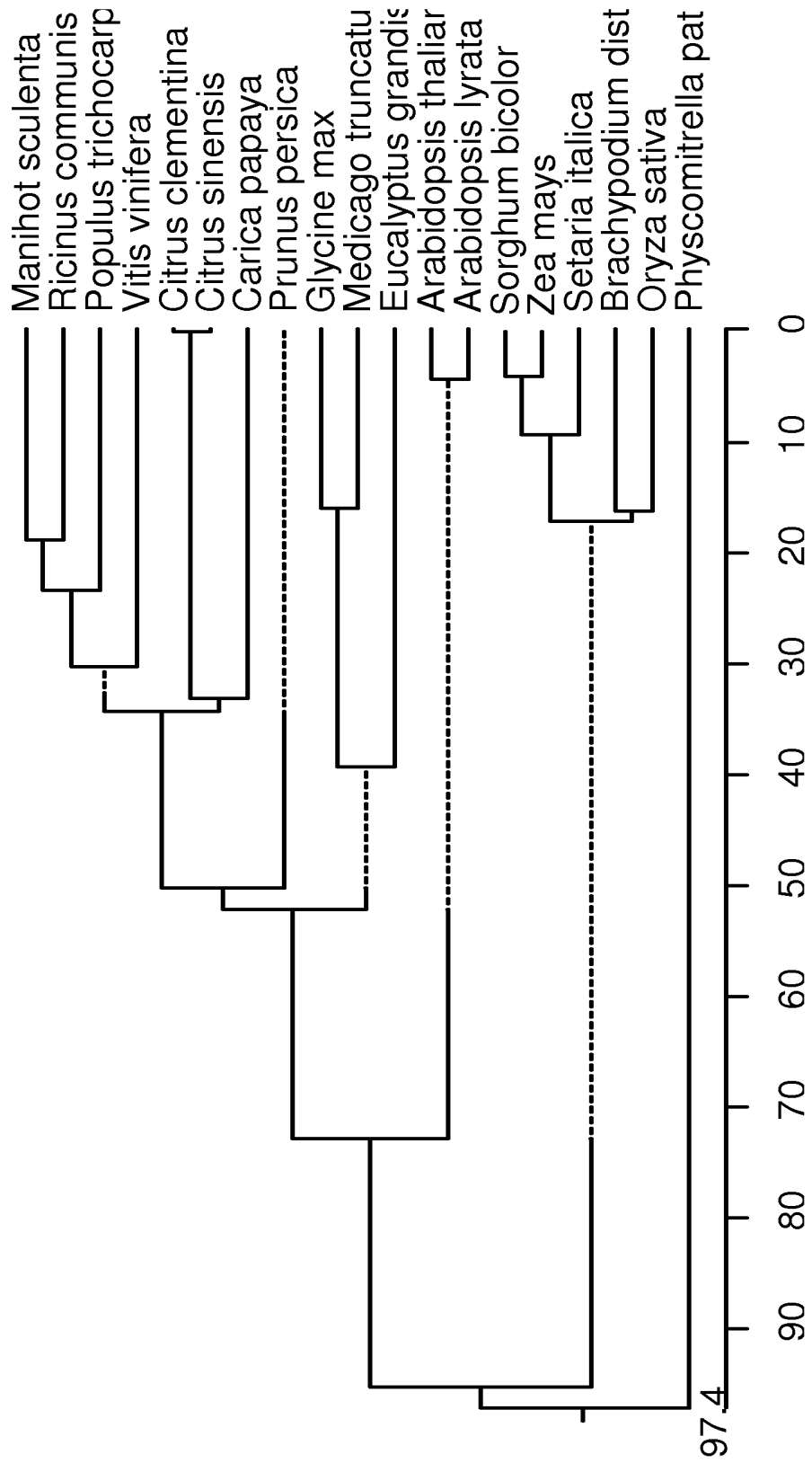


FIG. 15



1454	E K L K S A A L C G R R	Arabidopsis thaliana
1432	E K L K L A A L C G R R	Arabidopsis lyrata
1521	H R L K T S I D G	Brachypodium distachyon
1601	E K L A R T S	Citrus clementina
1601	E K L A R T S	Citrus sinensis
1547	E K L R S A F V K K F N S V K	Manihot sculenta
1479	R R L K T S I D R	Oryza sativa
1515	E K L K S A F V K K Q F V D R L	Populus trichocarpa
1499	E K L R Y S L K M K M L	Prunus persica
1541	E N L R S A F V K K L N	Ricinus communis
1527	Q R L K A S L G Q	Setaria italica
1541	Q R L R A C L G Q	Sorghum bicolor
1592	E K L K S S F R Q D Q	Vitis vinifera
1520	Q R L K A C L G Q	Zea mays
1463		Physcomitrella patens
1404		Calica papaya
1377	D K L K A V V K N S S	Glycine max
1456	K K L K D A A E K N S L	Medicago truncatula
1456	G K L S S K F Q L L Q R	Eucalyptus grandis

FIG. 17



(g)

T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - S S I I S V T H I A I A R K S P X C T T A I L K Y F K F V Q T
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - Y L V S I F I A I A R K S P Q S R A D A I L M C T M L V Q S
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C I I S I F I A I A R K S P I G A N A I L N C E R L I F E T S
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C I I S I F I A I A R K S P T C A E A I M N C Q G L V K T M E H O T S C A L E N T I N A
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - N L V S I L L G I A R K S P Q S A D A I M N C Q R L V Q S O R Y Z A S A T I V A
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C I I S I L L G I A R K S P T C A N A I M N C Q R L V Q S P O P U L U S T R I C H O C A R P A
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - Y I I S I L I A I A R K S P T G A N A I M N C Q G L I V T R I C I U S C O M M U N I S
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - V L V S V L I A I A R K S P Q S A N A I L N C P R L I Q S S E T A R I A I T A L I C A
 T I Q K D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C M F S I L I A I A R K S P T C A N A I M N C E R L I V Q T V I T I S V I N I F E R A
 T I V G D D A T V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - Y L V S V L V A L L A R K S P Q S A D A I M N C P R L I Q S S E A P H Y S C O M I T R E L L A P A T E N S
 T I V G D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C L I S V L I A I A R K S P T C A N A I M N C Q R L V Q T C A R I C A P E P Y A
 T I V G D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - C I V S I L I A I A R K S P S L C A N A V L L K C E R L I V Q T G L Y C I N E M A X
 T I V G D D I V F V A G G Q D V V A A G L V R M D I L P R I Y K L L E T E P P S A T A A L E D - R M I S I L I A I A R K S P I A S C A I M D C P R L I V Q T E U C A L Y P T U S G R A N D I S

Arabidopsis thaliana
 Arabidopsis lyrata
 Brachypodium distachyon
 Citrus sinensis
 Citrus sinensis
 Manihot esculenta
 Oryza sativa
 Populus trichocarpa
 Prunus persica
 Ricinus communis
 Setaria italica
 Sorghum bicolor
 Vitis vinifera
 Zea mays
 Physcomitrella patens
 Carica pepaya
 Glycine max
 Medicago truncatula
 Eucalyptus grandis

314 M S D G V G V E Y V S E K Q P Q C S M E D S L S C E L K G - - - - -
251 - - - - - K M K G G I C G R S D L S E N Q Q - - - - - P - - - - -
249 - - - - - T G K K K I C G V V K K K D K K D K K S - - - - - F - - - - -
260 - - - - - K G K K K S V V W G K K D L S S - - - - - R - - - - -
266 V R K K D D D S I Q Q L S S S F - - - - - R R S - - - - -
234 -
250 - - - - - G G K E - - I R E S L E K N Q - - - - - - - - - - -
292 - - - - - K E K V - - S Q K S S L E N K A - - - - - - - - - - -
274 - - - - - K G K K V G E G L L M K A - - - - - D - - - - - S V K - - - - -
248 - - - - - T K G K K G - - H I G K K K A - - - - - - - - - - - S L K - - - - -
250 - - - - - K E K A S S I G D Q K K K A P - - - - - - - - - - - L L S M L E - - - - -
311 L E E E R R K G G S S A K K K M V A A S S S R R A T Q K K G S K S T R R R P A G G V A - - - - -
314 L E E E R R K K S S A K K K M V A A S S S R R A T Q K K G S K S T R R R P A G G V A - - - - -
307 L E E E R R K G G S S A K K K M V A A S S S R R A T Q K K G S K S T R R R P A G G V A - - - - -
291 L E E E R R K K S S A K K K M V A A S S S R R A T Q K K G S K S T R R R P A G G V A - - - - -
299 L E E E R R K K S S A K K K M V A A S S S R R A T Q K K G S K S T R R R P A G G V A - - - - -
256 -

344 L Q T L D G K N T L P -
270 - - - - - - - - - - - S A K N -
273 - - - - - - - - - - - P K N -
285 - - - - - - - - - - - T P M I -
316 V H S I S I S I S E R R Q -
234 -
269 - - - - - - - - - - - S M K T -
312 - - - - - - - - - - - S S K T -
294 -
267 - - - - - - - - - - - S I K A -
273 - - - - - - - - - - - S E S K -
340 -
346 G K E V D F T T S T I I I I G D D A S T N T M R M M N Q V V V L S S S V L V D D N V K P S S S S S - - - - -
339 G H E V D F T T S T I I I I I M G D D A G S S L T N T M R M M N Q V V V L S S S V L V D D N V K P S S S S S - - - - -
323 G H E V G F T T S T I I I I M G D D A G S S L T N T M R M M N Q V V V L S S S V L V D D N V K P S S S S S - - - - -
331 G H E V G F T T S T I I I I M G D D A G S S L T N T M R M M N Q V V V L S S S V L V D D N V K P S S S S S - - - - -
268 - - - - - - - - - - - T I I -

381 - - - - - Y H A N S V E D G E E I T L A A E S Y E E R R K S A F Q K G A G N C S S S E I G V T K S C L L K K I S S G S K K
300 - - - - - A T P D S G T - - - - - N S N - - - - - N S R S L D T M F A Q N L M E N E D K K L P D Q K F G A G N C S S S E I G V T K S C L L K K I S S G S K K
302 - - - - - D A Y P -
315 - - - - - E I A V C - - - - - S N -
352 - - - - - D A V - - - - - A S -
259 - - - - - D D -
299 - - - - - D S Q C S -
342 - - - - - V S -
314 - - - - - D A - - - - - Q O T -
297 - - - - - Y - - - - - S -
303 -
368 - - - - - Q O O S S E K D S T A Y A E E Q L L C F E F S S E A V V N I G M R D D E F E T T S D D E K K M K P P A L L K K S S L M K K A G T
389 - - - - - S S S A K K D S T A Y A E E Q L L C F E F S S E A V V N I G M R D D E F E T T S D D E K K M K P P A L L K K S S L M K K A G T
382 - - - - - Q O O S S S A K K G P F M V T -
366 - - - - - Q O O Y A A I D S V T -
380 T Q O Y A A R D D L G -
298 - - - - - D -

425 K L S R S V T W A D D Q E N - - - - - D G R G D L C E V R R N M D N A T G P S S L Q R S A - - - - - Y S N D I E D - - -
339 K L G R R S V T W A D D E K K - - - - - T D C V A G G G K L L P E L R N D M K T T E K P P S S - - - - - - - - - - - T T S N G I V D K - - -
344 K L C T R R S V T W A D D E K K - - - - - D D A A D G S I G M N L L L P E L R N D M K T T E K P P S S - - - - - - - - - - - T T S N G I V D K - - -
357 K V T R R S V T W A D D E K K - - - - - T V I N S A D G S I G M N L L L P E L R N D M K T T E K P P S S - - - - - - - - - - - T T S N G I V D K - - -
391 K F S T R R S V T W A D D E K K - - - - - I S I D T G A G S S I L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
282 K E S T R R S V T W A D D E K K -
339 K S N T R R S V T W A D D E K K - - - - - I D G S S R R R N D L L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
383 Q L T R R S V T W A D D E K K - - - - - I V D S S T A G S S R R R N D L L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
355 K L N R R S V T W A D D E K K - - - - - I V D S S T A G S S R R R N D L L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
330 R S N R R S V T W A D D E K K - - - - - I V D S S T A G S S R R R N D L L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
342 K V I R R S V T W A D D E K K - - - - - M D S S A D S S R R R N D L L C E I F R K R E E F I M K K G V G D D D I N K K G A V L D E - - - - - V M P - - - - -
417 S S R Q O S S V T W A D D E K K -
438 S G R Q O S S V T W A D D E K K -
431 S G S Q O S S V T W A D D E K K -
415 N A G H Q S S V T W A D D E K K -
430 N K S S S V T W A D D E K K -
329 Q L N R S S V T W A D D E K K K F E Q S D K I E V L E K R E T L S N T S S S L V A L K S L E S T S Q S A T

615 G L S S S E F I I K Q E Q T I A G C L A R A A L P R V V T A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
536 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
538 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
546 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
585 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
473 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
525 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
576 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
587 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
523 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
535 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
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625 G R R S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
618 G H S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
603 G H S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
619 S Q L S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A
544 G L S S E F E I I K Q E Q T I A G G C C L L G T R A A L P P G G L V A S X L R L P L T I A I I E L E K G G S L L L E T M S L T G A A

665 V P S F R V K E W L V I V L L F L D A L S V S R R I P R I A P V I S N W R D - - - - I I L N E G S G I G M
586 L P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V R R R R I P P S L A S A X M S S R M L L Y K K V L L D G R A Q O I R S V
598 L P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
635 L P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
523 L P G F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
575 V P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
626 V P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
637 I P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
573 L P A F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
585 L P S F F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
654 L P S L R R S R Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
675 L P S L R R S R Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
668 L P S L R R S R Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
653 L P S L R R S R Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
669 L P P P L R S R Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M
594 I P P F R M K K Q Q W Q V V I V V L L F F I E A L L S V S V C C R R R R I P P V L A L T I S S Y M M T D R R A S F K K V L L Q S A A R R I R M

711	E E V E T M K D I L L P L G R V P Q F A T R S G A	Arabidopsis thaliana
638	E E V E T M K D L L I P L G R A P Q F S M Q S G D	Carica papaya
638	D E V E I M R D H I L P L G R R A P Q F S S A Q S G A	Cucumis sativa
648	E E V E I M K D L L I P L G R R A P Q F S S A Q S G A	Eucalyptus grandis
685	E E V E V L K D L V V P L G R R A P K I S S Q S G A	Glycine max
573	E E F E I M K D L I I P L G R R V P Q F S T Q S G G	Mimulus guttatus
625	E E V E V M K D L M I I P L G R R D P R - - A R S G A	Manihot sculenta
672	E E V E V M K D L M I I P L G R R A P Q F S S A Q S G A	Populus trichocarpa
687	E Q V E L M K D L I I P L G R R A P Q F S S A Q S G A	Prunus persica
623	E E V D I M K D F M V P L G R R D P R - - A R S G A	Ricinus communis
635	E E V E V M K D L I I P L G R R V P Q F S S A Q S G G	Vitis vinifera
703	E E V D S M M V D L F L P F G R S V A I T P M	Zea mays
724	E E V D S M M V D L F L P F G R S V A T T P M	Sorghum bicolor
717	E E V E S M M V D L F L P F F G R S I T F M Q I	Setaria italica
702	E E V D S M M V D L F L P F F G R S S T V Q A L P S	Oryza sativa
718	E E V D S M M V D L F L P F F G R S S T P P P S Q P V Q V P	Brachypodium distachyon
644	E E Y K T M K E L L T P L G R C P F S S Q S G G	Picea glauca

FIG. 20

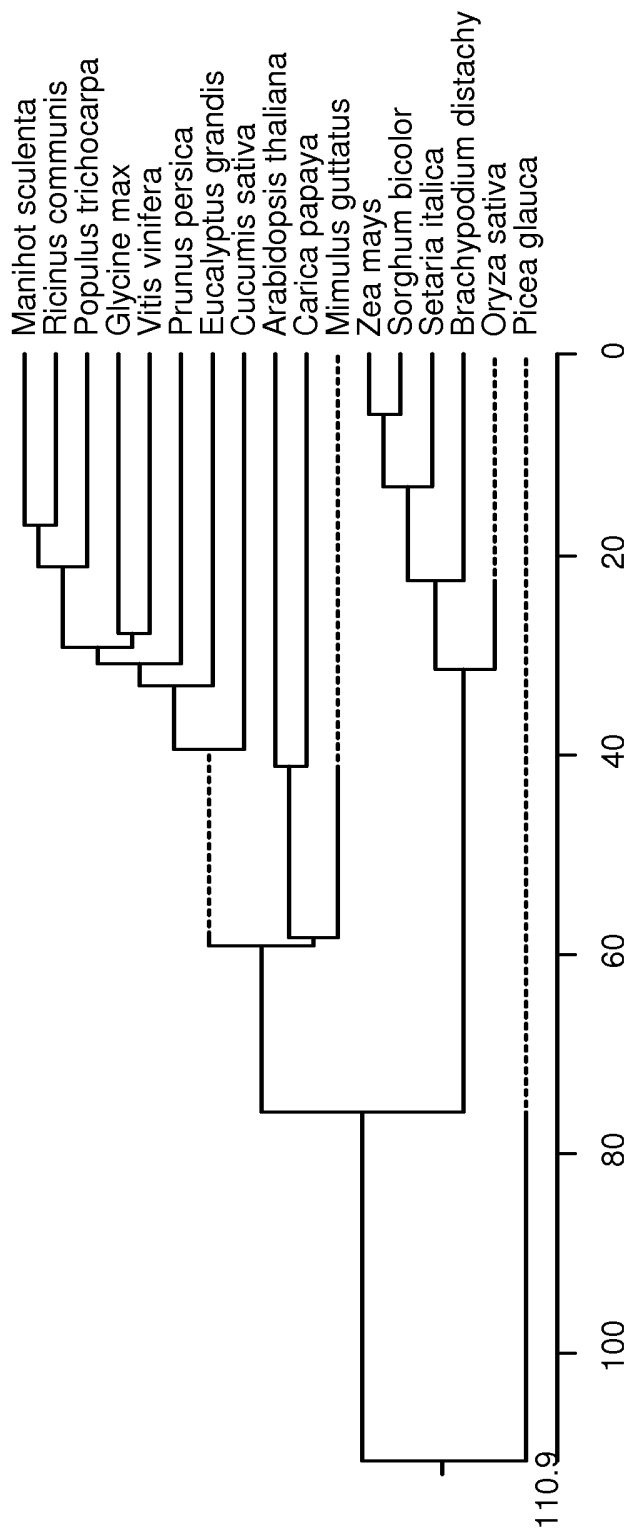


FIG. 21

(a)

D V V T E R A I A K L C G Y T L C Q R F L P S D - - - - - V S R R G M Y R I S L R D R K V Y D L Q E T S K F C S A G C Arabidopsis thaliana
D V V T E R T I S M L C G Y P L C M S L S S D - - - - - P Q R K G M Y M I S L K E K K V Y D L Q E L V R R F C S S S C Carica papaya
D V V T E R S I A D L C G Y P L C H S M L P S D - - - - - M T R R G R V R I S L K E K K V Y D L Q E F T Y K V C S S A C Cucumis sativa
D V V A E R S I A G L C G Y P L C A T P L P S D - - - - - R P R K G R V R I S L K E K K V Y D L Q E F T Y M Y C S P G C Eucalyptus grandis
D V V T E R S I T M W C G Y P L C S M A L P S D - - - - - R P R K G R V R I S L K E K K V Y D L Q E F T Y M F C S S M C Glycine max
D V V T E R T I A H V C G Y P L C V M S L P S E - - - - - P P R K G H Y R I S L K E K K V Y D L H E T M M Y C S T E C Mimulus guttatus
D V V I E R S I A N L C G Y P L C M S M P L D - - - - - R P H K G R V R I S L K E K K V Y D L H E F T Y M Y C S S S C Manihot sculenta
D V V T E R T I A N L C G Y P L C G M S L P S D - - - - - R P Q K G R V R I S L K E K K V Y D L H E F T Y M Y C S S S C Populus trichocarpa
D V V T E R T I A N L C G Y P L C S M A L P S D S S - - - - - R P H K G M Y R I S L K E K K V Y D L H E F T Y M Y C S S R C Prunus persica
D V V V E R S I S M L C G Y P L C M S L P S D - - - - - R P V K G R V R I S L K E K K V Y D L Q E F T Y M Y C S S S C Ricinus communis
D V V T E R T I A N L C G Y P L C S M S L P S E - - - - - R L E K G H Y R I S L K E K K V Y D L H E F T Y M Y C S S G C Vitis vinifera
D V V T E R T I S D V C G M P A C P M P L S S S S - - - - - A A T G P A F M I A L S E K R V Y D L E A R K F C S E R C Zea mays
D V V T E R T I A D A C G N P A C P M P L P S S S S - - - - - A A A T G P P F M I A L S E K R V Y D L E F A R K F C S D R C Sorghum bicolor
D V V T E R T I A D A C G M P A C P M P L P R A A T - - - - - T A G G P R F M I S L R E K R V Y D L E F A R K F C S E R C Setaria italica
D V V T E R S I A D A C G Y P A C P M P L P S E D - - - - - A R G K A A P R F M I S L R E K R V Y D L E F A R K F C S E R C Oryza sativa
D V V T E R S I A D A C G K P P C A S P L P A A A - - - - - A A A A A P P R F M I S L R E K R V Y D L E F A R K F C S E R C Brachypodium distachy
D V V T E R T I V N L C G Y P L C S M K L P A S E E Q Q Q Q R E K R G R Y R I S L K D R K V Y D L Q E T W L Y C S T P C Picea glauca

(b)

S	R	S	D	Y	E	D	V	V	T	E	R	A	I	A	K	L	C	G	V	T	L	C	<i>Arabidopsis thaliana</i>
S	R	S	D	Y	E	D	V	V	T	E	R	T	I	S	N	L	C	G	Y	P	L	C	<i>Carica Papaya</i>
S	R	S	D	Y	E	D	V	V	T	E	R	S	I	A	N	L	C	G	Y	P	L	C	<i>Cucumis sativa</i>
S	R	R	D	Y	E	D	V	V	A	E	R	S	I	A	G	L	C	G	Y	P	L	C	<i>Eucalyptus grandis</i>
S	R	S	D	Y	E	D	V	V	T	E	R	S	I	T	M	V	C	G	Y	P	L	C	<i>Glycine max</i>
S	Q	S	D	Y	E	D	V	V	T	E	R	T	I	A	H	V	C	G	Y	P	L	C	<i>Mimulus guttatus</i>
S	H	S	D	Y	E	D	V	V	T	E	R	S	I	A	N	L	C	G	Y	P	L	C	<i>Morhnia esculenta</i>
S	H	S	D	Y	E	D	V	V	T	E	R	T	I	A	N	L	C	G	Y	P	L	C	<i>Populus trichocarpa</i>
S	R	S	D	Y	E	D	V	V	T	E	R	T	I	A	N	L	C	G	Y	P	L	C	<i>Prunus persica</i>
S	R	S	D	Y	E	D	V	V	T	E	R	S	I	S	N	L	C	G	Y	P	L	C	<i>Ricinus communis</i>
S	R	S	D	Y	E	D	V	V	T	E	R	T	I	A	N	L	C	G	Y	P	L	C	<i>Vitis Vinifera</i>
S	R	A	D	Y	E	D	V	V	T	E	R	T	I	S	D	V	C	G	N	P	A	C	<i>Zea mays</i>
S	R	A	D	Y	E	D	V	V	T	E	R	T	I	A	D	A	C	G	N	P	A	C	<i>Sorghum bicolor</i>
S	R	A	D	Y	E	D	V	V	T	E	R	S	I	A	D	A	C	G	N	P	A	C	<i>Setaria italica</i>
S	R	A	D	Y	E	D	V	V	T	E	R	S	I	A	D	A	C	G	Y	P	A	C	<i>Oryza sativa</i>
S	R	A	D	Y	E	D	V	V	T	E	R	S	I	A	D	A	C	G	H	P	P	C	<i>Baccharidium distachyon</i>
S	R	S	D	Y	E	D	V	V	T	E	R	T	I	V	N	L	C	G	Y	P	L	C	<i>Picea glauca</i>

(c)

I	S	L	K	D	H	K	V	Y	D	L	Q	E	A	r	a	b	i	d	o	p	s	i	s	<i>Arabidopsis thaliana</i>	
I	S	L	K	E	H	K	V	Y	D	L	Q	E	C	a	r	i	c	a						<i>Carica Papaya</i>	
I	S	L	K	E	H	K	V	Y	D	L	Q	E	C	u	c	u	m	i	s					<i>Cucumis sativa</i>	
I	S	L	K	E	H	K	V	Y	D	L	Q	E	E	u	c	a	l	y	p	t	u	s		<i>Eucalyptus grandis</i>	
I	S	L	K	E	H	K	V	Y	D	L	H	E	E	G	l	y	c	i	n	e				<i>Glycine max</i>	
I	S	L	K	E	H	K	V	Y	D	L	H	E	E	M	i	m	u	l	u	s				<i>Mimulus guttatus</i>	
I	S	L	K	E	H	K	V	Y	D	L	H	E	E	M	o	r	h	n	i	a				<i>Morhnia esculenta</i>	
I	S	L	K	E	H	K	V	Y	D	L	H	E	E	P	o	p	u	l	u	s				<i>Populus trichocarpa</i>	
I	S	L	K	E	H	K	V	Y	D	L	H	E	E	P	r	u	n	u	s					<i>Prunus persica</i>	
I	S	L	K	E	H	R	V	Y	D	L	Q	E	E	R	i	c	i	n	u	s				<i>Ricinus communis</i>	
I	S	L	K	E	H	R	V	Y	D	L	H	E	E	V	i	t	i	s						<i>Vitis Vinifera</i>	
I	A	L	S	E	H	R	V	Y	D	L	E	E	E	Z	e	a								<i>Zea mays</i>	
I	A	L	S	E	H	R	V	Y	D	L	E	E	E	S	o	r	g	h	u	m				<i>Sorghum bicolor</i>	
I	S	L	R	E	H	R	V	Y	D	L	E	E	E	S	e	t	a	n	a					<i>Setaria italica</i>	
I	S	L	R	E	H	R	V	Y	D	L	E	E	E	O	r	y	z	a						<i>Oryza sativa</i>	
I	S	L	R	E	H	R	V	Y	D	L	E	E	E	B	a	c	h	a	r	i	d	i	u	m	<i>Baccharidium distachyon</i>
I	S	L	K	D	H	K	V	Y	D	L	Q	E	E	P	i	c	e	a						<i>Picea glauca</i>	

(d)

S V T R A D E Arabidopsis thaliana
 S V T R A D E Calcea Pappaya
 S V T R A D E Cucumis sativa
 S V T R A D E Eucalyptus grandis
 T V T R A D E Glycine max
 S V T R A D E Mimulus guttatus
 S V T R A D E Manihot esculenta
 S V T R A D E Populus trichocarpa
 S V T R A D E Prunus persica
 S V T R A D E Ricinus communis
 S V T R A D E Vitis vinifera
 S V T R A D E Zea mays
 S V T R A D E Sorghum bicolor
 S V T R A D E Setaria italica
 S V K R A D E Oryza sativa
 S V T R A D E Brachypodium distachyon
 S V T R A D E Picea glauca

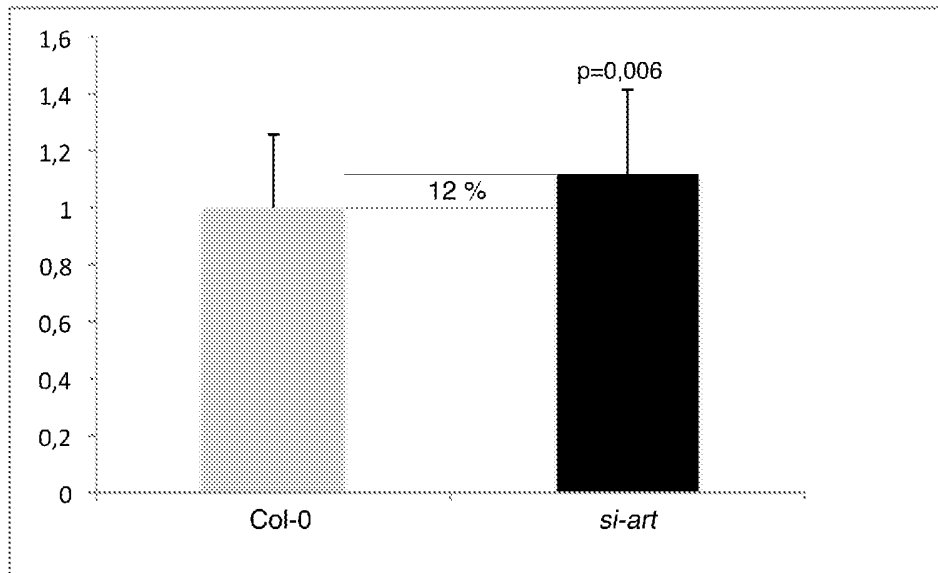
(e)

A E A C A A L S Q A A E A V S S G M S D A S D A T A K A G I I L L P Arabidopsis thaliana
 A E A C A A L S Q A A V E A V A S G M S D V S D I V S E A G I I L L P Calcea Pappaya
 A E A C A A L S Q A A K A I T S G Q S E V S D A V S E A G I I L L P Cucumis sativa
 A E A C A A L S Q A A E A A T S G E S D V S D A V S E A G L I I L L P Eucalyptus grandis
 A E A C A A L S S A S E A V A S G D S D V S D A V S E A G I I L L P Glycine max
 A E A C A A L S Q A A E A V A S G K T D A S D A V S E A G V I I L L P Mimulus guttatus
 A E A C A A L S Q A A E A V A S G D A D P S D A M S E A G V I I L L P Manihot esculenta
 A E A C A A L S Q A A E A V A S G D A D A S M A L S E A G L V I I L L P Populus trichocarpa
 A E A C A A L N Q A A E A V A S G E S D V S G A V S G A G I I L L P Prunus persica
 A E A C A A L S Q A A E A V A S G D A D V N K A M S E A G I I V L L P Ricinus communis
 A E A C A A L V E A A E A I S S G T A E A T D A V S E A G I I L L P Vitis vinifera
 A E A C A A L I E A A E A I S S G T A E T E D A V S M A G I I L L P Zea mays
 A E A C A A A F I E A A E A I S S G T S E V D D A V S K A G I I L L P Sorghum bicolor
 A E A C A A A L I E A A E A I S S G T S E V D D A V S K A G I I L L P Setaria italica
 A E A C A A A L I E A A E A I S S G T S E V E D A V S K A G I I L L P Oryza sativa
 A E V F A K A L T E A A M A V A S G E V D A S E A A S K V G I C I I P Brachypodium distachyon
 Picea glauca

(f)

Fig 22

a)



b)

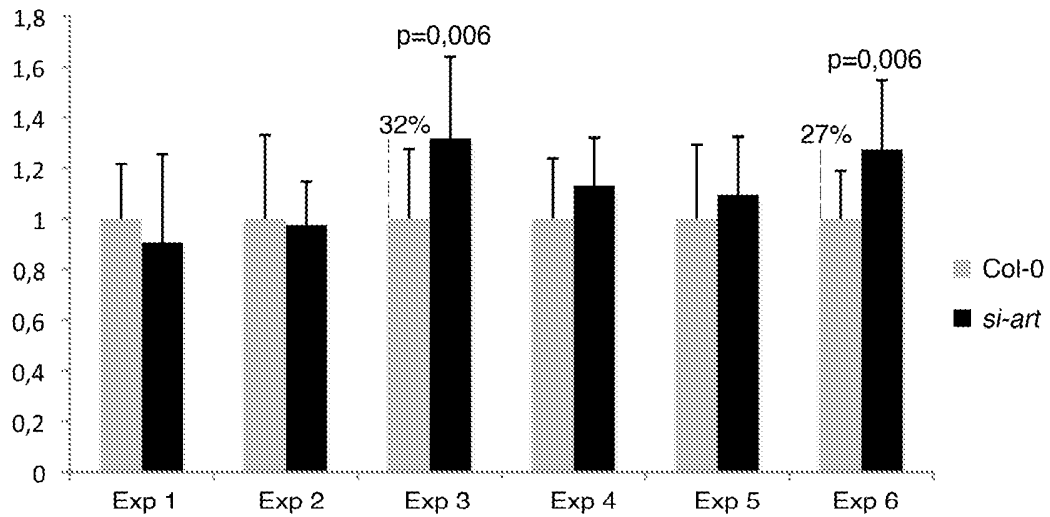
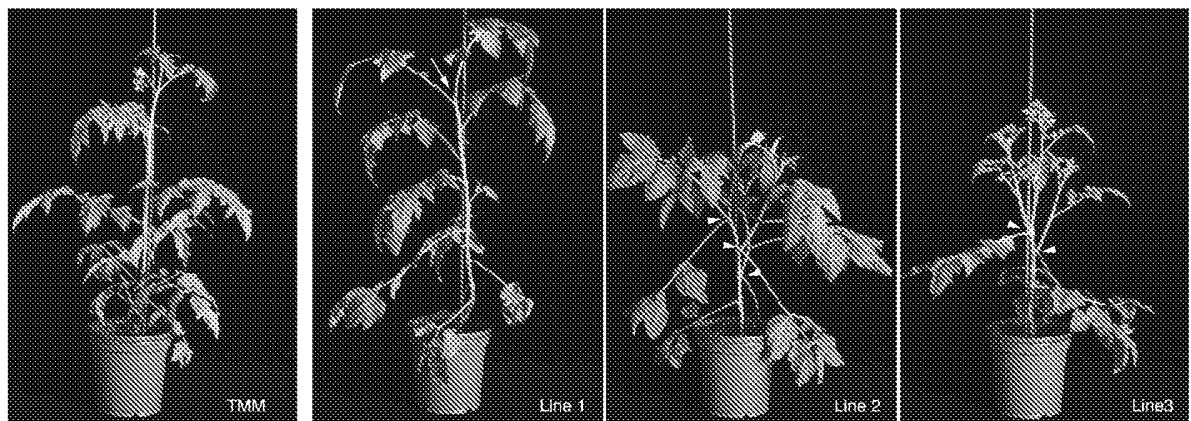


Fig 23



INTERNATIONAL SEARCH REPORT

International application No PCT/GB2012/051146

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/82 C07K14/415 A01H5/00 ADD.				
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) C07K C12N				
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) EPO-Internal, CHEM ABS Data, BIOSIS, Sequence Search, EMBASE, WPI Data				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
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Date of the actual completion of the international search	Date of mailing of the international search report			
23 August 2012	05/09/2012			
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Puonti-Kaerlas, J			

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