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(54) **Titre : METHODE DE MODIFICATION DE VEGETAUX AU MOYEN DE POLYPEPTIDES/PEPTIDES CLE**
(54) **Title: METHOD FOR THE ALTERATION OF PLANTS USING CLE POLYPEPTIDES/PEPTIDES**

(57) **Abrégé/Abstract:**

The present invention relates to altering the biomass and/or structure of a plant, in order to maximise its potential as a source of feedstock or increase its potential as a feedstock for the paper industry. CLE41 and/or CLE42 are used to manipulate growth and structure of the vascular tissue of the plant. The present invention also provides plants in which the levels of CLE41 and/or CLE42 are increased compared to those of a native plant grown under identical conditions, and parts of such plants. Also provided are methods for using such plants or plant parts in the production of plant derived products such as paper or biofuels.

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(54) Title: METHOD FOR THE ALTERATION OF PLANTS USING CLE POLYPEPTIDES / PEPTIDES

(57) Abstract: The present invention relates to altering the biomass and/or structure of a plant, in order to maximise its potential as a source of feedstock or increase its potential as a feedstock for the paper industry. CLE41 and/or CLE42 are used to manipulate growth and structure of the vascular tissue of the plant. The present invention also provides plants in which the levels of CLE41 and/or CLE42 are increased compared to those of a native plant grown under identical conditions, and parts of such plants. Also provided are methods for using such plants or plant parts in the production of plant derived products such as paper or biofuels.

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METHOD FOR THE ALTERATION OF PLANTS USING CLE POLYPEPTIDES/PEPTIDES

FIELD OF THE INVENTION

The present invention relates to manipulating the growth and/or structure of a plant through modulation of the amount of CLE41 and/or CLE42 expressed in the plant, and additionally or alternatively, modulating the amount of PXY in the plant. Manipulating the growth and/or structure of a plant can be used to alter the mechanical properties of a plant or plant derived product, or to maximise its potential for the production of plant derived products such as biofuels and paper.

BACKGROUND

In multi-cellular organisms, cells must communicate with each other in order for growth and development to occur in an ordered manner. In animals, it has long been known that polypeptides act as signalling molecules in mediating communication between cells, a common example being insulin in humans. These signalling molecules are responsible for initiating many cellular processes, typically by binding to a receptor at the cell surface, which in turn transmits a message to inside the cell via downstream signalling proteins such as membrane associated protein kinases (MAPK), tyrosine phosphatases and Ras proteins. In the cell, the cell signalling pathway end-point is usually a transcription factor target, which mediates a change in gene expression in the cell, thus causing a change in the growth and/or development of the cell in response to the initial extracellular signal.

In plants, it is also known that cell signalling occurs, and this was thought to be mediated by plant hormones such as auxin and cytokinin. More recently, the discovery of systemin has shown that polypeptides also play a role in cell-signalling in plants. One of the largest families of signalling polypeptides identified in plants is the Clavata3 (clv3)/Endosperm Surrounding Region (ESR)-related (CLE) family. These proteins are the most highly characterised family of small polypeptides in plants. The *Arabidopsis thaliana* genome contains 32 CLE genes. Clv3 is the best characterised CLE family member which acts together with a receptor kinase (CLAVATA 1) to play a role in regulating the proliferation of cells in the shoot (apical) meristem. At present, however, most of the CLE family remain functionally undefined.

The CLE gene family has been shown to be present in a variety of other plant species (Jun *et al* Cell. Mol. Life. Sci. 65 743-755 (2008) and Frickey *et al* BMC Plant Biology 2008, 8:1 10.1186/1471-2229-8-1) including rice, maize, tomato and alfalfa.

- 5 The polypeptides encoded by the CLE genes share common characteristics. They are less than 15kDa in mass and comprise a short stretch of hydrophobic amino acids at the amino terminus which serves to target the polypeptide to the secretory pathway. This conserved stretch of 14 amino acids is known as the CLE domain (Jun *et al* supra).
- 10 Higher plants show post-embryonic development at shoot and root tips, which are known as the apical meristems. Stem cells at these meristems produce cells which differentiate to become flower, leaf, stem or root cells. A loss-of-function mutant resulting in an excess of stem cells at the apical meristem suggests that Clv3 plays a role in regulation of growth and/or differentiation at the growing tip. Over expression of CLV3 results in
- 15 loss of apical stem cells, thus post-embryonic above ground parts of the plant are lost. The signalling pathway which CLV3 regulates has been elucidated and is described in Jun *et al* (supra). This pathway is thought to be conserved amongst other plants species.
- 20 Shiu and Bleecker suggest that the CLE family is likely to coordinate with a group of plant receptors known as the leucine-rich-repeat receptor-like (LLR-RLK) kinases (PNAS 98 10763-10768 (2001)).

- US Patent No. 7179963 describes a maize clv3-like nucleotide sequence, and its use in
- 25 modulating plant development and differentiation. US Patent No. 7335760 discloses nucleic acid sequences for use in genetically modifying a plant to increase plant yield and the mass of the plant, for example for biofuel production.

- Other CLE family members have been shown to inhibit cell differentiation. For example,
- 30 Frickey *et al* (supra) have looked at the CLE family and suggested that CLE family members CLE41 and/or 42 may play a role in vascular development. Ito *et al* (Science Vol 313 842-845 (2006)) show that dodecapeptides are important in preventing vascular cell differentiation.

- 35 In contrast, however, Strabala *et al* (Plant Physiology vol. 140 1331-1344 (2006)) show that CLE41 and/or 42 are genuine expressed members of the CLE family. Although

general over-expression of CLE42 throughout the plant results in a dwarf phenotype, Strabala *et al*/report that CLE42 is likely to be a functionally redundant molecule.

5 The source of biomass in plants is their woody tissue, derived from the vascular meristems of the plant such as the cambium and procambium, which divide to form the phloem and xylem cells of the vascular tissue within the plant stems and roots. The cambium and procambium (collectively known as the vascular meristems) are growth zones which enable the plant to grow laterally, thus generating the majority of biomass. Enhancing lateral growth by genetically altering the rates of procambial or cambial cell
10 division may lead to an increase in the plant biomass. This would provide an additional source of biomass for various industries dependent upon plant derived products, such as the biofuel or paper industries.

15 Increasing the yield of biomass of plants, for example for paper and fuel production has previously been done by breeding programs, but in recent years there is interest in the use of genetic manipulation or plant modification for such purposes.

The division of cells to form the vascular tissue is a highly ordered process. Prominent polarity of cells destined to become either phloem cells or xylem cells is observed, the
20 latter eventually forming the woody tissue of the plant. Xylem is principally water transporting tissue of the plant, and together with phloem, forms a vascular network for the plant. The cells of the xylem which are principally responsible for carrying water are the tracheary elements, of which there are two types- tracheids and vessels.

25 However, whilst there has been much investigation into the regulation of growth at the apical meristems, there is less understanding of the growth of the vascular tissue. Fisher *et al* (Current Biology 17 1061-1066 (2007)) report a loss of function mutant in which the spatial organisation of the vascular tissue is lost and the xylem and phloem cells are interspersed. The mutant is in a gene named PXY, which encodes a receptor-
30 like kinase.

Tracheary elements (TEs) are cells in the xylem that are highly specialized for transporting water and solutes up the plant. They are produced from xylem cells by a process which involves specification, enlargement, patterned cell wall deposition,
35 programmed cell death and cell wall removal. This results in adjacent TEs being joined together to form a continuous network for water transport.

Jun *et al* (supra) disclose that the CLE domain of CLE41 is identical to Tracheal Element Differentiation Inhibitory Factor (TDIF), which has been shown to inhibit cell differentiation, and CLE42 differs by only one amino acid from the TDIF sequence.

- 5 When exogenously applied to cell cultures, synthetic CLE41 and CLE42 suppressed the formation of tracheary element cells from the xylem (Ito *et al*, supra).

There remains a need for identification of genetic elements, the manipulation of which can be used to alter the growth and/or structure of the plant.

10

BRIEF SUMMARY OF THE INVENTION

In a first aspect the invention provides the use of a polypeptide selected from the group consisting of:

- 15 i) a CLE41 polypeptide;
ii) a CLE42 polypeptide;
iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
20 iv) a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of CLE41 of Figure 13A or CLE42 of Figure 14A;
v) a polypeptide encoded by a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of
25 Figure 14B;

in the manipulation of plant growth and/or structure.

In a second aspect the invention provides the use of a nucleic acid molecule selected from the group consisting of:

- 30 i) a nucleic acid molecule that encodes a CLE41 polypeptide;
ii) a nucleic acid molecule that encodes a CLE42 polypeptide;
iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
35 iv) a nucleic acid molecule which is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B;

- v) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii)

in the manipulation of plant growth and/or structure.

5 Preferably, the use of the first or second aspect is use of the polypeptide or nucleic acid in combination with a nucleic acid molecule selected from the group consisting of:

- i) a nucleic acid molecule that encodes a CLE41 receptor;
- ii) a nucleic acid molecule that encodes a CLE42 receptor;
- 10 iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof;
- iv) a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of Figure 15B or 15C;
- v) a nucleic acid molecule that is at least 70% identical to a nucleic acid
- 15 molecule of i) or ii);
- vi) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii).

20 Preferably, the use of the first or second aspect is use of the polypeptide or nucleic acid in combination with a polypeptide selected from the group consisting of:

- i) a CLE41 receptor;
- ii) a CLE42 receptor;
- 25 iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof
- iv) a polypeptide comprising an amino acid sequence that is at least 70% identical to the PXY sequence of Figure 15A;
- v) a polypeptide sequence comprising an amino acid sequence which is at least 70% identical to a sequence encoding i) or ii);
- 30 vi) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 70% identical to the PXY nucleotide sequence of Figure 15B or 15C.

Preferably said CLE41 or CLE42 receptor is PXY or a functional equivalent thereof.

Preferably said manipulation of the plant growth and/or structure is an increase or decrease in the amount of growth and/or division of the procambial and/or cambial cells in a plant, specifically the number of cells generated. More specifically, it is an increase or decrease in the rate of division of such cells. Thus, the manipulation of growth and/or structure can be said to be an increase or decrease in the secondary growth of the plant, and/or an increase or decrease in the degree of organisation of the secondary structure, at the cellular level. By secondary growth is preferably meant the woody tissue of a plant, or the vascular or interfascicular tissue. Preferably, where there is an increase in the number of procambial and/or cambial cells, these cells differentiate into xylem and/or phloem cells, preferably the former.

In a further aspect, the present invention provides a method of manipulating the growth and/or structure of a plant, comprising modulating the level of CLE41 and/or CLE42 or a functional equivalent thereof, in the plant.

15

Preferably the levels of CLE41 and/or CLE42 are modulated by introducing into a cell of the plant:

- i) a CLE41 polypeptide;
- ii) a CLE42 polypeptide;
- 20 iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
- iv) a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of CLE41 of Figure 13A or CLE42 of Figure 14A;
- 25 v) a polypeptide encoded by a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B.

30 Alternatively the levels of CLE41 and/or CLE42 are modulated by introducing into a cell of the plant:

- i) a nucleic acid molecule that encodes a CLE41 polypeptide;
- ii) a nucleic acid molecule that encodes a CLE42 polypeptide;
- 35 iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;

- iv) a nucleic acid molecule which is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B;
- v) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii).

5

Preferably, the levels of levels of CLE41 and/or CLE42 or a functional equivalent thereof are upregulated.

Optionally, the method further comprises introducing into a cell of the plant:

- 10 i) a nucleic acid molecule that encodes a CLE41 receptor;
- ii) a nucleic acid molecule that encodes a CLE42 receptor;
- iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof;
- 15 iv) a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of Figure 15B or 15C;
- v) a nucleic acid molecule that is at least 70% identical to a nucleic acid molecule of i) or ii);
- vi) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii).
- 20

Alternatively, the method further comprises introducing into cell of a plant:

- i) a CLE41 receptor;
- ii) a CLE42 receptor;
- 25 iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof
- iv) a polypeptide comprising an amino acid sequence that is at least 70% identical to the PXY sequence of Figure 15A;
- 30 v) a polypeptide sequence comprising an amino acid sequence which is at least 70% identical to a sequence encoding i) or ii);
- vi) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 70% identical to the PXY nucleotide sequence of Figure 15B or 15C.

35

Preferably said CLE41 and/or CLE42 receptor is PXY or a functional equivalent thereof.

In aspects where the levels of two or more of CLE41, CLE42 and PXY are to be manipulated in a plant, this may be achieved by:

- (i) manipulating the levels of CLE41 and/ or CLE42 as hereinbefore described, in a first plant;
- 5 (ii) manipulating the levels of a CLE41 and/or CLE42 receptor as hereinbefore described, in a second plant;
- (iii) crossing said first and second plants to obtain a plant in which the levels of CLE41 and/or CLE42 and said receptor are manipulated. Also provided in the present invention is the plant produced by the crossing of the first and second plants,
10 and progeny thereof which express the non-native nucleotide and/or polypeptide sequences.

In a further aspect, the present invention provides a plant cell manipulated to express:

- i) a CLE41 polypeptide;
- 15 ii) a CLE42 polypeptide;
- iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
- iv) a polypeptide comprising an amino acid sequence that is at least 70%
20 identical to the amino acid sequence of CLE41 of Figure 13A or CLE42 of Figure 14A;
- v) a polypeptide encoded by a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B;
- 25 optionally in combination with expression of a receptor for CLE41 and/or CLE42.

In a further aspect, the present invention provides a plant cell manipulated to express

- i) a nucleic acid molecule that encodes a CLE41 polypeptide;
- ii) a nucleic acid molecule that encodes a CLE42 polypeptide;
- 30 iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
- iv) a nucleic acid molecule which is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B;
- 35 v) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii).

Preferably, said plant cell is further manipulated to express a nucleic acid molecule selected from the group consisting of:

- i) a nucleic acid molecule that encodes a CLE41 receptor;
- 5 ii) a nucleic acid molecule that encodes a CLE42 receptor;
- iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof;
- iv) a nucleic acid molecule that is at least 70% identical to the nucleotide
10 sequence of Figure 15B or 15C;
- v) a nucleic acid molecule that is at least 70% identical to a nucleic acid molecule of i) or ii);
- vi) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii).

15

Alternatively, said plant cell is further manipulated to express a polypeptide selected from the group consisting of:

- i) a CLE41 receptor;
- ii) a CLE42 receptor;
- 20 iii) a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof
- iv) a polypeptide comprising an amino acid sequence that is at least 70% identical to the PXY sequence of Figure 15A;
- 25 v) a polypeptide sequence comprising an amino acid sequence which is at least 70% identical to a sequence encoding i) or ii);
- vi) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 70% identical to the PXY nucleotide sequence of Figure 15B or 15C.

30

Preferably said CLE41 and/or CLE42 receptor is PXY or a functional equivalent thereof.

In a further aspect, there is provided a nucleic acid molecule encoding a functional equivalent of PXY, preferably derived from *Arabidopsis thaliana*, poplar or rice, and more
35 preferably encoding the amino acid sequence of the consensus sequence of Figure 12. Also provided is a polypeptide sequence encoding a functional equivalent of PXY,

preferably derived from *Arabidopsis thaliana*, poplar or rice, and preferably comprising an amino acid sequence of the consensus sequence of Figure 12. Preferably, the amino acid sequence comprises the sequence of pttPXY, PXYL-1, PXYL-2 or Os02g02140.1, or Os03g05140.1 of Figure 12. Also included are sequences having
5 70% sequence identity or sequence homology thereto.

It is apparent that the levels of CLE41, CLE42 and or a receptor thereof, such as PXY, in each of the aspects of the present invention may be manipulated by altering the expression of native CLE41, CLE42 and or a receptor thereof within the plant cell. This
10 may be achieved by placing the native nucleotide sequence under the control of a nucleotide sequence which modifies expression of a native gene to allow modify expression thereof. The nucleotide sequence may be a regulatory sequence, as defined herein, or may encode a regulatory protein, such as a transcription factor, or may encode a DNA or RNA antisense sequence. As such, the nucleotide sequence or its
15 expression product can modify expression, amount and/or activity of a native gene/polypeptide. Methods of function of such regulatory proteins, expression products and antisense will be known to persons skilled in the art.

In a yet further aspect, the present invention provides a plant comprising a cell according
20 to the invention. Also provided are progeny of the plants of the invention.

In a further aspect, there is provided the use of a cell or plant of the invention in the production of a plant-derived product. A plant-derived product may include biomass, fibres, forage, biocomposites, biopolymers, wood, biofuel or paper. In addition, the
25 invention provides the use of a cell or a plant of the invention in altering the mechanical properties of a plant or a plant derived product.

In a further aspect, the present invention provides a method of manipulating the growth and/or structure of a plant, comprising the steps of:
30 i) providing a cell/seed according to the invention;
ii) regenerating said cell/seed into a plant; and optionally
iii) monitoring the levels of CLE41 and/or CLE42 or a receptor thereof, and or PXY or functional equivalents thereof in said regenerated plant.

35 In a further aspect, there is provided an expression construct comprising a first nucleic acid sequence selected from the group consisting of:

- i) a nucleic acid molecule that encodes a CLE41 polypeptide;
 - ii) a nucleic acid molecule that encodes a CLE42 polypeptide;
 - iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of Figure 10;
 - iv) a nucleic acid molecule which is at least 70% identical to the nucleotide sequence of CLE41 of Figure 13B or CLE42 of Figure 14B;
 - v) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii);
- and optionally a second nucleic sequence encoding a regulatory sequence capable of expressing the first nucleic sequence specifically in or adjacent to the vascular tissue of a plant.

Preferably, the regulatory sequence will be capable of directing expression of a nucleotide sequence specifically to the vascular tissue, preferably to the cambial/procambial cells and more preferably to tissue adjacent to the cambial/procambial cells i.e. the phloem and/or xylem tissue. Most preferably, a regulatory sequence used in the present invention will be capable of directing expression specifically to the phloem cells. Examples of suitable phloem specific regulatory sequences are SUC2 and APL, KAN1, KAN2, At4g33660, At3g61380, At1g79380. Xylem specific regulatory sequences may also be used in the present invention. Examples include REV, IRX1 COBL4, KOR, At2g38080, and At1g27440, the promoter sequence for the irregular xylem3 (irx3) (AtCESA7) gene, the promoter sequence for the irregular xylem7 (FRAGILE FIBER 8) gene, and the promoter sequence for the irregular xylem12 (ARABIDOPSIS LACCASE-LIKE MULTICOPPER OXIDASE 4) gene (Brown et al. The Plant Cell, Vol. 17, 2281–2295).

Optionally, the expression cassette may further comprise a third nucleic acid sequence selected from the group consisting of:

- i) a nucleic acid molecule that encodes a CLE41 receptor;
- ii) a nucleic acid molecule that encodes a CLE42 receptor;
- iii) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 70% identical to the consensus sequence of Figure 12, or a functional equivalent thereof;

- iv) a nucleic acid molecule that is at least 70% identical to the nucleotide sequence of Figure 15B or 15C;
- v) a nucleic acid molecule that is at least 70% identical to a nucleic acid molecule of i) or ii);
- 5 vi) a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence i) or ii)

Preferably, the expression cassette comprises a nucleic acid encoding PXY or a functional equivalent thereof. The third nucleic acid sequence may be provided on the
10 same expression cassette as the first and/or second nucleic acid sequence, or on a separate expression cassette to the first nucleic acid sequence. The third nucleic acid sequence may be under the control of fourth nucleic acid sequence encoding a regulatory sequence capable of expressing the third nucleic sequence specifically in or adjacent to the vascular tissue of a plant.

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The second nucleic acid sequence may be the same or different to the fourth nucleic acid sequence.

In a further aspect, there is provided a host cell or organism comprising an expression
20 construct of the invention.

According to a further aspect of the invention there is provided a transgenic plant seed comprising a cell according to the invention.

25 The present invention also provides a plant derived product produced by a method of the invention.

The present invention also provides a host cell or organism comprising an expression construct of the invention. A host cell or organism may be a plant cell, plant seed, plant,
30 or other plant material.

The present a method of producing a plant-derived product comprising:

- a) manipulating the growth and/or structure of a plant using the methods of the invention;
- 35 b) growing the plant until it reaches a pre-determined lateral size; optionally
- c) harvesting the plant derived product of the plant.

A plant-derived product may include biomass, fibres, forage, biocomposites, biopolymers, wood, biofuel or paper.

- 5 The present invention also provides a method of altering the mechanical properties of a plant or plant derived product comprising:
- a) manipulating the growth and/or structure of a plant using the methods of the invention;
 - b) growing the plant until it reaches a pre-determined size; and optionally
 - 10 c) harvesting a plant derived product of the plant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the cambial meristem of birch (A) and *Arabidopsis* (B). (A) Transverse section of the growing cambium in Birch. (B) Cross section of a vascular bundle from an *Arabidopsis* stem. Phloem (ph), xylem (xy), procambium (pc) and cambium (c) are indicated.

Figure 2 is a diagrammatic representation of vascular development by the procambium (in, for example, *Arabidopsis*) or by the cambium (for example in trees such as birch). New cells arise from division of procambial cells that subsequently differentiate into the phloem or xylem. In this model a ligand expressed in the xylem signals cell division in the procambium.

Figure 3 shows the effects of over-expressing CLE41 (B,C) and CLE42 (D) on *Arabidopsis* vascular development compared to the wild type (A) in 35 day old plants. See also Table 1.

Figure 4 is a graph showing the average number of cells in the vascular bundles of wild type compared to *35S::CLE41* and *35S::CLE42* *Arabidopsis* plants at 35 days.

30

Figure 5 shows the effect of over-expressing CLE41 on the stem vascular bundle of an *Arabidopsis* compared to the wild-type. Sections through stem vascular bundles of Wild type, *35S::CLE41* and *35S::CLE42* from 50 day old plants. A large number of the extra cells in *35S::CLE41* plants (c.f. Figs 3 and 4) have differentiated into xylem cells. *35S::CLE41* therefore has more xylem cells than wild type.

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Figure 6 shows the effects on plant stature of wild type compared to *35S::CLE41* and *35S::CLE42* in *Arabidopsis*.

Figure 7 shows the disrupted hypocotyl structure of a *Arabidopsis* plant where either
 5 CLE41 (M) or CLE42 (N) is over-expressed and compared with the wild type (L). In the transgenic lines (M, N), hypocotyls are much larger.

Figure 8 shows the effect of over expressing both CLE41 and/or CLE42 and PXY on the structure and amount of cells in the vascular bundle and interfascicular region of stems
 10 from an *Arabidopsis thaliana* plant. Simultaneous over-expression of PXY and CLE41/42 gives increased in vascular cell number compared to plants over expressing CLE41/42 alone (c.f. Fig. 3).

Figure 9 shows the effect of over expression of both CLE41 and/or CLE42 and PXY on
 15 the leaf structure of an *Arabidopsis thaliana* plant. Multiple midveins in *35S::CLE42* *35S::PXY* plants demonstrate that expression of CLE42 and PXY can initiate vascular tissue.

Figure 10 is an alignment of rice, poplar and *Arabidopsis thaliana* putative PXY ligands.
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Figure 11 shows the conservation of residues in the CLE signalling domain - the dashed line indicates the group 5 that contains all the putative PXY ligands.

Figure 12 is a comparison of the kinase domain of PXY (*Arabidopsis thaliana*) from
 25 proteins in rice (Os02g02140.1), poplar (PttPXY) and *Arabidopsis thaliana* (PXL1 and PXL2).

Figure 13 shows the amino acid sequence of the CLE41 proteins (A) and nucleotide
 30 sequence of the CLE41 gene (B).

Figure 14 shows the amino acid sequence of the CLE42 proteins (A) and nucleotide
 sequence of the CLE42 gene (B).

Figure 15 shows the amino acid sequence of the PXY proteins (A) and nucleotide
 35 sequence of the PXY gene without (B) or with (C) the intron.

Figure 16 shows preferred promoter and terminator sequences for use in the invention.

Figure 17 shows the diagram of the multisite gateway kit for cloning.

- 5 Figure 18 is an alignment of full length PXY and related PXY proteins from poplar (PttPXY) and *Arabidopsis thaliana* (PXYL1 and 2)

Figure 19 the heights of *Nicotiana* plants which over express CLE41, PXY or both are shown. A height defect is associated with plants carrying the 35S::CLE41 construct.

- 10 Normal plant height is restored when plants harbour both 35S::CLE41 and 35S::PXY cassettes.

Figure 20 shows the cross section of the *Nicotiana* plants showing tissue structure and size. 35S::CLE41 35S::PXY plants have hypocotyls larger than wild type

15

Figure 21 shows cell organisation in *Nicotiana* plants in transverse section. 35S::CLE41 and 35S::CLE42 plants have more vascular tissue than wild type, but it is not ordered. 35S::CLE41 35S::PXY plants have ordered vasculature. Given that these plants have larger hypocotyls than wild type (see Fig 20), and are of normal height (see Fig 19),
20 these plants clearly have more vascular tissue than wild type.

- Figure 22 shows the effect of phloem specific promoter SUC2 and xylem specific promoter IRX3 on cell organisation in *Arabidopsis*. IRX3::CLE41 plants have large vascular bundles. IRX3::CLE41 35S::PXY plants have large vascular bundles with large
25 amounts of secondary growth (c.f. Fig 8 wild type). SUC2::CLE41 and SUC2::CLE41 35S::PXY plants have vascular tissue that is highly ordered with many more vascular cells than wild type.

- Figure 23 is a graph showing the mean number of cells per vascular bundle. Expression
30 of CLE41 in phloem cells under SUC2 gives more cells per vascular bundle in plants 6 week old *Arabidopsis* plants.

Figure 24 is a graph showing the effect of over expression of CLE41 and CLE42 on hypocotyl diameter in *Arabidopsis*.

35

Figure 25 shows the effect of over expression of CLE41 in poplar trees using the SUC2 or 35S promoters. The bracket denotes xylem cells, of which there are more in *35S::CLE41* and *SUC2::CLE41* than wild type.

5 DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the finding that CLE41 and CLE42 function as ligands for the transmembrane receptor kinase PXY in plants, and modify and/or initiate the regulatory pathway which controls cell division and differentiation in the vascular tissue of a plant. Thus, by modulating the levels of CLE41 and/or CLE42 in a plant, optionally in combination with PXY, the growth and/or structure of the plant can be manipulated, as hereinbefore described.

In particular, the present invention is based upon the finding that individual over-expression of CLE41 and/or CLE42 leads to an excess of undifferentiated cells in the vascular meristem and a subsequent increase in the radial thickness of the xylem. Further, over-expression of PXY or a functional equivalent thereof, together with a PXY ligand such as CLE41 and/or CLE42, results in an excess of undifferentiated cells in the vascular meristem of the plant, which show a highly ordered structure. This excess of cells in the vascular meristem have been shown to then differentiate into xylem cells, thus increasing the radial thickness of the xylem and the biomass of the plant.

A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of without abolishing or, more preferably, without substantially altering a biological activity, whereas an “essential” amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the conserved potassium channel domain are predicted to be particularly non-amenable to alteration, except that amino acid residues in transmembrane domains can generally be replaced by other residues having approximately equivalent hydrophobicity without significantly altering activity.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine,

asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue
5 in protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of coding sequences, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis of a polypeptide, the encoded proteins can be
10 expressed recombinantly and the activity of the protein can be determined.

As used herein, a “biologically active fragment” of protein includes fragment of protein that participate in an interaction between molecules and non-molecules. Biologically active portions of protein include peptides comprising amino acid sequences sufficiently
15 homologous to or derived from the amino acid sequences of the protein, which include fewer amino acids than the full length protein, and exhibit at least one activity of protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the protein, e.g., the ability to modulate membrane excitability, intracellular ion concentration, membrane polarization, and action potential.

20

As used herein, the term “nucleic acid molecule” includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., a mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. The
25 nucleotide sequence may be RNA or DNA, including cDNA.

With regards to genomic DNA, the term “isolated” includes nucleic acid molecules that are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an “isolated” nucleic acid is free of sequences that naturally
30 flank the nucleic acid (i.e., sequences located at the 5'- and/or 3'-ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals
35 when chemically synthesized.

As used herein, a “naturally-occurring” nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

- 5 As used herein, the terms “gene” and “recombinant gene” refer to nucleic acid molecules which include an open reading frame encoding protein, and can further include non-coding regulatory sequences and introns.

In the present invention, CLE41 and CLE42 are polypeptides comprising the amino acid
10 sequence of Figure 13A or 14A, respectively. CLE41 and CLE42 are ligands which are able to activate a kinase receptor, and result in phosphorylation of itself or its target. References to CLE41 and/or CLE42 include functional equivalents thereof. By a functional equivalent of CLE41 or CLE42 is meant a polypeptide which is derived from the consensus sequence of Figure 10 by addition, deletion or substitution of one or more
15 amino acids, preferably non-essential amino acids. Preferably, a substitution is a conservative substitution. A functional equivalent of CLE41 and/or CLE42 for use in the present invention will be biologically active, and preferably have some or all of the desired biological activity of the native polypeptide, preferably the ability to bind to PXY or a functional equivalent thereof and regulate growth and/or differentiation of the
20 vascular tissue. Preferably, the equivalent is a signalling protein, preferably of less than 15kDa in mass, and preferably comprising a hydrophobic region at the amino terminus. Functional equivalents may exhibit altered binding characteristics to PXY compared to a native CLE41 and/or CLE42 protein, but will mediate the same downstream signalling pathway. Preferred functional equivalents may show reduced non-desirable biological
25 activity compared to the native protein. Preferably, the equivalent comprises a conserved region of 14 amino acids having a sequence which is at least 70% more preferably 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the conserved region of amino acids 124 to 137 of the consensus of Figure 10, and more preferably across the full length of the consensus sequence. A functional equivalent of CLE41 and/or CLE42
30 preferably also shares at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with CLE41 or CLE42. Preferably, a functional equivalent may also share sequence identity with the CLE41 and/or CLE42 sequence of Figures 13A and 14A, respectively. Preferably,
35 functional equivalents have at least preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% % sequence identity with the amino acid sequence of Figures 13A and 14A respectively.

References to CLE41 and/or CLE42 also include fragments of the CLE41 and/or CLE42 polypeptides or their functional equivalents. A fragment is a portion of a polypeptide sequence, preferably which retains some or all of the biological activity of the full length sequence. Preferably, fragments of CLE41 and/or CLE42 retain the ability to bind PXY and regulate the growth and/or differentiation of the vascular tissue of a plant. Preferably, a fragment may be at least 7 amino acids in length, preferably at least 8, 9, 10 or 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids in length, up to the full length CLE polypeptide. Most preferably, a fragment will comprise the conserved region consisting of amino acids 124 to 137 of the consensus sequence of Figure 10.

Nucleic acid molecules encoding CLE41 and CLE42 are preferably those which encode an amino acid sequence as defined by the consensus sequence of Figure 10, and preferably having the sequences as shown in Figure 13B or 14B. References to nucleic acid molecules encoding CLE41 and CLE42 also include variants of the sequences of Figures 13B or 14B. A variant sequence is derived from the sequence of Figure 13B or 14B by the addition, deletion or substitution of one or more nucleotide residues. The variant preferably encodes a polypeptide having CLE41 or CLE42 or a functional equivalent thereof, as defined herein. Preferably, a variant of a nucleotide sequence of Figure 13B or 14B will have at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence of Figure 13B or 14B. Alternatively, a variant sequence which is substantially identical to a sequence of Figure 13B or 14B may also be defined as one which hybridises under stringent conditions to the complement of a nucleotide sequence of Figure 13B or 14B.

Nucleic acid molecules encoding CLE41 and/or CLE42 may be derived from *Arabidopsis*, or may be derived from any other plant and will preferably share preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a CLE41 and/or CLE42 gene from *Arabidopsis thaliana*, as shown in Figure 13B or 14B.

Also encompassed by the present invention are fragments of the nucleic acid molecules encoding CLE41 and/or or CLE42. Preferably, such fragments encode a fragment of a CLE41 or CLE42 polypeptide as defined herein. A fragment of a nucleic acid molecules encoding CLE41 or CLE42 will preferably comprise at least 21 nucleotides in length, more preferably at least 24, 27, 30 or 33 nucleotides, up to the total number of nucleotide residues in a full length sequence of Figure 13B or 14B.

In the present invention, PXY is a polypeptide having the amino acid sequences shown in Figure 15A. References to PXY include functional equivalents thereof. By a functional equivalent of PXY is meant a polypeptide which is derived from the native PXY polypeptide sequence of Figure 15 by addition, deletion or substitution of one or more amino acids. A functional equivalent of PXY for use in the present invention will be biologically active, and preferably have some or all of the desired biological activity of the native polypeptide, preferably the ability to bind to CLE41 and/or CLE42 and regulate growth and/or differentiation of the vascular tissue. Functional equivalents may exhibit altered binding characteristics to CLE41 and/or CLE42 compared to a native PXY protein. Preferred functional equivalents may show reduced non-desirable biological activity compared to the native protein.

In the present invention, PXY and functional equivalents thereof are proteins found in undifferentiated procambial cells, which mediate activation of the a signalling pathway when bound by CLE41 and/or CLE42, resulting in division of the cambial cells. Preferably, PXY or its functional equivalents is a protein kinase, preferably comprising a leucine rich domain. More preferably, it comprises a LLR-RLK (Leucine Rich Repeat-Receptor-Like-Kinase) protein. Preferably, PXY or its functional equivalents are members of the XI family of *Arabidopsis thaliana* RLK proteins, and preferably comprise a conserved region in the kinase domain having the sequence comprising the consensus sequence of Figure 12 or a biologically active portion thereof, or a sequence having at least 30%, 40%, 50%, 55%, 60, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the consensus sequence of Figure 12. Most preferably, a functional equivalent thereof will preferably comprise an amino acid having at least 70% sequence identity to the consensus sequence of Figure 12 and preferably will bind a CLE 41 and/or CLE 42 polypeptide or fragment thereof. A functional equivalent of PXY preferably also shares preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,

or 100% sequence homology with native PXY. Preferably, a functional equivalent may also share sequence identity with the PXY sequence of Figure 15A, respectively

References to PXY also include fragments of the PXY polypeptides or its functional
5 equivalents. A fragment is a portion of a polypeptide sequence, preferably which
retains some or all of the biological activity of the full length sequence. Preferably,
fragments of PXY retain the ability to bind a ligand and regulate the growth and/or
differentiation of the vascular tissue of a plant. Preferably, a fragment will comprise at
least a portion of the kinase domain, preferably a biologically active portion thereof, up to
10 the full length kinase domain. Most preferably, a fragment will further comprise at least
a portion of the extracellular domain, and will preferably comprise at least a portion of
the LLR region.

Nucleic acid molecules encoding PXY are preferably those which encode an amino acid
15 sequence as defined in Figure 15A, and preferably having the sequences as shown in
Figure 15B or C. References to nucleic acid molecules encoding PXY also include
variants of the sequences of Figure 15B or C. A variant sequence is a nucleic acid
molecules which is derived from the sequence of Figure 15B or C by the addition,
deletion or substitution of one or more nucleotide residues. The variant preferably
20 encodes a polypeptide having PXY activity, as defined herein. Preferably, a variant of a
nucleotide sequence of Figure 15B or C will have at least 50%, even more preferably at
least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%,
87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%
sequence identity with a sequence of Figure 15B or C. Alternatively, a variant sequence
25 which is substantially identical to a sequence of Figure 15B or C may also be defined as
one which hybridises under stringent conditions to the complement of a nucleotide
sequence of Figure 15B or C.

Nucleotide sequences encoding PXY may be derived from *Arabidopsis*, or may be
30 derived from any other plant and will preferably share preferably at least 50%, even
more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%,
84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
99%, or 100% % sequence identity with a PXY gene from *Arabidopsis thaliana*, as
shown in Figure 15B or C. Genbank references are PXY = At5g61480 (TAIR), PXL1=
35 At1g08590 (TAIR), PXL2 = At4g28650 (TAIR).

Also encompassed by the present invention are fragments of the nucleic acid molecule encoding PXY. Preferably, such fragments encode a fragment of a PXY polypeptide as defined herein. A fragment of a nucleic acid molecule encoding PXY will preferably comprise at least 10, 20, 30, 40, 50, 60, 70, 80 or 90 or 100, 200 or 300 or more
5 nucleotides in length, up to the total number of nucleotide residues in a full length sequence of Figure 15A.

Also provided in the present invention are antisense sequences of the above mentioned nucleic acid molecules, which hybridise under stringent conditions to the nucleotide
10 sequences encoding CLE41 and/or CLE42 or PXY, or a functional equivalents thereof, as defined above. Such sequences are useful in down regulating expression of the CLE41 and/or CLE42 and/or PXY or functional equivalents thereof. Whilst in a preferred embodiment, both receptor and ligand will be either up-regulated (over-expressed) or down-regulated in a cell of a plant, it is envisaged that it may in certain circumstances be
15 desirable to up-regulate either the receptor whilst down-regulating the ligand, or vice versa.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and
20 can be found in available references (e.g., Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6). Aqueous and non-aqueous methods are described in that reference and either can be used. A preferred example of stringent hybridization conditions are hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2× SSC, 0.1% (w/v) SDS at 50°C.
25 Another example of stringent hybridization conditions are hybridization in 6× SSC at about 45°C, followed by one or more washes in 0.2× SSC, 0.1% (w/v) SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6× SSC at about 45°C, followed by one or more washes in 0.2× SSC, 0.1% (w/v) SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6× SSC at about 45°C,
30 followed by one or more washes in 0.2× SSC, 0.1% (w/v) SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5 molar sodium phosphate, 7% (w/v) SDS at 65°C, followed by one or more washes at 0.2× SSC, 1% (w/v) SDS at 65°C.
35 Preferably, an isolated nucleic acid molecule of the invention that hybridizes under

stringent conditions to the sequence of Figure 15 B or C corresponds to a naturally-occurring nucleic acid molecule.

Sequence identity is determined by comparing the two aligned sequences over a pre-determined comparison window, and determining the number of positions at which -
5 identical residues occur. Typically, this is expressed as a percentage. The measurement of sequence identity of a nucleotide sequences is a method well known to those skilled in the art, using computer implemented mathematical algorithms such as ALIGN (Version 2.0), GAP, BESTFIT, BLAST (Altschul *et al* J. Mol. Biol. 215: 403
10 (1990)), FASTA and TFASTA (Wisconsin Genetic Software Package Version 8, available from Genetics Computer Group, Accelrys Inc. San Diego, California), and CLUSTAL (Higgins *et al*, Gene 73: 237-244 (1998)), using default parameters.

Calculations of sequence homology or identity between sequences are performed as
15 follows:

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence
20 for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 75%, 80%, 82%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,
25 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity
30 between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two
35 sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined

using the Needleman *et al.* (1970) J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a BLOSUM 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers *et al.* (1989) CABIOS 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) J. Mol. Biol. 215:403-410). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, gapped BLAST can be utilized as described in Altschul *et al.* (1997, Nucl. Acids Res. 25:3389-3402). When using BLAST and gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <<http://www.ncbi.nlm.nih.gov>>.

Sequence comparisons are preferably made over the full-length of the relevant sequence described herein.

The polypeptide sequences and nucleic acid molecules used in the present invention may be isolated or purified. By "purified" is meant that they are substantially free from other cellular components or material, or culture medium. "Isolated" means that they may also be free of naturally occurring sequences which flank the native sequence, for example in the case of nucleic acid molecule, isolated may mean that it is free of 5' and 3' regulatory sequences.

The polypeptide and nucleic acid molecule used in the invention may be naturally occurring or may be synthetic. The nucleic acid molecule may be recombinant.

10

The present invention is based upon using either CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, to manipulate the growth and/or structure of a plant. By "manipulate" is meant altering the native growth pattern of a plant, compared to that of a non-manipulated plant of the same species, grown under identical conditions. The manipulation is preferably effected by altering the levels of said receptor and ligand in a cell of the plant.

A plant having increased levels of said CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof in a particular tissue and at a pre-selected developmental stage, compared to the native levels in the same tissue of a native plant of the same species, at the same developmental stage and grown in identical conditions.

Herein, the growth of a plant refers to the size of a plant, preferably the secondary growth, and preferably the amount of vascular and/or interfascicular tissue, more preferably the amount of xylem cells, also referred to as the woody tissue or biomass of a plant.

By identical conditions is meant conditions which are the substantially the same in terms of temperature, light, and availability of nutrients and water. By substantially is meant that the conditions may vary slightly, but not to an extent to which is known to affect the growth of a plant.

The structure of a plant refers to the organisation of tissue in a plant, preferably the vascular tissue, most preferably the polarity of the phloem and xylem cells.

35

The use of said CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof as defined herein to manipulate the growth and/or structure of a plant may be achieved in any manner which alters the regulation of the signalling pathway mediated by CLE41 and/or CLE42 binding to PXY. Preferably, the invention may be achieved in any manner
5 which up-regulates the signalling pathway. Preferably, the manipulation is mediated via a PXY ligand as defined herein, preferably CLE41 and/or CLE42, or via a CLE41 and/or CLE42 receptor, preferably PXY. For example, manipulation may comprise altering their expression pattern within the plant, altering the amount of said receptor and/or ligand within the plant, or altering the binding pattern thereof.

10

By modulation of the levels of the CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, is meant an increase or decrease in the levels of in the plant, preferably the levels localised in the vascular tissue, and preferably in the cambium or procambium of a plant, as compared to the levels in the same tissue in a native plant of
15 the same species at the same stage if developed and grown under identical conditions, and in which no modulation has been made. Preferably, the levels of CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, are increased. Preferred levels of PXY ligand are at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90% more or less relative to said native plant. Preferred levels of CLE41 and/or CLE42 receptor are 5, 10, 20, 30,
20 40, 50, 60, 70, 80, 90% more or less relative to said native plant.

The alteration in levels of CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, as defined above preferably increases or decreases the activity by at least about 2-fold compared to a basal level of activity. More preferably said activity is increased or
25 decreased by at least about 5 fold; 10 fold; 20 fold, 30 fold, 40 fold, 50 fold. Preferably said activity is increased or decreased by between at least 50 fold and 100 fold. Preferably said increase or decrease is greater than 100-fold.

It will be apparent that means to modulate the activity of a polypeptide encoded by a nucleic acid molecule are known to the skilled artisan. For example, and not by limitation, altering the gene dosage by providing a cell with multiple copies of said gene or its complement. Alternatively, or in addition, a gene(s) may be placed under the control of a powerful promoter sequence or an inducible promoter sequence to elevate expression of mRNA encoded by said gene. The modulation of mRNA stability is also a mechanism used to alter the steady state levels of an mRNA molecule, typically via alteration to the 5' or 3' untranslated regions of the mRNA.

It is envisaged that where a plant naturally expresses said CLE41 and/or CLE42 receptor and/or PXY ligand, their modulation may be achieved by altering the expression pattern of the native gene(s) and/or production of the polypeptide. This may be achieved by any suitable method, including altering transcription of the gene, and/or translation of the mRNA into polypeptide, and post-translational modification of the polypeptide.

Altering the expression pattern of a native gene may be achieved by placing it under control of a heterologous regulatory sequence, which is capable of directing the desired expression pattern of the gene. Suitable regulatory sequences are described herein. Alternatively, regulation of expression of the native gene may be altered through changing the pattern of transcription factors which mediate expression of the gene. This may require the use of modified transcription factors, whose binding pattern is altered to obtain a desired expression pattern of the gene. Alternatively, the copy number of the native gene may be increased or decreased, in order to change the amount of expression of the gene. Suitable methods for carrying out these embodiments of the invention are known to persons skilled in the art, and may employ the use of an expression construct according to the invention.

Plants transformed with a nucleic acid molecule or expression construct of the invention may be produced by standard techniques known in the art for the genetic manipulation of plants. DNA can be introduced into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transferability (EP-A-270355, EP-A-0116718, NAR 12(22):8711-87215 (1984), Townsend et al., US Patent No. 5,563,055); particle or microprojectile bombardment (US Patent No. 5,100,792, EP-A-444882, EP-A-434616; Sanford et al., US Patent No. 4,945,050; Tomes et al. (1995) "Direct DNA Transfer into Intact Plant Cells via

Microprojectile Bombardment", in *Plant Cell, Tissue and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe et al. (1988) *Biotechnology* 6: 923-926); microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. 91987) *Plant Tissue and Cell Culture*, Academic Press, Crossway et al. (1986) *Biotechniques* 4:320-334); electroporation (EP 290395, WO 8706614, Riggs et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606; D'Halluin et al. 91992). *Plant Cell* 4:1495-1505) other forms of direct DNA uptake (DE 4005152, WO 9012096, US Patent No. 4,684,611, Paszkowski et al. (1984) *EMBO J.* 3:2717-2722); liposome-mediated DNA uptake (e.g. Freeman et al (1984) *Plant Cell Physiol*, 29:1353); or the vortexing method (e.g. Kindle (1990) *Proc. Nat. Acad. Sci. USA* 87:1228). Physical methods for the transformation of plant cells are reviewed in Oard (1991) *Biotech. Adv.* 9:1-11. See generally, Weissinger et al. (1988) *Ann. Rev. Genet.* 22:421-477; Sanford et al. (1987) *Particulate Sciences and Technology* 5:27-37; Christou et al. (1988) *Plant Physiol.* 87:671-674; McCabe et al. (1988) *Bio/Technology* 6:923-926; Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182; Singh et al. (1988) *Theor. Appl. Genet.* 96:319-324; Datta et al. (1990) *Biotechnology* 8:736-740; Klein et al. (1988) *Proc. Natl. Acad. Sci. USA* 85: 4305-4309; Klein et al. (1988) *Biotechnology* 6:559-563; Tomes, US Patent No. 5,240,855; Buising et al. US Patent Nos. 5,322,783 and 5,324,646; Klein et al. (1988) *Plant Physiol* 91: 440-444; Fromm et al (1990) *Biotechnology* 8:833-839; Hooykaas-Von Slogteren et al. 91984). *Nature (London)* 311 :763-764; Bytebier et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349; De Wet et al. (1985) in *The Experimental Manipulation of Ovule Tissues* ed. Chapman et al. (Longman, New York), pp. 197-209; Kaeppler et al. (1990) *Plant Cell Reports* 9:415-418 and Kaeppler et al. (1992) *Theor. Appl. Genet.* 84:560-566; Li et al. (1993) *Plant Cell Reports* 12: 250-255 and Christou and Ford (1995) *Annals of Botany* 75: 407-413; Osjoda et al. (1996) *Nature Biotechnology* 14:745-750.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama et al. (1988) *Bio/Technology* 6: 1072-1074; Zhang et al. (1988) *Plant Cell rep.* 7379-384; Zhang et al. (1988) *Theor. Appl. Genet.* 76:835-840; Shimamoto et al. (1989) *Nature* 338:274-276; Datta et al. (1990) *Bio/Technology* 8: 736-740; Christou et al. (1991) *Bio/Technology* 9:957-962; Peng et al (1991) *International Rice Research Institute*, Manila, Philippines, pp.563-574; Cao et al. (1992) *Plant Cell*

Rep. 11: 585-591; Li et al. (1993) Plant Cell Rep. 12: 250-255; Rathore et al. (1993) Plant Mol. Biol. 21:871-884; Fromm et al (1990) Bio/Technology 8:833-839; Gordon Kamm et al. (1990) Plant Cell 2:603-618; D'Halluin et al. (1992) Plant Cell 4:1495-1505; Walters et al. (1992) Plant Mol. Biol. 18:189-200; Koziel et al. (1993). Biotechnology 11:194-200; Vasil, I.K. (1994) Plant Mol. Biol. 25:925-937; Weeks et al (1993) Plant Physiol. 102:1077-1084; Somers et al. (1992) Bio/Technology 10:1589-1594; WO 92/14828. In particular, Agrobacterium mediated transformation is now emerging also as an highly efficient transformation method in monocots. (Hiei, et al. (1994) The Plant Journal 6:271-282). See also, Shimamoto, K. (1994) Current Opinion in Biotechnology 5:158-162; Vasil, et al. (1992) Bio/Technology 10:667-674; Vain, et al. (1995) Biotechnology Advances 13(4):653-671; Vasil, et al. (1996) Nature Biotechnology 14: 702).

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, e.g. bombardment with Agrobacterium-coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

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Altering the production of a polypeptide may be achieved by increasing the amount of mRNA produced, increasing the stability of protein, altering the rate of post translational modification for example altering rates of proteolytic cleavage.

Altering the post-translational modification of a polypeptide may also affect its structure and function, and may be used to alter the expression of the native polypeptide. For example, the ligand is likely to be only a portion of the full length proteins and the active ligand is probably released by proteolysis.

Alternatively, a polypeptide or nucleic acid molecule as defined herein may be introduced into the plant, by any suitable means such as spraying, uptake by the roots, or injection into phloem. To down-regulate said receptor or ligand in a plant, an enzyme may be introduced which inhibits or digests one or both of the receptor or ligand.

In addition, modulating the activity mediated by CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, by altering their binding pattern, in order to up-or-down-

regulate the downstream signalling pathway. The binding pattern may be altered in any suitable way, for example by altering the structure, binding affinity, temporal binding pattern, selectivity and amount available for binding on the cell surface of CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof.

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The binding pattern may be altered by making appropriate variations to the ligand polypeptide, for example to change its binding site to the receptor, using known methods for mutagenesis. Alternatively, non-protein analogues may be used. Methods for manipulating a polypeptide used in the present invention are known in the art, and
10 include for example altering the nucleic acid sequence encoding the polypeptide. Methods for mutagenesis are well known. Preferably, where variants are produced using mutagenesis of the nucleic acid coding sequence, this is done in a manner which does not affect the reading frame of the sequence and which does not affect the polypeptide in a manner which affects the desired biological activity.

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In selecting suitable variants for use in the present invention, routine assays may be used to screen for those which have the desired properties. This may be done by visual observation of plants and plant material, or measuring the biomass of the plant or plant material.

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Thus, for use in altering the expression of the CLE41 and/or CLE42 and/or a PXY, or functional equivalents thereof, in a cell of a plant, there is provided an expression cassette comprising a regulatory sequence to modulate the expression of the native CLE41 and/or CLE42 or PXY genes in a plant. Preferably, the regulatory sequences are
25 designed to be operably linked to the native gene, in order to direct expression in a manner according to the present invention.

The nucleic acid molecules as described herein, and/or a regulatory sequence are preferably provided as part of an expression cassette, for expression of the sequence in
30 a cell of interest. Suitable expression cassettes may also comprise 5' and 3' regulatory sequences operably linked to the sequences of interest. In addition, genes encoding, for example, selectable markers and reporter genes may be included. The expression cassette will preferably also contain one or more restriction sites, to enable insertion of the nucleotide sequence and/or a regulatory sequence into the plant genome, at a pre-
35 selected position. Also provided on the expression cassette may be transcription and translation initiation regions, to enable expression of the incoming genes, transcription

and translational termination regions, and regulatory sequences. These sequences may be native to the plant being transformed, or may be heterologous and/or foreign.

Heterologous sequences are sequences which in nature are not operably linked to each other and/or are not found next to each other in a native sequence. In contrast, homologous sequences refer to sequences which share sequence similarity, which may be described as sequence homology. Homology is usually in a fragment of the sequence, typically in a functional domain of the sequence.

10 A foreign sequence is one which is not found in the native genome of the plant being transformed.

A regulatory sequence is a nucleotide sequence which is capable of influencing transcription or translation of a gene or gene product, for example in terms of initiation, rate, stability, downstream processing, and mobility. Examples of regulatory sequences include promoters, 5' and 3' UTR's, enhancers, transcription factor or protein binding sequences, start sites and termination sequences, ribozyme binding sites, recombination sites, polyadenylation sequences, sense or antisense sequences. They may be DNA, RNA or protein. The regulatory sequences may be plant- or virus derived, and preferably may be derived from the same species of plant as the plant being modulated.

By "promoter" is meant a nucleotide sequence upstream from the transcriptional initiation site and which contains all the regulatory regions required for transcription. Suitable promoters include constitutive, tissue-specific, inducible, developmental or other promoters for expression in plant cells comprised in plants depending on design. Such promoters include viral, fungal, bacterial, animal and plant-derived promoters capable of functioning in plant cells.

Constitutive promoters include, for example CaMV 35S promoter (Odell et al. (1985) Nature 313, 9810-812); rice actin (McElroy et al. (1990) Plant Cell 2: 163-171); ubiquitin (Christian et al. (1989) Plant Mol. Biol. 18 (675-689); pEMU (Last et al. (1991) Theor Appl. Genet. 81: 581-588); MAS (Velten et al. (1984) EMBO J. 3. 2723-2730); ALS promoter (U.S. Application Serial No. 08/409,297), and the like. Other constitutive promoters include those in U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680, 5,268,463; and 5,608,142.

Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induced gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1a promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena et al. (1991) *Proc. Natl. Acad. Sci. USA* 88: 10421-10425 and McNellis et al. (1998) *Plant J.* 14(2): 247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz et al. (1991) *Mol. Gen. Genet.* 227: 229-237, and US Patent Nos. 5,814,618 and 5,789,156).

Where enhanced expression in particular tissues is desired, tissue-specific promoters can be utilised. Tissue-specific promoters include those described by Yamamoto et al. (1997) *Plant J.* 12(2): 255-265; Kawamata et al. (1997) *Plant Cell Physiol.* 38(7): 792-803; Hansen et al. (1997) *Mol. Gen. Genet.* 254(3): 337-343; Russell et al. (1997) *Transgenic Res.* 6(2): 157-168; Rinehart et al. (1996) *Plant Physiol.* 112(3): 1331-1341; Van Camp et al. (1996) *Plant Physiol.* 112(2): 525-535; Canevascni et al. (1996) *Plant Physiol.* 112(2): 513-524; Yamamoto et al. (1994) *Plant Cell Physiol.* 35(5): 773-778; Lam (1994) *Results Probl. Cell Differ.* 20: 181-196; Orozco et al. (1993) *Plant Mol. Biol.* 23(6): 1129-1138; Mutsuoka et al. (1993) *Proc. Natl. Acad. Sci. USA* 90 (20): 9586-9590; and Guevara-Garcia et al (1993) *Plant J.* 4(3): 495-50.

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter. In a preferred aspect, the promoter is an inducible promoter or a developmentally regulated promoter.

The promoters which control the expression of CLE41 and/or CLE42 are preferably tissue or organ specific, such that expression of CLE41 and/or CLE42 can be directed to a particular organ or tissue, such as the vascular tissue, preferably the cambium or

procambium, and most preferably phloem or xylem tissue. The promoters may be constitutive, whereby they direct expression under most environmental or developmental conditions. More preferably, the promoter is inducible, and will direct expression in response to environmental or developmental cues, such as temperature, chemicals,
5 drought, and others. The promoter may also be developmental stage specific.

Examples of suitable promoter sequences include those of the T-DNA of *A. tumefaciens*, including mannopine synthase, nopaline synthase, and octopine synthase; alcohol dehydrogenase promoter from corn; light inducible promoters such as ribulose-
10 biphosphate-carboxylase small subunit gene from various species and the major chlorophyll a/b binding protein gene promoter; histone promoters (EP 507 698), actin promoters; maize ubiquitin 1 promoter (Christensen et al. (1996) Transgenic Res. 5:213); 35S and 19S promoters of cauliflower mosaic virus; developmentally regulated promoters such as the waxy, zein, or bronze promoters from maize; as well as synthetic
15 or other natural promoters including those promoters exhibiting organ specific expression or expression at specific development stage(s) of the plant, like the alpha-tubulin promoter disclosed in U.S. Pat. No. 5,635,618. Preferred phloem specific promoters include SUC2, APL, KAN1, KAN2, At4g33660, At3g61380, and At1g79380. Preferred xylem specific promoters include REV, IRX1 COBL4, KOR, At2g38080, and
20 At1g2744.

Suitable expression cassettes for use in the present invention can be constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other
25 sequences as appropriate. For further details see, for example, Molecular Cloning: Laboratory Manual: 2nd edition, Sambrook et al. 1989, Cold Spring Harbor Laboratory Press or Current Protocols in Molecular Biology, Second Edition, Ausubel et al. Eds., John Wiley & Sons, 1992. The expression cassettes may be a bi-functional expression cassette which functions in multiple hosts. In the case of GTase genomic DNA this may
30 contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

An expression cassette including a nucleic acid molecule according to the invention
35 need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the gene.

Suitable selectable marker or reporter genes may be used to facilitate identification and selection of transformed cells. These will confer a selective phenotype on the plant or plant cell to enable selection of those cells which comprise the expression cassette.

5 Preferred genes include the chloramphenicol acetyl transferase (cat) gene from Tn9 of *E. coli*, the beta-glucuronidase (gus) gene of the uidA locus of *E. coli*, the green fluorescence protein (GFP) gene from *Aequoria victoria*, and the luciferase (luc) gene from the firefly *Photinus pyralis*. If desired, selectable genetic markers may be included in the construct, such as those that confer selectable phenotypes such as resistance to

10 antibodies or herbicides (e.g. kanamycin, hygromycin, phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones and glyphosate).

Reporter genes which encode easily assayable marker proteins are well known in the art. In general, a reporter gene is a gene which is not present or expressed by the

15 recipient organism or tissue and which encodes a protein whose expression is manifested by some easily detectable property, e.g. phenotypic change or enzymatic activity.

The selectable marker or reporter gene may be carried on a separate expression

20 cassette and co-transformed with the expression cassette of the invention. The selectable markers and/or reporter genes may be flanked with appropriate regulatory sequences to enable their expression in plants.

The expression cassette may also comprise elements such as introns, enhancers, and

25 polyadenylation sequences. These elements must be compatible with the remainder of the expression cassette. These elements may not be necessary for the expression or function of the gene but may serve to improve expression or functioning of the gene by affecting transcription, stability of the mRNA, or the like. Therefore, such elements may be included in the expression construct to obtain the optimal expression and function of

30 CLE41 and/or CLE42 and/or PXY in the plant.

The expression cassette comprising the heterologous nucleic acid may also comprise sequences coding for a transit peptide, to drive the protein coded by the heterologous gene into a desired part of the cell, for example the chloroplasts. Such transit peptides

35 are well known to those of ordinary skill in the art, and may include single transit peptides, as well as multiple transit peptides obtained by the combination of sequences coding for at least two transit peptides. One preferred transit peptide is the Optimized

Transit Peptide disclosed in U.S. Pat. No. 5,635,618, comprising in the direction of transcription a first DNA sequence encoding a first chloroplast transit peptide, a second DNA sequence encoding an N-terminal domain of a mature protein naturally driven into the chloroplasts, and a third DNA sequence encoding a second chloroplast transit peptide.

In the present invention, any plant species may be used, including both monocots and dicots. Preferred plants for use in the present invention are those which are targets for biomass, and/or are readily grown, exhibit high growth rates, are easily harvested, and can be readily converted to a biofuel. Preferred plants include grasses, trees, crops, and shrubs.

Suitable plants for use in the present invention are those which in their native form produce a high yield of feedstock, for paper or fuel production. Examples of suitable plant types include perennial fast growing herbaceous and woody plants, for example trees, shrubs and grasses. Preferred trees for use in the invention include poplar, hybrid poplar, willow, silver maple, black locust, sycamore, sweetgum and eucalyptus. Preferred shrubs include tobacco. Perennial grasses include switchgrass, reed canary grass, prairie cordgrass, tropical grasses, *Brachypodium distachyon*, and *Miscanthus*. Crops include wheat, soybean, alfalfa, corn, rice, maize, and sugar beet.

In yet still a further preferred embodiment of the invention said plant is a woody plant selected from: poplar; eucalyptus; Douglas fir; pine; walnut; ash; birch; oak; teak; spruce. Preferably said woody plant is a plant used typically in the paper industry, for example poplar.

Methods to transform woody species of plant are well known in the art. For example the transformation of poplar is disclosed in US4795855 and WO9118094. The transformation of eucalyptus is disclosed in EP1050209 and WO9725434.

In a still further preferred embodiment of the invention said plant is selected from: corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis*

hypogaea), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus tree (*Citrus* spp.) cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), oats, barley, vegetables and ornamentals.

10 Preferably, plants of the present invention are crop plants (for example, cereals and pulses, maize, wheat, potatoes, tapioca, rice, sorghum, millet, cassava, barley, pea, and other root, tuber or seed crops. Important seed crops are oil-seed rape, sugar beet, maize, sunflower, soybean, and sorghum. Horticultural plants to which the present invention may be applied may include lettuce, endive, and vegetable brassicas including
15 cabbage, broccoli, and cauliflower, and carnations and geraniums. The present invention may be applied in tobacco, cucurbits, carrot, strawberry, sunflower, tomato, pepper, chrysanthemum.

Grain plants that provide seeds of interest include oil-seed plants and leguminous plants.
20 Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, Brassica, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

25

In a preferred embodiment of the invention said seed is produced from a plant selected from the group consisting of: corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), flax (*Linum usitatissimum*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*),
30 potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus tree (*Citrus* spp.) cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium*
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occidentale), macadamia (*Macadamia intergrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), oats, barley, vegetables.

The present invention has uses in methods which require increased biomass in plants, for example where plant biomass is used in the manufacture of products such as biofuels and paper. The invention is not limited to methods of making these particular products, and it is envisaged that the invention will be applicable to the manufacture of a variety of plant based products. In addition, the invention is also useful in altering the characteristics of plant material, such that the plant material can be adapted for particular purposes. In one such embodiment, over expression of the ligand and/or receptor as defined herein may be used to increase the number of cells in the vascular tissue of a plant, but without increasing the actual biomass of the plant (i.e. the number of cells may be increased, but the size of these cells is smaller). This has the effect of increasing the density of the vascular tissue, and therefore producing a harder wood. Thus, the invention includes methods for the production of a wood product having a particular density. In addition, it is envisaged that by manipulating plant cells to differentiate their vascular tissue, and therefore grow, environmental growth signals may be bypassed and the present invention may be used to extend the growth season of plants, beyond that which would be possible in a native plant.

20

The embodiments described in relation to the each aspect apply to the other aspects of the invention, *mutatis mutandis*.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be

understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

The present invention will now be described with reference to the following non-limiting
5 examples:

EXAMPLES

DNA manipulation was carried out using standard methods. Over expression (35S)
10 constructs for plant transformation were generated by cloning CLE41, CLE42, and PXY
genomic DNA sequences into pK2GW7,0 (M. Karimi, D. Inze, A. Depicker, *Trends in
Plant Science* 7, 193 (2002)) using gateway technology (invitrogen) with primers listed in
the table. Sequences were amplified by PCR and cloned into pENTR-D-TOPO.
Subsequently, reactions containing LR clonase II and pK2GW7,0 and the relevant
15 TOPO vector, sequences were used to transfer sequences in pENTR-D-TOPO to the
binary plasmid. *pIRX3::CLE41/42* were constructed using the p3HSC Gateway
destination vector (Atanassov et al. 2008) derived from pCB1300 by insertion of the 1.7
kb promoter sequences of *irx3*, the frame A (attR1/CmR/ccdB/attR2) cassette
(Invitrogen) and the NOS terminator region from pGPTV-BAR. For *SUC2::CLE41* we
20 used overlapping PCR. The *SUC2* promoter and *CLE41* coding sequence were
amplified separately with overlapping ends. These products were mixed, annealed and
elongated prior to amplification with *SUC2* and *CLE41* forward and reverse oligos
respectively. The resulting PCR product was cloned into pTF101.gw1 (Paz et al. 2004)
via pENTR-D-TOPO. For tissue specific expression, promoters known to give xylem
25 (*IRX3*; (Gardiner et al. 2003) or phloem (*SUC2*; (Truernit and Sauer 1995) specific
expression were used. Plasmids were sequenced and transformed into *Arabidopsis*
using the method of Clough and Bent (S. J. Clough, A. F. Bent, *Plant Journal* 16, 735
(Dec, 1998)),

In order to understand if over expression of *PXY* and *CLE41* had the same effect in
30 poplar, plants were transformed in tissue culture using *Agrobacterium* to transfer the
constructs into poplar tissue using the method of Meilan and Ma (R. Meilan, C. Ma,
Methods in Molecular Biology 344, 143 (2006). *35S::CLE41*, *SUC::CLE41* and wild type
were grown in magenta boxes in the growth cabinet under the same conditions.
Similarly, plasmids were transformed into *Nicotiana* using the method of Horsch et al (R.
35 B. Horsch *et al.*, *Science* 227, 1229 (March 8, 1985, 1985). Maize was transformed with
the plasmids by contracting out transformation services. Similar phenotypes were

confirmed in 10 independent transgenic lines for *35S::CLE* constructs in *Arabidopsis*. Increases in expression were confirmed in 5 lines per construct by RT-PCR. RT-PCR analysis was carried out using the gene-specific primers listed in the table. RNA was isolated using Trizol reagent (Invitrogen). cDNA synthesis, following DNase treatment, was performed using Superscript III reverse transcriptase (Invitrogen). Expression levels of *CLE41* in wild type were compared to that of *35S::CLE41* by qRT-PCR. All samples were measured in technical triplicates on biological triplicates. The qRT-PCR reaction was performed using SYBR Green JumpStart Taq ReadyMix (Sigma) using an ABI Prism 7000 machine (Applied Biosystems). PCR conditions were as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 60 s. A melting curve was produced at the end of every experiment to ensure that only single products were formed. Gene expression was determined using a version of the comparative threshold cycle (Ct) method. The average amplification efficiency of each target was determined using LinReg (M. Hardstedt *et al.*, *Xenotransplantation* 12, 293 (2005)).

Arabidopsis lines which carried *35S::CLE41 35S::PXY* and *35S::CLE42 35S::PXY* were generated by crossing and identified in the F2 population. *IRX3::CLE41 35S::PXY* and *SUC2::CLE41 35S::PXY* lines were generated by directly transforming plants carrying the *35S::PXY* construct with *pIRX3::CLE41* or *pSUC2::CLE41*. *SUC2::CLE41* and *35S::CLE41* cell counts were carried out on 10 independent T2's (2 bundles/plant) and 6 independent T1's (3 bundles/plant) respectively. 5 week plants were used. *Nicotiana* lines carrying *35S::CLE41 35S::PXY* were also generated by crossing.

Table 1. Oligonucleotides used in Invention.

Oligo Name	Sequence (5'-3')	Used for
CLE41F	CACCATGGCAACATCAAATGAC	35S::CLE41 construct
CLE41R	AAACCAGATGTGCCAACTCA	35S::CLE41 construct and genotyping
CLE42F	CACCATGAGATCTCCTCACATC	35S::CLE42 construct
CLE42R	TGAATCAAACAAGCAACATAACAA	35S::CLE42 construct and genotyping
PXY_ORF_f	CACCTTAAATCCACCATTGTCA	35S::PXY construct
PXY_ORF_r	CCAAGATAATGGACGCCAAC	35S::PXY construct
SUC2promFtopo	caccaacacatgttgccgagtca	SUC2::CLE41 overlap PCR entry clone
SUC2pro/CLE41(1)	GTCATTTGATGTTGCCATgaaattctttgagagggttttg	SUC2::CLE41 overlap PCR entry clone
SUC2pro/CLE41(2)	caaaaaccctctcaaagaaatttcATGGCAACATCAAATGAC	SUC2::CLE41 overlap PCR entry clone
CLE41_RTf	CCATGACTCGTCATCAGTCC	RT-PCR
CLE41_RTR	TTTGGACCACTAGGAACCTCA	RT-PCR
CLE42_RTf	TCCAAACCCATCAAAGAACC	RT-PCR
CLE42_RTR	ATTGGCACCGATCATCTTTC	RT-PCR
PXY1_RTf	AACCTAGCAATATCCTCCTCGAC	RT-PCR
PXY1_RTR	GGTTCACCGATCTTTTCC	RT-PCR

ACT8-1	ATGAAGATTAAGGTCGTGGCA	RT-PCR control
ACT8-2	CCGAGTTTGAAGAGGCTAC	RT-PCR control
qCLE41f	TCAAGAGGGTTCTCCTCGAA	qRT-PCR
qCLE41r	TGTGCTAGCCTTTGGACGTA	qRT-PCR
18s rRNA F	CATCAGCTCGCGTTGACTAC	qRT-PCR control
18s rRNA R	GATCCTTCCGCAGGTTTAC	qRT-PCR control
35S promoter F	CGCACAATCCCACTATCCTT	Genotyping
pxy-3-r	TTACCGTTTGATCCAAGCTTG	Genotyping

Histology

Analysis of tobacco, poplar and *Arabidopsis* vasculature was carried out using thin transverse sections cut from JB4 resin embedded material as described previously (Pinon et al. 2008). Tissue was fixed in 3% glutaraldehyde or FAA, dehydrated through an ethanol series to 100% ethanol and embedded in JB4 resin (Agar Scientific). Embedded tissue was sectioned at 3 µm and subsequently stained with 0.02% Toluidine Blue. For hand cut sections, tissue was stained with either aqueous 0.02% Toluidine Blue or 0.05M Anniline blue in 100 mM Phosphate buffer, pH7.2. Stems were analyzed at 8 weeks for *Arabidopsis*, 50 days for *Nicotiana* and four weeks after transfer to rooting medium for poplar.

Comparison of cell numbers in 35S::CLE41/42 lines in *Arabidopsis*.

At the base of 6 week old inflorescence stems, lines over-expressing either *CLE41* or *CLE42* had, on average, more undifferentiated cells in vascular tissue (105.7 and 89.1, respectively) than those of wild type (58.6). When assaying cell numbers in vascular bundles from multiple insertion lines, both 35S::*CLE41* and 35S::*CLE42* plants had more vascular cells, although only in the case of 35S::*CLE41* plants was this result statistically significant. There was no difference in the number of differentiated vascular cells in either 35S::*CLE41* or 35S::*CLE42* plants compared to the wild type (Table 1). We analysed progeny from two of the stronger transformed lines which were also used in subsequent genetic analysis. Stems from these lines had significantly more cells per vascular bundle (318.7 and 373.7 for 35S::*CLE41* and 35S::*CLE42*, respectively) than wild type (273.7) clearly demonstrating that these genes are capable of increasing

procambial cell divisions. In the case of *35S::CLE42* lines there was also a statistically significant increase in the number of differentiated cells.

In order to determine whether these extra procambial cells would remain undifferentiated or would differentiate into xylem and phloem, we looked at the base of plant stems at senescence. In all genotypes the vast majority of vascular cells in the stem were fully differentiated (Fig. 5), including areas in *35S::CLE41/42* where large numbers of undifferentiated cells were present at earlier stages of development. Therefore, early on *35S::CLE41/42* plants have more undifferentiated cells but these ultimately become differentiated in inflorescence stems.

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Mean vascular cell number from 19 independent transgenic lines.

	Col (<i>n</i> =10)	<i>35S::CLE41</i> (<i>n</i> =9)	<i>35S::CLE42</i> (<i>n</i> =10)
Total Cells	311.6 ± 15.6	373.2 ^φ ± 24.3	341.8 ± 19.1
Undifferentiated Cells	58.6 ± 4.4	105.7* ± 9.8	89.0* ± 7.2
Differentiated Cells (Xylem and Phloem)	253 ± 12.6	267.5 ± 15.2	252.8 ± 14.4

* Significantly different from Col *P*<0.001.
^φ Significantly different from Col *p*<0.05.
± Standard error.

Over-expression of CLE41 and CLE42 in conjunction with PXY further enhances effects on secondary growth

We addressed the consequences of expressing *PXY* and *CLE41* by using a *35S::PXY* construct in a *35S::CLE41/42* background. The stems of *35S::CLE41/42 35S::PXY* plants were characterised by dramatic increases in cell number in both the vascular bundle and in the interfascicular region such that a continuous ring of additional tissue within the stem. New cells were generated between the xylem and phloem in vascular bundles and also outside the interfascicular cells making the phenotype characteristic of dramatically increased secondary growth (Fig. 8). These results provide strong genetic evidence that *CLE41/42* and *PXY* are sufficient for induction of vascular cell division within the procambium and elsewhere.

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Interestingly, the majority of increased cell divisions occurring when both CLE41/42 and PXY are over-expressed were relatively ordered, although aberrant cells divisions are still present. We made lines harbouring both *IRX3::CLE41* and *35S::PXY* constructs. We found that vascular organisation was disrupted in *35S::PXY IRX3::CLE41* plants (Fig 25?), but increased secondary growth was also observed. *35S::PXY SUC2::CLE41* plants also demonstrated enhanced secondary growth (Fig 22), but in contrast to *35S::PXY IRX3::CLE41*, vascular tissue was highly ordered.

An additional phenotype was observed in the leaves of *35S::CLE41/42 35S::PXY* plants. In Col, *35S::CLE41/42* (Fig. 9) and *35S::PXY*, leaves have a single midvein, however, in a minority of *35S::CLE42 35S::PXY* plants the leaves appeared to exhibit increased vascular development. This additional vascular tissue develops together with the associated lamina suggesting development of ectopic vascular tissue.

Identification of CLE and PXY homologues

Identification of *Populus Trichocarpa* CLE family was carried out by subjecting CLE41/42 to a WU-BLAST search against green plant GB genomic (DNA) datasets using TBLASTN: AA query to NTdb parameters on the TAIR website (www.arabidopsis.org). All *Populus trichocarpa* hits (genomic region) with probability value (P) less than 1 were selected. These hits were subsequently on the Populus gene map (http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=3694). ± 1kb from the WU-BLAST hit region was then analyzed with the NCBI ORF finder (<http://www.ncbi.nlm.nih.gov/projects/gorf/>) and all coding regions containing similar 12 AA sequences to the output CLE sequence were examined. All putative proteins were aligned using the ClustalW algorithm using default settings.

The PXY homolog in *Oryza Sativa* was identified by locating PXY (At5g61480) in the homology tree from Shiu et al. (S.-H. Shiu et al., *Plant Cell* 16, 1220 (May 1, 2004, 2004)). The putative homolog was Osi056321.1 (*Oryza Sativa Indica*). This sequence was then subjected to a BLASTP protein search against *O.sativa* (japonica cultivar-group) Non-RefSeq protein. The top hit was EAZ41508.1 : hypothetical protein OsJ_024991 and was confirmed as being the PXY orthologue by performing a BLASTP OsJ_024991 against Non-RefSeq protein database, *Arabidopsis Thaliana*, NCBI.

Table 2

CLE	Representative	Synonyms	Organism	ID (representative seq)	Protein/Gene Annotation
41	AT_GEN_At3g24770.1	CLE41	<i>Arabidopsis thaliana</i>	AT_GEN_At3g24770.1	At3g24770.1 68416.m03109 CLE41, putative CLAVATA/ESR- Related 41 (CLE41)
				AATEST_TC255991_+1	TC255991
				AT-TA_TA13011_3702_+1	TA13011 3702 Putative CLE41 protein related cluster
42	AATEST_NP1098871_+1	CLE42	<i>Arabidopsis thaliana</i>	AATEST_NP1098871_+1	NP1098871 putative CLAVATA3/ESR-related 42 precursor [<i>Arabidopsis thaliana</i>]
44	AT_GEN_At4g13195.1	CLE44	<i>Arabidopsis thaliana</i>	AT_GEN_At4g13195.1	At4g13195.1 68417.m02052 expressed protein
				AATEST_TC275167_+3	TC275167 GB AAO11557.1 27363276 BT002641 At4g13194/At4g13194 { <i>Arabidopsis thaliana</i> ;} , complete
51	GMEST_TC171126_+3		<i>Glycine max</i>	GMEST_TC171126_+3	TC171126 similar to PIR S61040 S61040 probable membrane protein YDL172c - yeast (<i>Saccharomyces cerevisiae</i>), partial (11%)
				GM-TA_TA36215_3847_-3	TA36215 3847
53	GMEST_TC162846_+3		<i>Glycine max</i>	GMEST_TC162846_+3	TC162846 homologue to GP 21618281 gb AAM67331 .1 unknown { <i>Arabidopsis thaliana</i> }, partial (27%)
				GMEST_TC162847_+1	TC162847 homologue to GP 21618281 gb AAM67331 .1 unknown { <i>Arabidopsis thaliana</i> }, partial (19%)
				GM-TA_BQ627547_-1	BQ627547 Hypothetical protein CBG22664 related cluster
				GM-TA_TA14900_3847_+1	TA14900 3847
				GM-TA_TA8421_3847_+3	TA8421 3847 SPBC215.13 protein related cluster
60	GMEST_BE658554_-3		<i>Glycine max</i>	GMEST_BE658554_-3	BE658554 homologue to PIR H72173 H7217 D5L protein - variola minor virus (strain Garcia- 1966), partial (23%)
				GM-TA_BE658554_-3	BE658554
61	GMEST_BM085374_+2		<i>Glycine max</i>	GMEST_BM085374_+2	BM085374 homologue to GP 16945432 emb related to GLUCAN 1 3-BETA- GLUCOSIDASE PRECURSOR protein { <i>Neurospora</i>

					{Neurospora crassa}, partial (1%)
				GM-TA_TA39380_3847_+1	TA39380 3847
62	GMEST_TC171467_+2		<i>Glycine max</i>	GMEST_TC171467_+2	TC171467
				GM-TA_TA31733_3847_+2	TA31733 3847
63	GMEST_BU763224_+1		<i>Glycine max</i>	GMEST_BU763224_+1	BU763224 similar to GP 21592472 gb CLE gene family putative {Arabidopsis thaliana}, partial (15%)
				GM-TA_BU763224_+1	BU763224
64	MT_GEN_IMGA AC137080_19.1		<i>Medicago truncatula</i>	MT_GEN_IMGA AC137080_19.1	IMGA AC137080_19.1 AC137080.13 104569-102347 E EGN_Mt041209 20041210 hypothetical protein
				MT_GEN_IMGA AC147499_5.1	IMGA AC147499_5.1 AC147499.5 26650-24428 E EGN_Mt041209 20041210 hypothetical protein
				MTEST_BI311733_+1	BI311733
				MT-TA_BI311733_+1	BI311733
90	OS_GEN_Os02g56490.1		<i>Oryza sativa</i>	OS_GEN_Os02g56490.1	Os02g56490.1 11972.m333 18 protein expressed protein
				OSEST_TC278386_+3	TC278386 Oryza sativa (japonica cultivar-group) cDNA clone:J033127D10, full insert sequence
				OS-TA_TA21276_4530_+2	TA21276 4530 Hypothetical protein OJ1520 C09.33 related cluster
116	PT_GEN_63277		<i>Populus trichocarpa</i>	PT_GEN_63277	jgi Poptr1 63277 fgenes h1_pg.C_LG_I000629
119	PT_GEN_569594		<i>Populus trichocarpa</i>	PT_GEN_569594	jgi Poptr1 569594 eugen e3.00120247
148	ZMEST_DR801316_+1		<i>Zea mays</i>	ZMEST_DR801316_+1	DR801316
				ZM-TA_DR801316_-1	DR801316 Hypothetical protein P0617C02.125 related cluster
149	ZMEST_BM350390_-2		<i>Zea mays</i>	ZMEST_BM350390_-2	BM350390 similar to UP Q4NZF2 9DELT (Q4NZF2) PE-PGRS family protein, partial (4%)
				ZM-TA_BM350390_+2	BM350390 PE-PGRS family protein related cluster
166	STEST_TC114822_+3		<i>Solanum tuberosum</i>	STEST_TC114822_+3	TC114822 TIGR Ath1 At4g13195.1 68417.m02052 expressed protein, partial (12%)
				ST-TA_TA8910_4113_+3	TA8910 4113 Hypothetical protein MTH423 related cluster
167	STEST_BF187584_+1		<i>Solanum tuberosum</i>	STEST_BF187584_+1	BF187584
				ST-TA_TA17128_4113_+	TA17128 4113 Cluster related to

				+2	UPI0000517AA3
168	BN-TA_CX187708_+3		<i>Brassica napus</i>	BN-TA_CX187708_+3	CX187708 F20P5.29 protein related cluster
169	PV-TA_CV532906_+1		<i>Phaseolus vulgaris</i>	PV-TA_CV532906_+1	CV532906 Hypothetical protein related cluster
172	STEST_TC129811_+3		<i>Solanum tuberosum</i>	STEST_TC129811_+3	TC129811 similar to TIGR Ath1 At4g13195.1 68417.m02052 expressed protein , partial (12%)
				ST-TA_TA11604_4113_+3	TA11604 4113 Hypothetical protein related cluster
173	STEST_CV500295_+3		<i>Solanum tuberosum</i>	STEST_CV500295_+3	CV500295
				ST-TA_TA19709_4113_+3	TA19709 4113 Hypothetical protein related cluster

***Nicotiana* over expressing CLE41/42 and PXY**

In order to observe the phenotypic differences between the transgenic plants and wild type in *Nicotiana*, 35S::*PXY*, 35S::*CLE41*, 35S::*CLE42* and wild type plants were grown in individual pots and places in the growth cabinet under same conditions. The height (from soil surface to the plant top in cM), hypocotyl width and stem width (diameter in mm) were measured when plants were 50 days old. The results of mean, standard error (SE), standard deviation (STD), minimum (Min) and maximum (Max) value were summarised in tables 3, 4, and 5. Single ANOVA between transgenic lines and wild type have been analysed and the P-value were given in the tables as well.

In table 3, the results show that the height of transgenic lines are highly significant difference between wild type ($P < 0.001^{****}$), the means of 35S::*CLE41* and 35S::*CLE42* are similar. 35S::*CLE41* and 35S::*CLE42* are also 20 cM and 12 cM shorter than wild type and 35S::*PXY* respectively. The results are consistent with the phenotypes being induced by over expression of CLE41 and CLE42.

Table 3. Height of *Nicotiana* at 50days (cM)

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Name of plant	N	Mean	± SE	STD	Min	Max	P-value
35S:: <i>PXY</i>	10	26.70	1.10	3.49	18	30.5	<0.001****
35S:: <i>CLE41</i>	10	14.40	1.37	4.34	5.5	20	<0.001****
35S:: <i>CLE42</i>	8	15.98	3.32	9.38	6	29	<0.001****
Wild type	10	35.35	1.20	3.79	27	39	

Table 4, shows that Hypocotyl width is not significantly different between 35S::*PXY* and wild type, however, there is a highly significant difference between 35S::*CLE41* or

35S::CLE42 and Wild type ($P < 0.0001^{***}$). The means of *35S::CLE41* and *35S::CLE42* are about 2.3 mm thicker than wild type. The maximum hypocotyls width is 10.29mm in *35S::CLE41* compared to 7.1mm in wild type, there is a 3.19mm difference, demonstrating that overexpression of *CLE41* and *CLE42* increases hypocotyl width.

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Table 4. Hypocotyl width at 50 days (mm) (Nicotiana)

Name of plant	N	Mean	$\pm$ SE	STD	Min	Max	P-value
35S::PXY	10	6.48	0.20	0.63	5.2	7.41	>0.05
35S::CLE41	10	8.71	0.30	0.96	6.87	10.29	<0.0001 ^{***}
35S::CLE42	8	8.93	0.35	0.99	7.1	10.06	<0.0001 ^{***}
Wild type	10	6.34	0.14	0.45	5.3	7.1	

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In table 5, the results show that there is no significant difference between *35S::PXY* and wild type stem width, however, there is highly significant difference between *35S::CLE41* or *35S::CLE42* and Wild type ($P < 0.0001^{****}$). The maximum stem width is 8.62mm in *35S::CLE41* compared with 5.92 mm in wild type, there is 2.7 mm different. The results shown that *35S::PXY* did not affect the stem width, while the overexpression of *CLE41* and *CLE42* made the stem thicker than wild type.

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Table 5. Stem width at 50days (mm) (Nicotiana)

Name of plant	N	Mean	$\pm$ SE	STD	Min	Max	P-Value
35S::PXY	10	5.23	0.22	0.68	4.3	6.67	>0.05
35S::CLE41	10	7.06	0.33	1.03	5.24	8.62	<0.001 ^{****}
35S::CLE42	8	6.89	0.40	1.13	4.96	8.06	<0.001 ^{****}
Wild type	10	5.49	0.11	0.36	4.8	5.92	

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In summary, over expression of *PXY* results in a significant change to the plant height compared to the wild type. However, over expression of *CLE41* and *CLE42* significantly alter the plants phenotype in terms of height, hypocotyl width and stem width.

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2. Nicotiana Images

In order to observe the phenotypic changes between the transgenic lines and wild type, the hypocotyl sections of transgenic lines *35S::PXY*, *35S::CLE41*, *35S::CLE42*, *35S::CLE41 35S::PXY* and wild type were cut when the plants were 50 days old. The images of whole plants and hypocotyl cross sections in Fig 19 and 20 illustrate the phenotypic differences between the lines. Fig 19, shows that the over expression *CLE41* results in a dwarf phenotype as documented in the table 4. The photographs demonstrate that this defect is much less when both *CLE41* and *PXY* are over expressed.

In Fig 20, cross sections of hypocotyls of plants demonstrates that plants over expressing *PXY* and *CLE41* have thicker hypocotyls.

3. Histological analysis of Nicotiana hypocotyl sections.

Fig 21, wild type (A), *35S::CLE41* (B), *35S::CLE42* (C), *35S::PXY* (D), *35S::CLE41 35S::PXY*(E) Organisation is lost in plants over expressing *CLE41/CLE42*. Organisation is restored in plants over expressing both *CLE41* and *PXY*.

A further experiment illustrates differences between single over expression of *PXY*, *CLE41* and both *PXY* and *CLE41*. 10 plants of genotypes *35S::PXY*, *35S::CLE41*, *35S::CLE41 35S::PXY* and wild type were planted in an individual pots and grown in a growth cabinet in identical conditions. The height (from soil surface to the plant top in cm), hypocotyl width and stem width (diameter in mm) were measured when plants were 42 days old. The results of mean, standard error (SE), standard deviation (STD), minimum (Min) and maximum (Max) value are presented in tables 6, 7, and 8. Single ANOVA between transgenic lines and wild type have been analysed and the P-value were given in the tables.

Table 6. The height of *35S::CLE41* plants are significantly smaller than wild type ($P < 0.001^{****}$), however, there is no difference between *35S::CLE41 35S::PXY* and wild type in height.

Table 6 Height at 42 days (cM)

Name	N	Mean	SE	STD	Min	Max	P-value
35S::CLE41	10	9.6	0.42	1.34	7	11.5	$P < 0.0001^{****}$

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35S::PXY	10	23.8	1.34	4.26	16	31.5	P<0.01**
35S::CLE41 35S::PXY	10	16.95	1.45	4.58	12	23	P>0.05
Wild type	10	19.12	0.62	1.98	16.5	22	

Table 7. Hypocotyl width was significantly larger in *35S::CLE41* and *35S::CLE41 35S::PXY* compared to Wild type ($P<0.0001^{***}$). The mean of *35S::CLE41* is about 2.4 mm thicker than wild type. The mean of *35S::CLE41 35S::PXY* is 2.7 mm thicker than wild type.

Table 7 Nicotiana Hypocotyls at 42 days (mm)

Name	N	Mean	SE	STD	Min	Max	P-value
35S::CLE41	10	9.81	0.35	1.11	8.27	11.58	$P<0.0001^{****}$
35S::PXY	10	8.31	0.39	1.25	6.47	9.86	$P>0.05$
35S::CLE41 35S::PXY	10	10.22	0.39	1.24	8.19	11.58	$P<0.0001^{****}$
Wild type	10	7.45	0.27	0.87	5.99	8.87	

Table 8. There is highly significant difference between *35S::CLE41* and *35S::CLE41 35S::PXY* compared to wild type ($P<0.0001^{****}$). There is also a significant difference between *35S::PXY* and Wild type. The maximum stem width is 9.43mm in *35S::CLE41* compare 6.74 mm in wild type, a difference of 2.7 mm. The results demonstrate that overexpression of both *CLE41* and *PXY* increase stem thickness compared to wild type.

Table 8 Stem width at 42 days (mm)

Name	N	Mean	SE	STD	Min	Max	P-value
35S::CLE41	10	8.49	0.22	0.70	7.33	9.43	$P<0.0001^{****}$
35S::PXY	10	6.81	0.37	1.19	4.58	8.27	$P<0.05^*$
35S::CLE41 35S::PXY	10	8.18	0.36	1.14	6.86	9.69	$P<0.0001^{****}$
Wild type	10	5.88	0.16	0.53	5.14	6.74	

Poplar harbouring 35S::CLE41 or SUC2::CLE41 constructs generate more vascular tissue than wild type.

Poplar transformed with 35S::CLE41 or SUC2::CLE41 were in JB4 sections were found to have more xylem tissue (see brackets in figure 25) demonstrating increases in vascular tissue.

CLAIMS

1. A use of a heterologous polypeptide of paragraph I) or a heterologous nucleic acid of paragraph II) in combination with a heterologous nucleic acid of paragraph III) or heterologous polypeptide of paragraph IV), for increasing vascular tissue growth of a plant, wherein said heterologous polypeptides and heterologous nucleic acids are introduced into the plant; and wherein

I) is a polypeptide for mediating a native CLE41 and/or CLE42 downstream signalling pathway when bound to a CLE41 or CLE42 receptor selected from the group consisting of:

i) a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of CLE41 (SEQ ID NO: 21) or CLE42 (SEQ ID NO: 23) measured over a comparison window which is the length of SEQ ID NO: 21 or SEQ ID NO: 23, respectively; and

iii) a polypeptide encoded by a nucleic acid molecule that is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively;

II) is a nucleic acid molecule selected from the group consisting of:

i) a nucleic acid molecule that encodes a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a nucleic acid molecule which is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the sequence of SEQ ID NO: 22 or SEQ ID NO: 24, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24));

III) is a nucleic acid molecule that encodes a polypeptide for mediating a native CLE41 and/or CLE42 receptor downstream signalling pathway when bound to a CLE41 or CLE42 polypeptide selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide which binds a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a nucleic acid molecule that encodes a polypeptide which binds a polypeptide of paragraph I) and is at least 80% identical to the nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27; and

IV) is a polypeptide that mediates a native CLE41 and/or CLE42 receptor downstream signalling pathway when bound to a CLE41 or CLE42 polypeptide, selected from the group consisting of:

i) a polypeptide which binds to a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a polypeptide which binds to a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the leucine-rich repeat protein kinase family protein (PXY) sequence of SEQ ID NO: 25 measured over a comparison window which is the length of SEQ ID NO: 25; and

iii) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 80% identical to the PXY nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively,

wherein the increasing is relative to a corresponding native plant in which the heterologous polypeptides and heterologous nucleic acids are not introduced.

2. The use according to claim 1, for increasing radial growth of the plant, wherein the increasing is relative to the corresponding native plant in which the heterologous polypeptides and heterologous nucleic acids are not introduced.

3. The use according to claim 1 or 2, wherein said increase comprises increasing the growth and/or division of cambial and/or pro-cambial cells, wherein the increasing is relative to the corresponding native plant in which the heterologous polypeptides and heterologous nucleic acids are not introduced.

4. The use according to any one of claims 1 to 3, wherein said CLE41 or CLE42 receptor is PXY (Phloem Intercalated with Xylem).

5. The use according to claim 4, wherein the PXY is a polypeptide comprising the amino acid sequence of SEQ ID NO: 25.

6. A method of modifying a plant to increase the vascular tissue growth compared to a non-modified plant of the same species grown under identical conditions, the method comprising introducing into a cell of the plant:

I) a polypeptide for mediating a native CLE41 and/or CLE42 downstream signalling pathway when bound to a CLE41 or CLE42 receptor selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence of SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of CLE41 (SEQ ID NO: 21) or CLE42 (SEQ ID NO: 23) measured over a comparison window which is the length of SEQ ID NO: 21 or SEQ ID NO: 23, respectively; and

iii) a polypeptide encoded by a nucleic acid molecule that is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively;

or

II) a nucleic acid selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a nucleic acid molecule which is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24);

and

III) a nucleic acid molecule that encodes a polypeptide for mediating a native CLE41 and/or CLE42 receptor downstream signalling pathway when bound to a CLE41 or CLE42 polypeptide selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide which binds a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a nucleic acid molecule that encodes a polypeptide which binds a polypeptide of paragraph I) and is at least 80% identical to the nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27; or

IV) a polypeptide for mediating a native CLE41 and/or CLE42 downstream signalling pathway when bound to a CLE41 or CLE42 receptor, selected from the group consisting of:

i) a polypeptide which binds to a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof,

ii) a polypeptide which binds to a polypeptide of paragraph I) and comprises an amino acid sequence that is at least 80% identical to the PXY sequence of SEQ ID NO: 25 measured over a comparison window which is the length of SEQ ID NO: 25; and

iii) a polypeptide which binds to a polypeptide of paragraph I) and comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 80% identical to the PXY nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively.

7. The method according to claim 6, wherein said CLE41 and/or CLE42 receptor is PXY (Phloem Intercalated with Xylem).

8. The method according to claim 7, wherein PXY is a polypeptide comprising the sequence of SEQ ID NO: 25.

9. A plant cell modified to express:

I) a heterologous polypeptide for mediating a native CLE41 and/or CLE42 downstream signalling pathway when bound to a CLE41 or CLE42 receptor selected from the group consisting of:

i) a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of CLE41 (SEQ ID NO: 21) or CLE42 (SEQ ID NO: 23) measured over a comparison window which is the length of SEQ ID NO: 21 or SEQ ID NO: 23, respectively; and

iii) a CLE41 or CLE42 polypeptide encoded by a nucleic acid molecule that is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively;

and

II) a heterologous polypeptide which binds a polypeptide of paragraph I) for mediating a native CLE41 and/or CLE42 downstream signalling pathway when bound to a CLE41 or CLE42 receptor, selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20;

ii) a polypeptide comprising an amino acid sequence that is at least 80% identical to the PXY sequence of SEQ ID NO: 25 measured over a comparison window which is length of SEQ ID NO: 25; and

iii) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule that is at least 80% identical to the PXY nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively.

10. The plant cell of claim 9, wherein the plant cell comprises:

I) a heterologous nucleic acid selected from the group consisting of:

i) a nucleic acid molecule that encodes a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a nucleic acid molecule which is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24);

and

II) a nucleic acid molecule that encodes a polypeptide for mediating a native CLE41 and/or CLE42 receptor downstream signalling pathway when bound to a CLE41 or CLE42 polypeptide, selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 9, paragraph I, and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 9, paragraph I, and is at least 80% identical to SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27.

11. A use of the cell according to claim 9 or 10 in the production of plant biomass, fibres, forage, biocomposites, biopolymers, wood, biofuel and/or paper.

12. A method of increasing the vascular tissue growth of a plant, comprising the steps of:

- i) providing the cell according to claim 9 or 10;
- ii) regenerating said cell into a plant, and
- iv) growing the regenerated plant,

wherein the increasing is relative to a corresponding native plant, the cells of which are free of the heterologous polypeptides and heterologous nucleic acids.

13. The method according to claim 12, comprising:

monitoring the levels of CLE41 and/or CLE42 or a receptor thereof, and/or PXY in said regenerated plant.

14. An expression construct comprising a first nucleic acid sequence selected from the group consisting of:

- i) a nucleic acid molecule that encodes a CLE41 or CLE42 polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of amino acids 124 to 137 of the consensus sequence SEQ ID NO: 12 measured over a comparison window which is the length of said amino acids 124 to 137 of SEQ ID NO: 12;

ii) a nucleic acid molecule which is at least 80% identical to the nucleotide sequence of CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24) measured over a comparison window which is the length of SEQ ID NO: 22 or SEQ ID NO: 24, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence CLE41 (SEQ ID NO: 22) or CLE42 (SEQ ID NO: 24).

15. The expression construct according to claim 14, further comprising a regulatory sequence for expressing the first nucleotide sequence specifically in or adjacent to the vascular tissue of a plant.

16. The expression construct according to claim 14, further comprising a second nucleotide sequence selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 9, paragraph I, and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 9, paragraph I, and is at least 80% identical to the nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27.

17. An expression construct comprising a nucleotide sequence selected from the group consisting of:

i) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 6, paragraph I, and comprises an amino acid sequence that is at least 80% identical to the consensus sequence SEQ ID NO: 20 measured across the full length thereof;

ii) a nucleic acid molecule that encodes a polypeptide which binds to a polypeptide according to claim 6, paragraph I, and is at least 80% identical to the nucleotide sequence of SEQ ID NO: 26 or SEQ ID NO: 27 measured over a comparison window which is the length of SEQ ID NO: 26 or SEQ ID NO: 27, respectively; and

iii) a nucleic acid molecule that hybridizes at 6x SSC at 45°C followed by one or more washes in 0.2x SSC, 0.1% (w/v) SDS at 50°C to the complement of nucleotide sequence SEQ ID NO: 26 or SEQ ID NO: 27.

18. A host cell comprising the expression construct of any one of claims 14 to 17.
19. A method of producing a product from a plant comprising:
 - a) modifying the plant according to the method of any one of claims 6 to 8; and
 - b) growing the plant.
20. The method according to claim 19, further comprising harvesting the product of the plant.
21. A method of increasing the vascular tissue growth of a plant comprising:
 - a) modifying a plant according to the method of any one of claims 6 to 8; and
 - b) growing the plant,

wherein the increasing is relative to a corresponding native plant that is not modified according to step a).

22. The method according to claim 21, further comprising harvesting a product of the plant produced in step b).
23. The method of any one of claims 19, 20, and 22, wherein the product comprises biomass, fibres, forage, or wood.
24. The method of any one of claims 19, 20, and 22, which further comprises using the product to form a biocomposite, a biopolymer, a biofuel, or paper.

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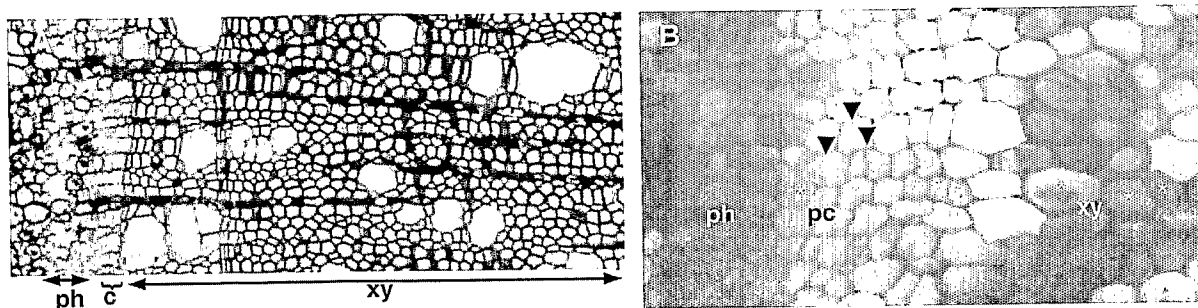


Fig. 1

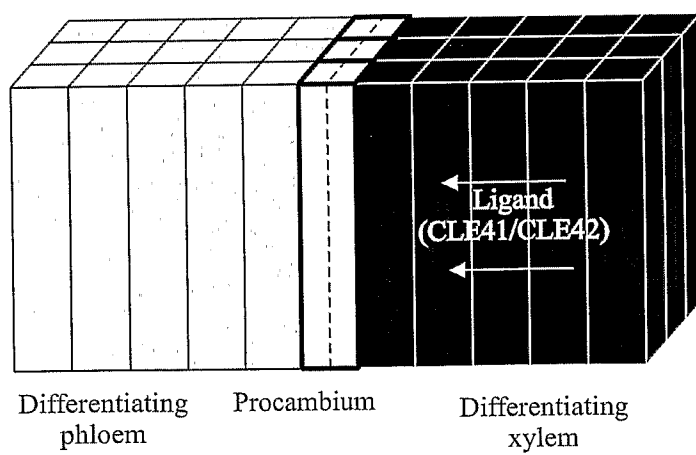


Fig. 2

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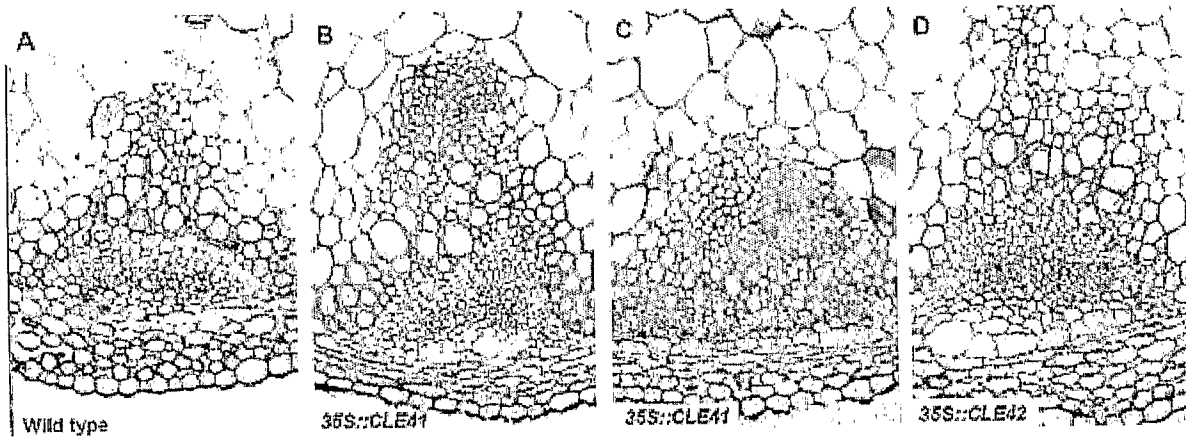


Fig. 3

Total number of cells in stem vascular bundle

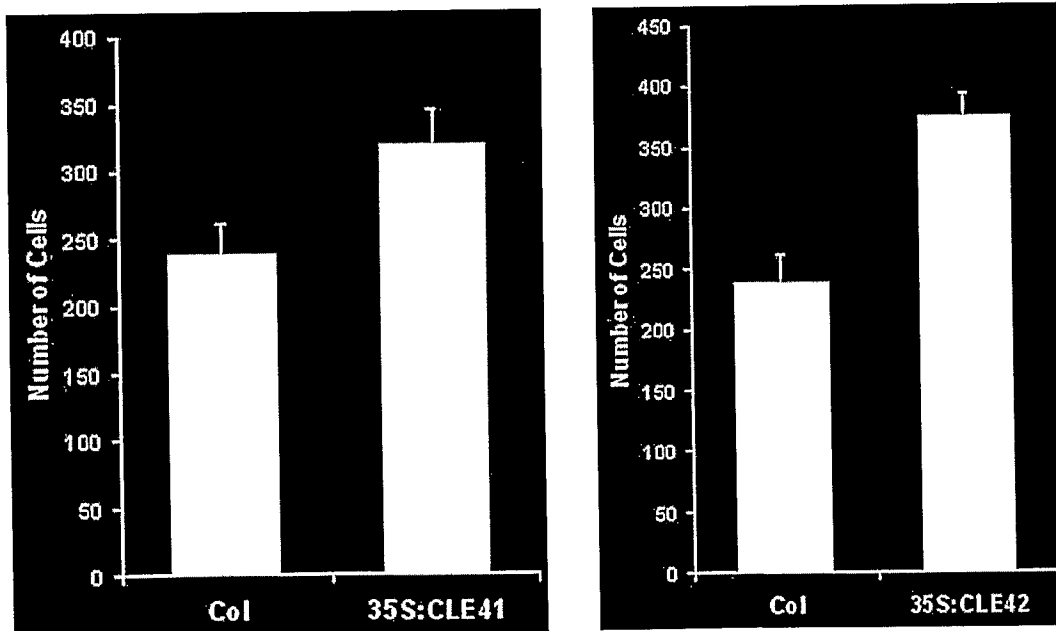


Fig. 4

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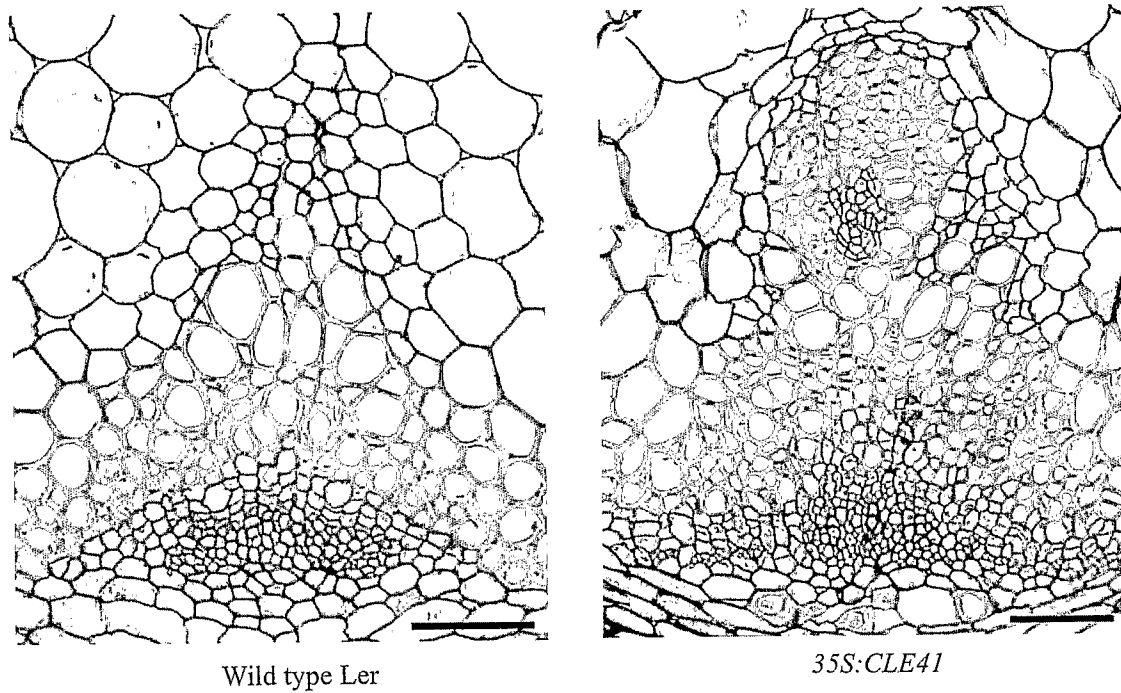


Fig. 5

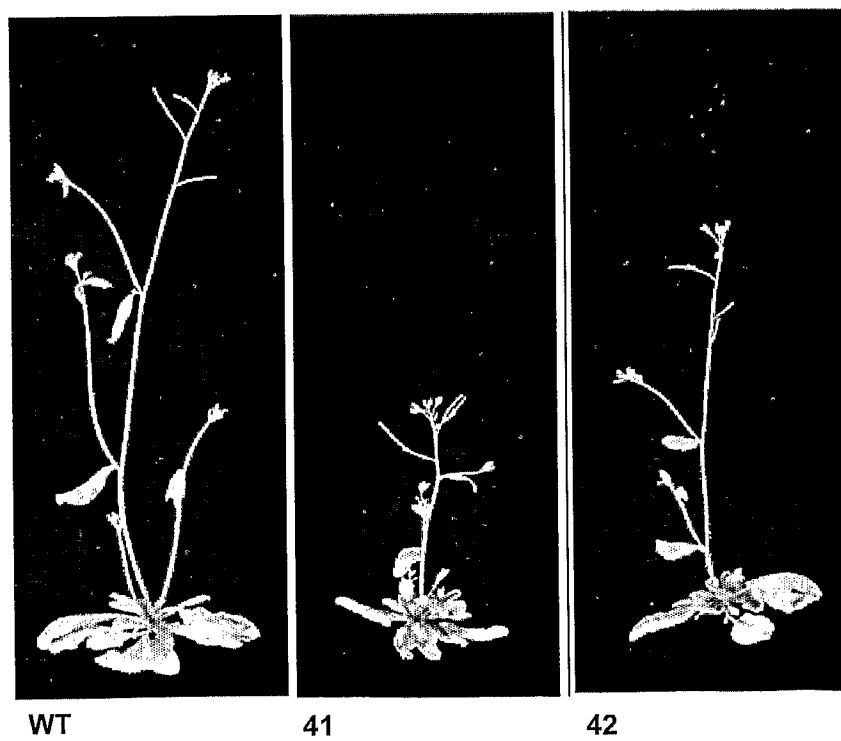


Fig. 6

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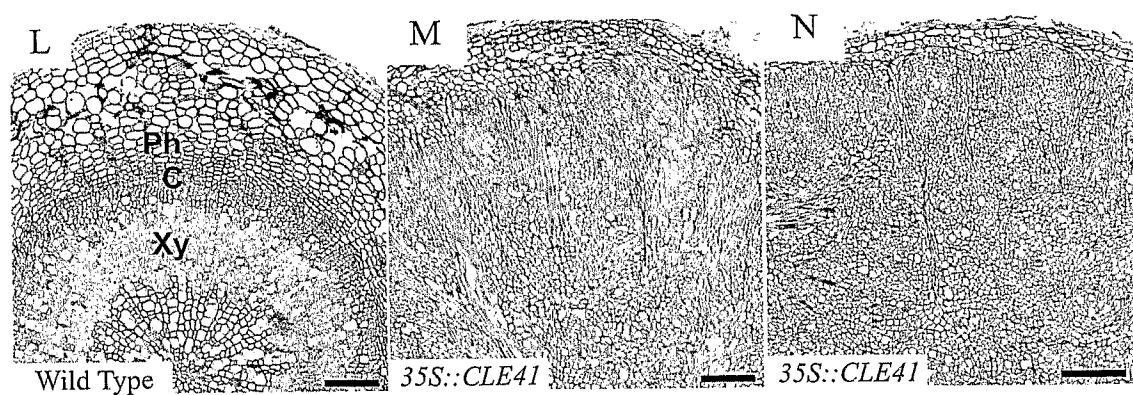


Fig. 7

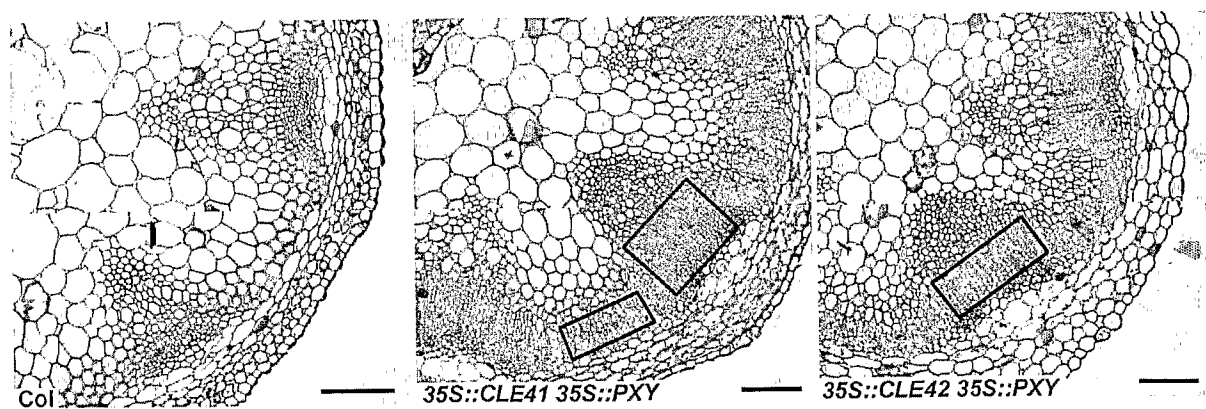


Fig. 8

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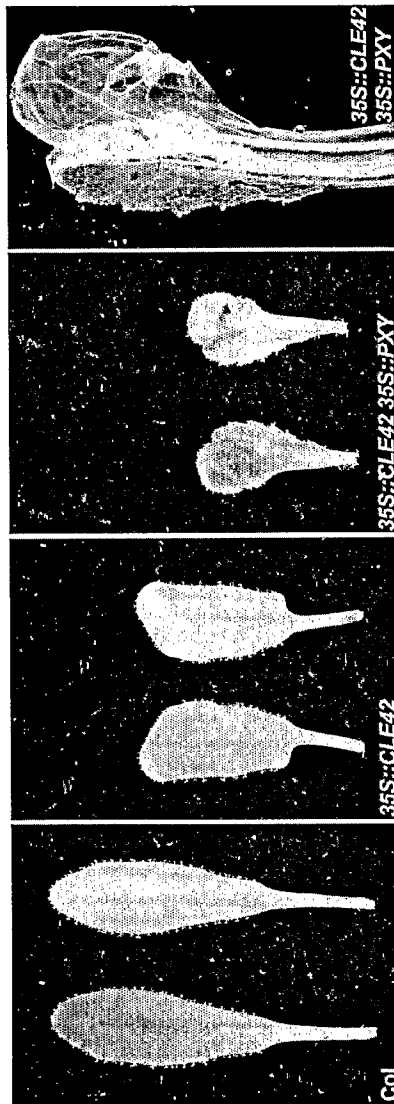


Fig. 9

(1)	MDI	EPLWALGGWFLFSI	TCATPKSGSTIS---	ETFKRSHHFFLFLALLFVFI	LLTSPSK---	PI NPTNTVASI	SI	KRLLESSESEPASTITMNL	HPKHTQGTRTSSSSPPSSKSTRKFGACAHEVPSGPNPI	SNR	1395
(1)											5110
(1)											Cle41
(1)											Cle44
(1)											148
(1)											1849
(1)											428
(1)											Cle46
(1)											OsCLE205
(1)											Cle42
(1)											OsCLE102
(1)											Consensus

Fig. 10

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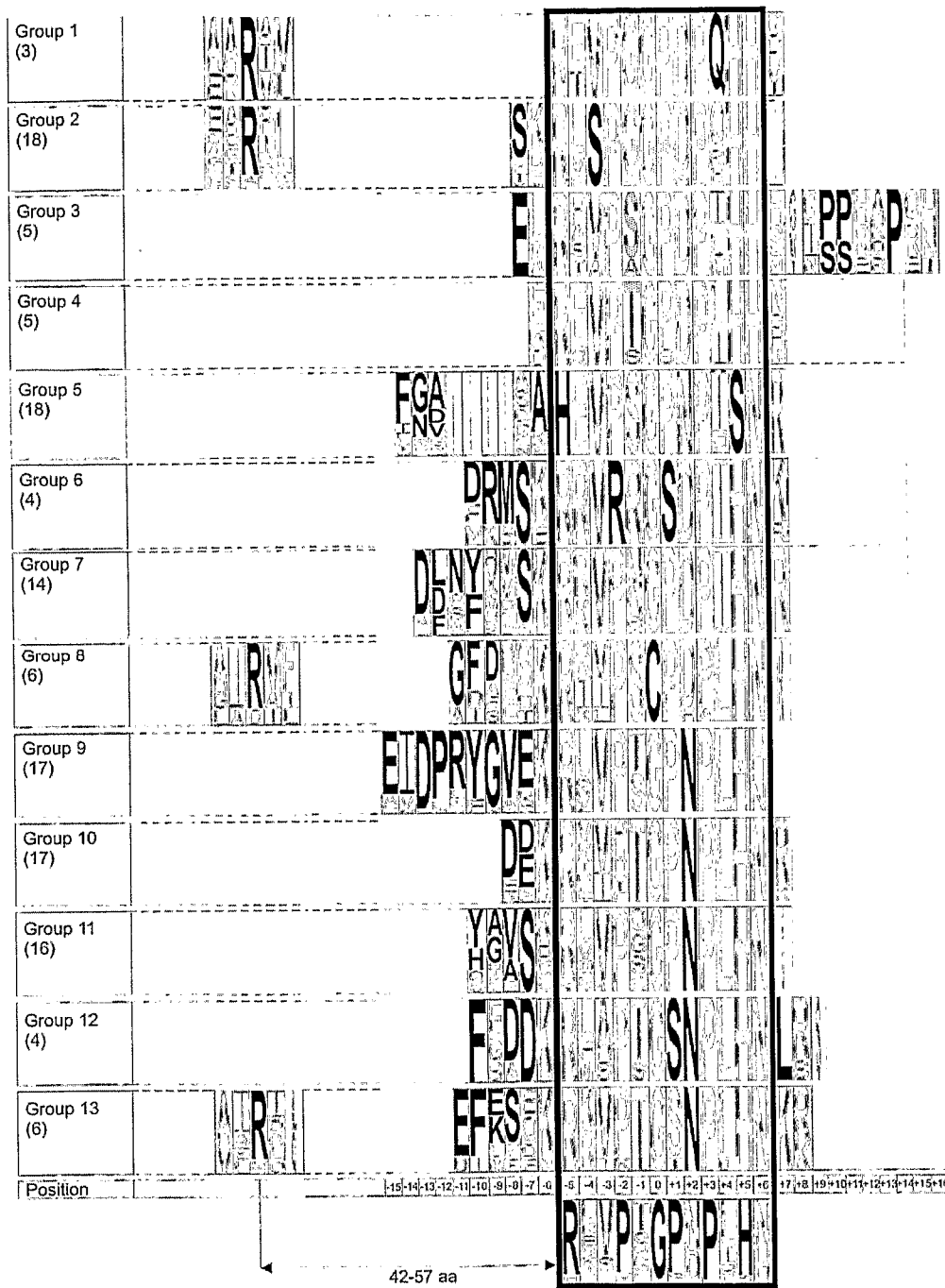


Fig. 11

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							Section 1
	(1)	1	10	20	30	40	55
Os02g02140.1	(1)	VKEANIVVG	MGATGVVYKAELPR	ARAVIAVKKELWRF	FAAAA	-----	EAAAAAP
Os03g05140.1	(1)	IKEANIVG	MGGTGVVYRADMPR	HHAVVAVKKELWRA	AGCP	-----	EEATTVD
OsPXY	(1)	-GSDGIVG	AGSSGTIVYRAKMPN	--GEVIAVKKELWQAP	AAQKEAAAPTEONQKLRQ		
PttPXY	(1)	-MSDKILCMG	STGTIVYKAEMPG	--GEIIAVKKELWGG	-----	HKEN--IRR	
PXY	(1)	-KTDNII	LGMSSTGTIVYKAEMPN	--GEIIAVKKELWGG	-----	NKENGKIRR	
PXL1	(1)	IKESNII	LGMSAIGIVYRAEVMRRPLLT	VAVKKELWRS	SPSPQ	-----	NDIEDHH
PXL2	(1)	-KESNMII	GMGATGIVYKAEMSR	-SSTVLAVKKELWFS	AAAD	-----	
Consensus	(1)	KEANIVG	MGATGIVYKAEMPR	VI	AVKKELWRA	A	E
							Section 2
	(56)	56	70	80	90	100	110
Os02g02140.1	(47)	-----	ELTAEVLKEVGLLGR	LRHRNIVRLLGYMH	NEADAMMLYEY	FMPNGSLWEAL	
Os03g05140.1	(47)	GRTDVEAGGE	FAAEVKLLGR	LRHRNIVRLLGYV	SNNLDTMVI	YEYHVNGLSWDAL	
OsPXY	(53)	DSGGGGG	GKFTVAEEVVLGHL	LRHRNIVRLLGWCTH	GESTMLLYEY	FMPNGSLDELL	
PttPXY	(41)	RR-----	GVLAEVDVLGNV	RRNIVRLLGCCSH	RECTMLLYEY	FMPNGSLDHL	
PXY	(43)	RKS-----	GVLAEVDVLGNV	RRNIVRLLGCCCT	NRDCTMLLYEY	FMPNGSLDHL	
PXL1	(48)	--QEEDEED	DILREVNLLGGL	LRHRNIVKILGYV	HNEREVMVMVYEY	FMPNGSLGTAL	
PXL2	(38)	--IEDGTTG	DFVGEVNLLGKL	LRHRNIVRLLGFLY	NDKNMMIVYEY	FMPNGSLGTAL	
Consensus	(56)		DVLAEV	LLG	LRHRNIVRLLGYVSN	TMLLYEYMPNGSL	DAL
							Section 3
	(111)	111	120	130	140	150	165
Os02g02140.1	(97)	HGPPE	R-ETLVDWVSRYDVAAGVAQGLAYLHHD	CHFFVIHRDIKSN	NIILLDANME		
Os03g05140.1	(102)	HGQRKG	-KMLMDWVSRYNVAAGVAAGLAYLHHD	CRPPVIHRDVKSSN	VLLDDNMD		
OsPXY	(108)	HG--AAAMAR	PGWDARYKIAGVAGGVYLLHHDCL	FATAHRDIKPSN	ILLDDME		
PttPXY	(89)	HGKNKG	DNLVGDWLTTRYKIALGVAQGCIC	YLHHDCCDPVIVHRD	LEKPSNILLDGE		
PXY	(92)	HGGDKT	MTAAAEWTALYQIATGVAQGCIC	YLHHDCCDPVIVHRD	LEKPSNILLDGE		
PXL1	(101)	HSKDEK	-FLRDWLSRYNVAVGVQGLNYLHND	CYPPPIHRDIKSN	NIILLDANLE		
PXL2	(91)	HGKNAG	GELLVDWVSRYNIALGVAHGLAYLHHD	CHFFVIHRDIKSN	NIILLDANLE		
Consensus	(111)	HG	A	KLL	DWVSRYNIALGVAQGLAYLHHD	C	PIIHRDIKSSNILLDANME
							Section 4
	(166)	166	180	190	200	210	220
Os02g02140.1	(151)	ARIADFG	LARALGRAGESVSVVAGSYGYIAPEYGY	TMKVDQKSDIYSYGV	VLMEL		
Os03g05140.1	(156)	AKIADFG	LARVMARAHETVSVVAGSYGYIAPEYGY	TLKVDQKSDIYSYGV	VLMEL		
OsPXY	(161)	ARVADFG	VAKALQS-AAFMSV	VAGSCGYIAPEYTYTLEVN	EKS	SDVYSGV	VLLLEI
PttPXY	(144)	ARVADFG	VAKLIQS-DESM	SVIAGSYGYIAPEYAYTLQV	DEKSDIYSYGV	VLMEL	
PXY	(147)	ARVADFG	VAKLIQT-DESM	SVVAGSYGYIAPEYAYTLQV	DKSDIYSYGV	VLLLEI	
PXL1	(155)	ARIADFG	LAKNMLHKNETVSMVAGSYGYIAPEYGY	TLKIDEKSDIYSYGV	VLLLEI		
PXL2	(146)	ARIADFG	LARMHARKKETVSMVAGSYGYIAPEYGY	TLKIDEKSDIYSYGV	VLLLEI		
Consensus	(166)	ARTADFG	LAKLM	R	SVSVVAGSYGYIAPRYGYTLKVDKSDIYSYGV	VLLLEI	
							Section 5
	(221)	221	230	240	250	260	275
Os02g02140.1	(206)	LTGRR	AVEAAFGEGQDI	VGVNRKIRS-NTVED	HLDGQLVGA-GCP	HVREEMLLV	
Os03g05140.1	(211)	LTGRR	PIEFEGESQDI	VGVNRKIRS-NTG	VEELLDA	SVGG-RVD	HVREEMLLV
OsPXY	(215)	LTGRR	SVAEYEGENI	VDWVRKIVAG-GGV	GDVIDAAAWADND	VGGTR	DEMALA
PttPXY	(198)	ISGKR	SVDAEFGDGN	SIVDWVRKIRAK	GDVNDILDKDAGAS	--IASV	REEMHOM
PXY	(201)	ITGKR	SVPEFEGEN	SIVDWVRKIRAK	GDVNDILDKDAGAS	--IASV	REEMHOM
PXL1	(210)	VTGKM	PIDPSEDS	IDVVEVIRKVKK-MES	LEEMIDASIAG-DCK	HVIEEMLLA	
PXL2	(201)	LTGRR	PIEFEGES	VDIWEVVRKIRK-NIS	LEEALDPNVG--NCRY	VQEEEMLLV	
Consensus	(221)	ITGRR	SVPEFEGEN	DIWDVVR KIK	N	V	E LD AAVA C HVREEMLLV
							Section 6
	(276)	276	290	300	310	320	330
Os02g02140.1	(259)	LRI	AVLCTARLPDR	DSMRDVTMLGEAK	FRKSGSS	TGSASAKAPT	PAPPAVAA
Os03g05140.1	(264)	LRV	AVLCTAKSPKDR	PTMRDVTMLGEAK	FRKSSS	-----	ATVAAT
OsPXY	(269)	LRV	ALLCTSRCPQER	PONREVLMLQEAR	PKRKNSAKKQVK	-----	
PttPXY	(251)	LRI	ALLCTSRNPADR	PSMRDVTMLQEAR	FRKLP	SGSIVSVGSGD	HIVTVDGAIA
PXY	(252)	-----					
PXL1	(263)	LRI	ALLCTAKLPKDR	PSIRDTVITML	-----		
PXL2	(253)	LQI	ALLCTTKLPKDR	PSMRDVISMLG	-----		
Consensus	(276)	LRI	ALLCTAK	PKDRPSMRDVTML	EAKPKRK		

Fig. 12

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FIGURE 13A CLE41 protein sequence

MATSNDQTNTKSSHSRTLFLFIFLSLLLFSSLTIPMTRHQSTSMVAPFKRVLLESS
VPASSTMDLRPKASTRRSRTSRREFGNDHEVPSGPNPISN

FIGURE 13B CLE41 DNA sequence

ATGGCAACATCAAATGACCAAACCAATACTAAATCATCACATTCTCGTACTCT
TCTCCTTCTCTTCATCTTCTTATCCCTCCTTCTCTTCAGTAGCCTTACAATCCCC
ATGACTCGTCATCAGTCCACATCTATGGTTGCTCCCTTCAAGAGGGTTCTCC
TCGAATCTTCAGTTCCAGCTTCATCAACAATGGATCTACGTCCAAAGGCTAGC
ACACGACGCAGCCGCACTTCTAGAAGGAGAGAGTTTGGAAATGATGCTCATG
AGGTTCTAGTGGTCCAAACCCTATTCCAACCTAG

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FIGURE 14A CLE42 protein sequence

MRSPHITISLVFLFFLFLIIQTHQRTIDQTHQIGSNVQHVSDMAVTSPEGKRRER
FRVRRPMTTWLKGKMIGANEHGVPSGPNPISNR

FIGURE 14B CLE42 DNA sequence

ATGAGATCTCCTCACATCACCATTTCACCTTGTTTTCTTGTTCTTTCTTTTCT
AATCATCCAAACCCATCAAAGAACCATTGATCAAACCTCACCAGATTGGCT
CCAATGTTCAACATGTCAGTGACATGGCGGTGACTTCGCCTGAAGGGAAA
AGAAGAGAGAGGTTTAGAGTTTCGGCGGCCGATGACGACATGGCTGAAGG
GAAAGATGATCGGTGCCAATGAACATGGAGTCCCAAGTGGTCCAAATCCC
ATCTCCAATAGGTAG

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FIGURE 15A PXY protein sequence

MKKKNISPSLVLHPLLLLLLPFFAFNSLALKFSPQLLSLLSLKTSLSGPPSAFQDWKVPVNGQND
AVWCWSGSGVVCNDVTAQVISLDLSHRNLSGRIPIQIRYLSSLLYLNLSGNSLEGSFPTSIFDLTKL
TTLDISRNSFDSSFPFGISKLFKLVFNAFSNNFEGLLPSDVSRLRFLEELNFGGSYFEGEIPAAY
GGLQRLKFIHLAGNVLGGKLPPRLGLLTELQHMEIGYNHFNGNIPSEFALLSNLKYFDVSNCSLS
GSLPQELGNLSNLETFLFQNGFTGEIPESYSNLKSLKLLDFSSNQLSGSIPSGFSTLKNLTWLS
LISNNLSGEVPEGIGELPELTTLFLWNNNFTGVLP HKLGSNGKLETMDVSNNSTGTIPSSSLCHG
NKLYKLILFSNMFEGELPKSLTRCESLWRFRSQNNRLNGTIPIGFGSLRNLT FVDLSNNRFTDQIP
ADFATAPVLQYLNLSNFFHRKLPENIWKAPNLQIFSASFNLIGEIPNYVGCKSFYRIELQGNSL
NGTIPWDIGHCEKLLCLNLSQNHNLNGIIPWEISTLPSIADVDLSHNLLTGTIPSDFGSSKTITTFNV
SYNQLIGIPSGSFAHLNPSFFSSNEGLCGDLVGKPCNSDRFNAGNADIDGHHKEERP KKTAGA
IVWILAAAIGVGFFVLVAATRCFQKSYGNRVDGGGRNGGDIGPWKLTA FQRLNFTADDVVECLS
KTDNILGMGSTGT VYKAEMPNGEIIAVKKLWGKNKENGKIRRRKSGVLA EVDVLGNVRHRNIVR
LLGCCTNRDCTMLLYEYMPNGSLDDLLHGGDKTMTAAAEWTALYQIAIGVAQGICYLHHD CDPV
IVHRDLKPSNILL DADFEARVADFGVAKLIQTDESMSV VAGSYGYIAPEYAYTLQVDKKS DIYSYG
VILLEITGKRSVEPEFGE GNSIVDWVRSLKLTKE DVEEVLDKSMGRSCSLIREEMKQMLRIALL
CTSRSP TDRPPMRDVLLILQEAKPKRKT VGDNVIVVGDVNDVN FEDVCSVDVGHDKCQRIGV

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FIGURE 15B PXY DNA sequence without intron

ATGAAAAAGAAGAACATTTCTCCTTCTCTTGTTCCTTCATCCCTTCTCCTTCTTCTACTTCCT
TTCTTTGCTTTCAATTCCTTAGCTCTCAAGTTTTACCTCAACTCTTGTCTCTCCTTTCCCTT
AAAACATCTCTCTCTGGCCCTCCCTCTGCCTTTCAAGACTGGAAAGTCCCCGTTAACGGTC
AAAACGACGCCGTTTGGTGTCTTGGTCCGGTGTAGTCTGTGATAATGTAACGGCTCAAGT
CATTTCCCTCGACCTCTCTCACCGGAACCTCTCTGGTCTGATTCTATACAGATTCTGTTACT
TGTCGAGCTTACTCTACTTAAATCTCAGTGGGAATTCTTTGGAAGGTTCTGTTTCCAATTCT
ATCTTTGATCTCACCAAGCTCACTACCTCGACATCAGCCGTAACCTCGTTCGACTCGAGTT
TTCCTCCCGGAATCTCCAAGCTTAAAGTTCTTAAAAGTCTTCAATGCGTTTCAGCAACAACCTT
GAAGGTCTATTACCTAGTGACGTGTCTCGTCTTCTGTTTCTTGGAAAGAGCTTAACTTTGGTG
GAAGTTACTTTGAAGGAGAGATTCCAGCAGCTTACGGTGGTTTACAGAGATTGAAGTTTAT
TCATTTAGCTGGAAATGTCCTCGGAGGTAAACTACCTCCTAGATTAGGACTCTTAACAGAGC
TCCAACACATGGAAATCGGTTATAATCACTTCAACGGAAACATACCTTCGGAGTTTGCCTTA
CTCTCAAATCTCAAGTACTTTGACGTTTCCAATTGCAGCCTCTCTGGTTCTCTGCCTCAAG
AACTCGGGAATCTCTCAAACCTAGAGACTTTATTTCTATTCCAAAACGGTTTACCGGTGAA
ATCCCAGAGAGTTATAGCAACTTGAAATCCCTCAAGCTTCTCGATTTTTCGAGTAATCAGCT
TTCTGGTAGTATCCCATCAGGCTTCTCGACCTTGAAGAACCTCACATGGCTAAGCTTAATCA
GCAATAACCTCTCAGGTGAAGTACCTGAAGGAATCGGTGAACTCCCTGAGCTTACTACATT
GTTTCTATGGAACAATAACTTCACCGGAGTTTTGCCACACAAGCTTGGATCAAACGGTAA
CTTGAGACAATGGACGTCTCTAACAATTCATTACCGGAACAATCCCTTCTCTCTGCCA
TGGAACAAGCTATACAACTCATCCTCTTCTCCAACATGTTTGAAGGTGAGCTACCAAAG
AGCTTGACTCGTTGCGAATCTCTATGGCGGTTTCGGAGTCAAACAATCGATTAAACGGCA
CAATCCGATCGGATTCCGCTCTCTACGTAACCTCACTTTCGTTGATTAAAGCAACAACAGA
TTCACCGATCAAATTCGGGCGGATTTCCGCCACCGCTCCTGTTCTTCAGTACTTGAATCTCT
CAACCAATTTCTTCCACAGGAACTACCGGAAAACATATGGAAAGCTCCGAATCTACAGAT
CTTCTCAGCGAGTTTTCAGCAATTTGATCGGTGAAATCCCAAATTACGTTGGATGCAAAAGC
TTCTACAGGATTGAACTACAAGGAACTCACTCAACGGAACGATTCCATGGGACATCGGAC
ATTGCGGAGAAGCTTCTCTGTTTGAATCTCAGCCAAAATCATCTCAACGGAATCATTCCATGG
GAGATTTCAACTCTTCCGTCAATCGCCGACGTAGATCTTCTCATAATCTCTTAACCGGAAC
AATCCCTTCCGATTTCCGAAGCTCTAAGACGATCACAACCTTCAACGTTTCGTATAATCAGC
TAATCGGTCCGATTCCAAGTGTTCTTTCGCTCATCTGAATCCGTCGTTCTTCTCCTCAAAC
GAAGGACTCTGTGGAGATCTCGTCGGAAAACCTTGCAATTCTGATAGGTTTAAACGCCGA
AATGCAGATATAGACGGTCATCATAAAGAGGAACGACCTAAGAAAACAGCCGGAGCTATTG
TTTGGATATTGGCGGCGGCGATTGGGGTTGGATTCTTCGTCCTTGTAGCCGCCACTAGATG
CTTCCAGAAAAGCTACGGAAACAGAGTCGACGGTGGTGGAAAGAAACGGCGGAGATATAG
GACCGTGGAAGCTAACGGCTTTTTCAGAGACTAACTTCACGGCGGATGATGTGGTTGAGT
GTCTCTCAAAGACTGATAACATCCTCGGAATGGGATCAACAGGAACAGTGTACAAAGCAGA
GATGCCTAATGGAGAAATAATCGCCGTGAAAAAATTTGGGGAAAAAACAAGAGAACGG
CAAAATCCGGCGGCGGAAGAGCGGCGTATTGGCGGAGGTTGATGTTCTAGGGAACGTAC
GTCACCGGAACATCGTTCGTCTCCTTGGATGTTGCACGAATCGAGATTGCACGATGCTTTT
ATACGAATACATGCCTAATGGAAGCTTAGACGATCTTCTTCACGGTGGGGATAAGACGATG
ACCGCGGCGGCGGAATGGACGGCTTTGTATCAGATCGCGATTGGAGTGGCTCAAGGGAT
CTGTTATCTCCACCATGATTGTGATCCGGTGATTGTACACCGTGACCTGAAACCTAGCAATA
TCCTCCTCGACGCCGATTTTCGAGGCGCGTGTGGCGGACTTCGGCGTCGCGAAGCTTATT
CAAACCGACGAATCCATGTCCGTCTGTCGCCGGTTTCGTACGGTTACATTGCACCAGAATATG
CTTACACTTTACAAGTGGATAAAAAGAGTGATATCTATAGCTATGGAGTGATTTTATTAGAGAT
AATCACCGGAAAAAGATCGGTGGAACCGGAATTTGGAGAAGGTAACAGTATCGTGGATTG
GGTTAGATCAAAGTTGAAGACGAAAGAAGATGTAGAAGAAGTTCTAGACAAAAGCATGGGT
AGGTCGTGTAGTCTTATAAGAGAAGAGATGAAACAAATGTTGAGAATTGCGTTGTTGTGTAC
AAGCCGGAGTCCGACAGACAGACCGCCGATGAGAGATGTGTTGTTGATTCTTCAAGAGGC
AAAGCCAAAGAGGAAGACAGTAGGGGATAATGTGATCGTCGTTGGTGATGTTAATGATGTC
AATTTCAAGATGTTTGTAGTGTTGATGTTGGTCATGATGTTAAATGTCAAAGGATTGGGGT
GTGA

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FIGURE 15C PXY DNA sequence with intron

ATGAAAAAGAAGAACATTTCTCCTTCTCTTGTTCCTTCATCCCCTTCTCCTTCTTCTACTTCCTT
 TCTTTGCTTTCAATTCCTTAGCTCTCAAGTTTTACCTCAACTCTTGTCTCTCCTTTCCCTTAA
 AACATCTCTCTCTGGCCCTCCCTCTGCCTTTCAAGACTGGAAAGTCCCCGTTAACGGTCAA
 AACGACGCCGTTTGGTGTTCTTGGTCCGGTGTAGTCTGTGATAATGTAACGGCTCAAGTCAT
 TTCCTCGACCTCTCTCACCGGAACCTCTCTGGTTCGATTTCCTATACAGATTTCGTTACTTGTG
 GAGCTTACTCTACTTAAATCTCAGTGGGAATTCTTTGGAAGGTTTCGTTTCCAACCTTCTATCTT
 TGATCTCACCAAGCTCACTACCCTCGACATCAGCCGTAACCTCGTTCGACTCGAGTTTTCTC
 CCGGAATCTCCAAGCTTAAGTTCTTAAAAGTCTTCAATGCGTTCAGCAACAACCTTCGAAGGT
 CTATTACCTAGTGACGTGTCTCGTCTTTCGTTTCTTGGAAAGAGCTTAACTTTGGTGGAAGTTA
 CTTTGAAGGAGAGATTCCAGCAGCTTACGGTGGTTTACAGAGATTGAAGTTTATTCAATTTAGC
 TGGAAATGTCCTCGGAGGTAACTACCTCCTAGATTAGGACTCTTAACAGAGCTCCAACACA
 TGGAAATCGGTTATAATCACTTCAACGGAAACATACCTTCGGAGTTTGCCTTACTCTCAAATC
 TCAAGTACTTTGACGTTTCCAATTGCAGCCTCTCTGGTTCCTCTGCCTCAAGAACTCGGGAAT
 CTCTCAAACCTAGAGACTTTATTTCTATTCCAAAACGGTTTACCGGTGAAATCCCAGAGAG
 TTATAGCAACTTGAAATCCCTCAAGCTTCTCGATTTTTTCGAGTAATCAGCTTTCTGGTAGTATC
 CCATCAGGCTTCTCGACCTTGAAGAACCTCACATGGCTAAGCTTAATCAGCAATAACCTCTC
 AGGTGAAGTACCTGAAGGAATCGGTGAACTCCCTGAGCTTACTACATTGTTTCTATGGAACA
 ATAACCTCACCGGAGTTTTTGCACACAAGCTTGGATCAAACGGTAAACTTGAGACAATGGAC
 GTCTCTAACAAATTCATTACCGGAACAATCCCTTCTTCTCTGCCATGGAACAAGCTATAC
 AAACCTCATCCTCTTCTCCAACATGTTTGAAGGTGAGCTACCAAAGAGCTTGACTCGTTGCGA
 ATCTCTATGGCGGTTTTCGGAGTCAAAACAATCGATTAAACGGCACAATTCCGATCGGATTGCG
 GCTCTCTACGTAACCTCACTTTCGTTGATTTAAGCAACAACAGATTCACCGATCAAATTCCGG
 CGGATTTCCGCCACCGCTCCTGTTCTTTCAGTACTTGAATCTCTCAACCAATTTCTTCCACAGG
 AAACCTACCGGAAAACATATGGAAGCTCCGAATCTACAGATCTTCTCAGCGAGTTTCAGCAA
 TTTGATCGGTGAAATCCCAAATTACGTTGGATGCAAAAGCTTCTACAGGATTGAACTACAAG
 GAACTCACTCAACGGAACGATTCCATGGGACATCGGACATTGCGAGAAGCTTCTCTGTTT
 GAATCTCAGCCAAAATCATCTCAACGGAATCATTCCATGGGAGATTTCAACTCTTCCGTCAAT
 CGCCGACGTAGATCTTTCTCATAATCTCTTAACCGGAACAATCCCTTCCGATTTCCGAAGCT
 CTAAGACGATCACAACCTTCAACGTTTTCGTATAATCAGCTAATCGGTCCGATTCCAAGTGGTT
 CTTTCGCTCATCTGAATCCGTGTTCTTCTCCTCAAACGAAGGACTCTGTGGAGATCTCGTC
 GGAAAACCTTGCAATTCTGATAGGTTTAAACGCCGGAATGCAGATATAGACGGTCATCATAAA
 GAGGAACGACCTAAGAAAACAGCCGGAGCTATTGTTTGGATATTGGCGGCGGCGATTGGG
 GTTGGATTCTTCGTCCTTGTAGCCGCCACTAGATGCTTCCAGAAAAGCTACGGAAACAGAG
 TCGACGGTGGTGGAAAGAACGGCGGAGATATAGGACCGTGGAAGCTAACGGCTTTTCAGA
 GACTAACTTCACGGCGGATGATGTGGTTGAGTGTCTCTCAAAGACTGATAACATCCTCGGA
 ATGGGATCAACAGGAACAGGTGTACAAAGCAGAGATGCCTAATGGAGAAATAATCGCCGTGA
 AAAAATTTGGGGAAAAAACAAGAGAACGGCAAAATCCGGCGGCGGAAGAGCGGCGTAT
 TGGCGGAGGTTGATGTTCTAGGGAACGTACGTCACCGGAACATCGTTTCGTCTCCTTGGATG
 TTGCACGAATCGAGATTGCACGATGCTTTTATACGAATACATGCCTAATGGAAGCTTAGACGA
 TCTTCTTCACGGTGGGGATAAGACGATGACCGCGGCGGCGGAATGGACGGCTTTGTATCA
 GATCGCGATTGGAGTGGCTCAAGGGATCTGTTATCTCCACCATGATTGTGATCCGGTGATTG
 TACACCGTGACCTGAAACCTAGCAATATCCTCCTCGACGCCGATTTTCGAGGCGCGTGTGGC
 GGACTTCGGCGTCGCGAAGCTTATTCAAACCGACGAATCCATGTCCGTCTGTCGCCGGTTC
 GTACGGTTACATTGCACCAGgtacccttaacttttttgattattctttactttccccaaattttaaatttgactttttgtcccttggtt
 ttattattcgaattttgtccgttggtaaacattcttttggggatgacaacatctgacaaatagactaaaattttaatttggttgtttggttaca
 gAATATGCTTACACTTTACAAGTGGATAAAAAGAGTGATATCTATAGCTATGGAGTGATTTTATT
 AGAGATAATCACCGGAAAAAGATCGGTGGAACCGGAATTTGGAGAAGGTAACAGTATCGTG
 GATTGGGTTAGATCAAAGTTGAAGACGAAAGAAGATGTAGAAGAAGTTCTAGACAAAAGCAT
 GGGTAGGTCGTGTAGTCTTATAAGAGAAGAGATGAAACAAATGTTGAGAATTGCGTTGTTGT
 GTACAAGCCGGAGTCCGACAGACAGACCGCCGATGAGAGATGTGTTGTTGATTCTTCAAGA
 GGCAAAGCCAAAGAGGAAGACAGTAGGGGATAATGTGATCGTCGTTGGTGATGTTAATGAT
 GTCAATTTGGAAGATGTTTGTAGTGTGATGTTGGTCATGATGTTAAATGTCAAAGGATTGGG
 GTGTGA

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FIGURE 16

1. 35S promoter

CCTGTCCTCTCCAAATGAAATGAACTTCCTTATATAGAGGAAGGGTCTTGCGAAGGATAGTGGGA
 TTGTGCGTCATCCCTTACGTCAGTGGAGATATCACATCAATCCACTTGCTTTGAAGACGTGGTTG
 GAACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGTCCATCTTTGGGACCACTGTCGGC
 AGAGGCATCTTGAACGATAGCCTTTCTTTATCGCAATGATGGCATTGTAGGTGCCACCTTCCTT
 TTCTACTGTCCTTTTGATGAAGTGACAGATAGCTGGGCAATGGAATCCGAGGAGGTTTCCCGATA
 TTACCCCTTTGTTGAAAAGTCTCAATAGCCCTTTGGTCTTCTGAGACTGTATCTTTGATATTCTTGG
 AGTAGACGAGAGTGTCTGTCTCCACCATGTTGACGAAGATTTCTTCTTGTCAATTGAGTCGTAA
 AGACTCTGTATGAACTGTTCCGCCAGTCTTCACGGCGAGTTCTGTTAGATCCTCGATCTGAATTTT
 TGACTCCATGGGAATTC

2. lRX3 promoter

AAAAATAAGTAAAAGATCTTTTAGTTGTTTGCTTTGTATGTTGCGAACAGTTTGATTCTGTTTTCT
 TTTTCCTTTTTTTGGGTAATTTTCTTATAACTTTTTTCATAGTTTCGATTATTTGGATAAAATTTTCAG
 ATTGAGGATCATTTTATTTATTTATTAGTGTAGTCTAATTTAGTTGTATAACTATAAAATTGTTGTTTGT
 TTCCGAATCATAAGTTTTTTTTTTTTTTGGTTTTGTATTGATAGGTGCAAGAGACTCAAATTTCTGG
 TTTTCGATGTTAACAGAAATCAAGTAGCTGCCCACTTGATTGATTTGTTTTGTATTTGGAAACAAC
 CATGGCTGGTCAAGGCCCGACCCGTTGTGCTTCTGAACCTGCCTAGTCCCAGTCCCATGACTAGATCTT
 TATCCGCAGACTCCAAAAGAAAAGGATTGGCGCAGAGGAATTGTCATGGAACAGAAATGAACA
 AGAAAGGGTGAAGAAGATCAAAGGCATATATGATCTTTACATTCTCTTTAGCTTATGTATGCAGAAA
 ATTCACCTAATTAAGGACAGGGAACGTAACCTTGCTTGCACCTCCTCTCACCAAACCTTACCCCT
 AACTAATTTTAATTCAAATTAAGTATTTTGGCCGATCA
 CTTTATATAATAAGATACCAGATTTATTATATTTACGAATTATCAGCATGCATATACTGTATATAGTTTT
 TTTTTGTTAAAGGGTAAAATAATAGGATCCTTTTGAATAAAATGAACATATATAATTAGTATAATGAA
 AACAGAAGGAAATGAGATTAGGACAGTAAGTAAAATGAGAGAGACCTGCAAAGGATAAAAAAGA
 GAAGCTTAAGGAAACCGCGACGATGAAAGAAAGACATGTCATCAGCTGATGGATGTGAGTGATG
 AGTTTGTGTCAGTTGTGTAGAAATTTTACTAAAACAGTTGTTTTTACAAAAAGAAATAATATAAAA
 CGAAAGCTTAGCTTGAAGGCAATGGAGACTCTACAACAACTATGTACCATACAGAGAGAGAAAC
 TAAAAGCTTTTACACATAAAAACCAAACCTTATTCGTCTCTCATTGATCACCGTTTTGTTCTCTCAA
 GATCGCTGCTAATCT

3. LRR promoter

GTGTTTTTGGACATAATGATTTGAATGATATTAACAAAAAAAGGCATCTTATTGTAATTAATTAAT
 AAAGTAATGGACGTTTTTGTAGTGAGACGCCTGAACCTGTCATGACATACAGACATACCAATTCC
 ATTCATGCAGGAGAATGTTGAATAATGTGAGGGAACGTGAATAGAGGAACATTTTTTGGGTAAATG
 CAATCACATCCTCTCATTCTCAAACCTCAAACCAAATGTGTAGTTTTCTTCTCTCCAAGAAAC
 AATACTCTTATGACTGCAATACCACACTTTAGTCTGTGTGTGTTTTTAAATAGAATGGTATAAGAA
 GCATACTGAAATGGTGTTTTTAAATGAAAGATCAAATCACAACAATATCCAAAACCTAAAATAAAAA
 GATTGTGGGCTTATTTATTAGGCCCATAGTCTTTATTCTTTAAGGGCAAACCTTCAGAGATGCGGG
 TCTACATTATTTGGCCCGAACCAGTAAAACCTGCAGGCTTCTAGAGAAACCCATACGAATGGCGA
 GTTACCGGTCAGAAGCGTAATCACCGGTCCTTAAATTTCAATTCAGGCGAACGATTTGAAAAAG
 TCGGTGTCGTTAAGAAAAGACAATTCCTACCCTTTTTGTGTCGTCATTTGTTTGCTAATTGCTACCTT
 ATAATGTAAGGGAGGAGTGGAGTTGCTATTATTATTCGACATTTAAGTGTTATGTGTTTGTGCGAT
 CGGATTCTATCTTAAGAATCTTATCTTATTCCTCCCGAATATCATTGACTTTGTCTTAAATCTTAAT
 TGTTATGTTTGCACCTAACTCTCTTTTTTCGTTAGTGCACACAACTCGTATTCCATCAAATCAA
 TGATAAAATAATTCTTACCAAATTAACAAAAAGTGAATAAATATCACATTATTCATACTAATCTTCATG
 ATATCATCACAAGAATAATGTGTGCACACAAAAAACCTGTTTGTTCGCGGTGAAGTGAACCTCT
 CAAGAGTCTCCAATGTTGACCAAATCAAACAACCTCTTAACCTCTTTGACCAACAATTTTAAACT
 CATGAAATAAGTTACATACGAAGACTTGACTTGTCTTTCTCTTAAATACTCAAAGTTTAACTGCTT
 CAGAGTTAATTTTCAATGTGTCCATATACAATTCATTTAAATCTAAAGCAAATTCCTCTCTTTTTT
 TTCCTTTAATTTATTTTATTTTATGGAGTGAGTTGAGTTCTGTATACATTCTTTGTAAATGGAAAA
 AAATAAAAAAACAGCTTGATTAAAAAAATAAAGAAATTGAGAAAAAGACAAGAATTTAAATAATAAT
 AAAATAATGTAAAAAGAAAGTGAACAACAAAAAAGACACAAAAAGTAAAAGTAAAAGGAGTAT
 TTCTCTGTCATTTCCACACCAATCGCATAATCGATTTCTTCCAACCTCAATAAAGGGGAACCAAC
 GTAACCTAATTTTGTCTTCTCTCTTTGTTTCAAGAAATTTTCCCTTTACTCTCAAATTCCTTTTCTG
 ATTTCCCTCTCTTAAACCTCCGAAAGCTCAC

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4. Nodulin promoter

GAGGGTTGTCATGTCAGCTAAACAGCAGAAAAATAAAGAATGTGGGAGACAGGCT
TTGCCCTTTTACACATTTTAAAGCTCTCTTCTTTATTTTCTAAAAACACTATAGTACACA
GTAAAAAATTTGCGGGGCCCTTTGTTTAATCGATTTAGATTTAACCAACAGCTTCATAG
TCGTTTCTTCTTGTTAGAAATCAATAAATGAAAAGTTGGTTGTTTTGAAAGCATATAAT
TAACTAACAATGTATCATGTTGTGAAACAAAAGCTGATGATAAATGTTAAATATAGTGT
CCAAAATTTTGGGAATGTCTTCAAATTTTGATTTTAAAACATATGAGATGTACCAACAT
CCACAAAATGTTTGTGAAGTTGTGACTTAGTGAGACGTTGTCACCTTTATTATCTCGTT
TTGGTAACGGTAAGTGCATGTGAACGTGTTTGATTATAAGTTTAACCCGACTTTGTTT
ACGTGGTCATAGTTTCCAATAAGGCTAAGTAGAATAGTGTATGATAGTTTTCAATTCAA
GAGTCTTTTTTTTCCAAGAAAATCCGAAAACACAATCGTTTATTTTAATATCTCAAAAG
AAATCATATTTCTTAGGTAAACTATCATACGTTGAGTTTTCTTTCTCCTTTTGCGATT
TTCCCTGAAATATATTTATGTTGTGTGTGTGCTTTGTAAACAAAATAATAATGAACGTA
ATAATAGTAAACAAAAAGTAACCTATATTATTATCATACTTGATATAACCCAATAGAGA
AATAATAATATAAATTAAATTTTATTTCCCAATTCAAAAATCATAATCAAGGAGTGAAT
GCTGCCAGTAGACCAAAGTAACAATATTTGGTGTGTACATCAAATGATAGGAAACAA
CAAAAACCTCATTAATATACCTAAATCCATATATATATATATATATATATATTTCAATATTTCA
CATTGTTATAATTAGGTTTAATAGATACCATATTAGAAATCTCAGTATGGTGGTTC

5. 35S Terminator

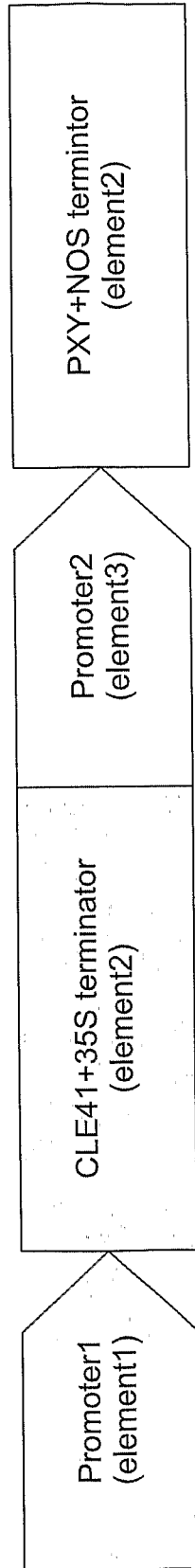
GGAATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTC
TTATATGCTCAACACATGAGCGAAACCCTATAAGAACCCTAATTCCCTTATCTGGGAA
CTACTCACACATTATTATAGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTTCAG
CG

6. NOS Terminator

GATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTG
CGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAAT
GCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAA
TACGCGATAGAAAACAAAATATAGCGCGCAAACCTAGGATAAATTATCGCGCGCGGTG
TCATCTATGTTACTAGATCGG

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Fig. 17



Please see the list of constructs for the details of information

- Element1 (35S promoter or IRX3 promoter)
- Element2 (CLE41+35S terminator)
- Element3 (35S promoter or LRR promoter or Nodulin promoter)
- Element4 (PXY + NOS terminator)

CLV-1 1 ---- NAMRLKTHLLFLHLFLFFSPCFAYT--- DMEVILNLKSSM GPKGHGLHDWI HSSSPDAH----- CSFSGVSCD- DDAVRI SLD
PXY 1 MKKKNI SPSLVLHPLLLLLPPFAFNSLAKFSPQLLSL LSLKTSLSGPPSPA- QDDWKVPVNGND- AVYCSGSGVCDNVTACVI SLD
PXYL-1 1 ---- MAI PRLFLFYI GFALFPFVSSETFQNSEQEI LAFKSDLPDPSSN- LQDWKRPENATTFSELVCHVHTGVHCD- ANGVAKLL
PXYL-2 1 ---- MKMKI I VLFLFYIYI GSTSSSVLASI DNVNELSVI LSVKSLTLDPLNF- LKDWKLS- DTSDH----- CNUYGVRCN- SGNVKELD
OsPXY 1 ---- MEATVPVLLLLTVLSLI LPSGI GAAAGADERSAL LKAKAGFVDTVA- LADWTGGKASPH----- CKWTGVGCN- AAGLVDRL
pt t PXY 1 ---- MMSLLQKPFMSFLRVLFF----- LLNLCI I PSFAFPSN-----
consensus 1
77 VSFTPLFGTI SPEI GMLTHLVNLTAAINFTGELPLEMKSLTSLKVLNI SNNGNLTGTFPGEI LKAWVDLEVLDTYNNNFNCKLPPENSE
PXY 88 LSHRNLSGRI PI GI RYLSLLYLNLGSGLEGGFPTSI FDLTKLTLLDI SRN- SFDSSFP- YGI SKLKFLKVFNAFNSNNFELLPDSVSR
PXYL-1 84 LSNMNLSGNVSDQI QSPFSLQALDLSNIAFESSLPKSLNLSLTKVI DSVSN- SFFGTFF- YGLGMATGLTHVNASSNFSELPEDLGN
PXYL-2 78 LAGMNLTKGI SDSI SQLSLSLVLSI SONGFESSLPKSI P- PLKSI DI SCN- SFGSGLF- AFLGSGGLVLHVLNAGNNNLNLTDELGN
OsPXY 79 LSGKNLSGKVADVDVRLPALAVLNI SNAFATTLPKSLPSLPSLKVFDVSGN- SFEQGFF- LGSNCCADLVAVNAGNNFACPLPEDLAN
pt t PXY 35 ---- SSATSFGAAYKEAAEGNEEAALLKVRASLDDSHSQSVLSS----- WVGSSPKCVLGI TCDSNGS
consensus 91
CLV-1 167 LKKLKYSFSGGNFFSGEIPESYGGI QSLEYLQAGNAGLSGKSPAFSLRNLKRENYI GYVLSYTTGVPFEGGLTKLEI LDMASCTLTCE
PXY 176 LRFLEELNFGGSYFEGEIPAAAGGLGRKFI HLAGNVLGGKLPRLGLLTTELQHEI G-YHFNENI SEFALLSNLYKFDVNSGSLSGS
PXYL-1 172 ATTLELDFRGGYFEGSVPSFKNLKNLKFLGSGNNNGGKVPKVI GELSSLEI I LG-YHFNENI DEEFGLKTLQYLDLAVGNLTQ
PXYL-2 163 LVSLLEVLDRGNFVGGSLSFKNLKLKFLGSGNNLTGELPSVLGDLQPLETAI LG-YHFNENI PEFNGI NSLYKFLDLAI GKLSCE
OsPXY 167 ATSLTEI DMRGSPFGGAI PAAYRRLTKLKFGLSGNNI TGKI PPEI GEMESLESI I G-YHFNENI PELGNLANLYLDLAVGNLQ
pt t PXY 95 VANFSLPHFG----- LRGTLHSFNFSSFPNLTPNLRN----- N G V P HSHI SNLTKI I NNLNCHNHNFS
consensus 181
CLV-1 257 I I TSLSNLKHHTLFIHI NNLTCIHPELSGLVSIKSLDLSEI NLTCEIHSFI NLGNI TLI NFRNNIYCI PEAI GELPKVEFVEVME
PXY 265 LPSLGLGNSNLETFLI FQNGFTGEI PYSYNSNLSKLDLDFSNQLSESI PSFSGTTLKNLTLVSLI SNNLSGVEPEGI GELPELTFLFLVN
PXYL-1 261 I PSSLGGLGKSTTYVYIYNGRTIKCLPRLRGMTSIVFLDLSDNI TGEI PVEGVELKNLQLLNIRNQLTGI I PSKI AELPNLEVLWLQ
PXYL-2 222 I PSELGGLKSTLTYVYIYNGFTTGEI PREI GSI TIKVLDLDFSDNALTGEI PVEI TKLKNLQLLNIRNQLSGSI PAI SSLACQVLELVN
OsPXY 256 I PSELGGLKSTLTYVYIYNGFTTGEI PELGNI TIKVLDLDFSDNALTGEI PVEI TKLKNLQLLNIRNQLSGSI PAI SSLACQVLELVN
pt t PXY 158 LPEPMNNITHIMVHLFNSNFTCHLPRDLCLGGLLVNFTASVYHFSFGI PKSLRNCTSLFVRVLDVNLQITGNI SEDFGVLPNNIYVLDLH
consensus 271
CLV-1 347 N N F T G V I P A N L C R N G N I K L D V S D H L T L I K D L C R G E K I E M L I S N I F F F C P I P E E L G K C K S I T K I R V K N L C T V I A G L F N P L V T
PXY 355 N N F T G V I P H K L S G N K I E T D V S N S F T G I P S S L C H G N K I Y K L I F S N F F E G E L P K S L T R C E S I W R F R S Q N I R L N G T I I G F G S I R N L T
PXYL-1 351 N S L M G S I P V H L C K N S P I K W L D V S S N K L S E D I P S G L C Y S R N I T K L I L F N I F S F C G I P E E I F S C P T I V R V R I Q K N I S C S I A G S G D C P M L Q
PXYL-2 342 N T L S G E I P S D L C K N S P I K W L D V S S N K L S E S T L C N K G N I T K L I L F N I T F T G I P A T L S T C G S I V R V R M Q N L L N C S I I G F G K E K L Q
OsPXY 346 N S L T G E I P A S I C R S S P I Q W V D V S N K L S E G P A G I C D G K A T I K M I M F N I G F T G I P A G L A S C A S I Y M R V H G L R N L C T I V A G S G D C P M L Q
pt t PXY 248 N N L G S I T P W K V G E F N N I T S L K S L S N N I T G E I P S E I A K A T G I Q M I D L S S I N L K E T I P K E L G K L A C I Y N L T L H N I H L F C V V F E I Q M S Q L R
consensus 361
CLV-1 437 I I E I T D I F F S G E L P V T W S G D V D I Y L S N N F S G E I P P A I G N F P N L Q T L F L D R N R F R E N I P R E I F E L K H S I T S A N N I T G E I H D S I S
PXY 445 F V D I S N N R F T D I P A D F A T S P I Q Y L N L S T F F H R K L P E N I W A K P N L Q I F S A S F S N L I C E I P N Y V G K S F Y R I E L Q S N L G T I P D S I G
PXYL-1 441 H L E I A K N L T G K I P D D I A T A L S L F I D S F N H L S L S S I F S P N L Q T F I A S H N N F A C I P N Q I Q D R P S L V L D S F N H F S G E P E R I A
PXYL-2 432 R L E I A G N L S G G I P G D I S D S V L S F I D F S R N Q I R S S L P S T I L S I H N L Q A F L V A D N F I S C V P D Q F C D C P S L N L D S S N L T G T I P S I A
OsPXY 436 R L E I A G N L S G G I P G D L A S S V L S F I D V S R N H L Q Y S I P S S L F T I P T L Q S F L A S D N F I S C E L P D Q F C D C P A A L D L S N R L A G I P S S I A
pt t PXY 338 A L N I A S N N L G I P G D L A S S V L S F I D V S R N H L Q Y S I P S S L F T I P T L Q S F L A S D N F I S C E L P D Q F C D C P A A L D L S N R L A G I P S S I A
consensus 451
CLV-1 526 R C S T I S V D L S R N I C E I P K G I N N V K N L G T L N I S G V A T S I P T G I G N N T S L T L D L S F D I S C R V I L G G Q F L V F N E T S F A G I T Y L C P
PXY 534 H E K I L C L N I S N H N G I P W E I S T L P S I A D V D L S N S I T C I P S D F G S S K T I T F N V S Y Q I C P I P S G S F A H I P K V F S S E G L C G D
PXYL-1 530 S F E K I V S L N I K S N G L V E I P K A L A G M H L A V L D L S N S I T C I P A D L G A S P T L E M N V S F K N I D E P I P S M L F A I P D K V L G N G L C G D
PXYL-2 522 S C E K I V S L N I K S N G L V E I P R Q I T T S A L A V L D L S N S I T C I P U S E I G T S P A L L E L N V S Y N I T C P V I N G F L K I N P D D L R G S L C G D
OsPXY 526 S C Q R I V S L N I K S N G L A E I P R S L A N P A L A I L D L S N S I T C I P E N F G S S P A E L T L N V S Y N I T C P V I N G F L R S I N P E L A G A G L C G D
pt t PXY 380 F L H F I G D L S G L L A C E I S E I G Q L K Q L E T M N L S I K S E I P T A F V D L V S L T T V D I S Y N E I C P I K I K G I E A P L E A F M N I S G L C G N
consensus 541
CLV-1 616 H R V S C P T R P G Q T S D H N H T A L F S P S R I V T V I A A I T G L I L I S V A I ----- R Q M N K N Q K S L A V K L T A F Q K L D F K
PXY 623 L V G P C S N D R F A N A G N D I D G H K H E R P K T A G A I V M I L A A I G V G F V L V A A T R C F Q K S Y G N R V D G G R N G D I G P W K L T A F Q R L N T
PXYL-1 620 L V P P C S K L S A K A G R N P G R I H N N A V F G I V G T S V I V A A M G M F L A G R V I T R Y D L Y S N F A R E Y I F C K K P R E E P V R L V A F Q R L G F T
PXYL-2 612 L V P P C S K F G R A T S ----- S H S L H G K I V A G W I G I A S V L A L G I L T I V T R T Y L K W Y S N - F G C G D E T A S - K G E V P R L M A F H R L C T
OsPXY 616 L V P P C S R S T A A G P R S R G A R L R I A V G W L V G M A V V - A A F A A L - F G H Y A Y R W Y V D G A G C D D E N L G G E S G A M P V R L T A F Q R L G F T
pt t PXY 470 A N G L K P C T L L T S R K K S N K I V I L F P L L G S L L L L I M V G C L Y F H ----- H Q T S R E R I S C L G E R G S P L S F V V W G H E E I
consensus 631
CLV-1 685 S E D V L E C L K E E ----- N I C K C G A C I V Y R G S M P N - N V D V I K R L V G ----- R G T G R S D H G ----- F T A E I Q T I G R I R H N I V R L L G Y V A
PXY 711 A D D V V E C L S K T D ----- N I L G S T G E I V K A E M P N - G E I I V K L V G K N K E N G I R R R K S ----- V L A E V D V I G N R H N I V R L L G C C T
PXYL-1 707 A G D I L S H I K E S ----- N I L G C A I G I V K A E V M R R P L T I V K L W R S P S P N D I E D H H Q E E D E D D I L R E V N L G G L R H N I V I L G Y V H
PXYL-2 692 A S D I L A C I K E S ----- N I L G C A I G I V K A E M R - S S T V L A V K L W R - S A A D I E D G T T G ----- D F V G E N L G K L R H N I V R L L G F Y
OsPXY 703 C A E V L A C V K E A ----- V Y V G C A T C V V K A E L P R - A R A V I V K L W R - P A A A A E A A A A P E L T A E V L G V G

SUBSTITUTE SHEET (RULE 26)

CLV-1 934 YPLTSTVHVFKI AMMVEEEAAATITM EVVHMTNP JK - - - - - SVANLI AF -
 PXY 964 LI REEMKQMLR ALLCTSRSPDTDPMPDVLII LGEAKPKRKTVGDVNI VVGVDVNDVN - - - - - FEDVCSVDVGHGDVKQRI GV - DVARI SLN
 PXYL-1 967 HVI EEMLLALR ALLCTAKLPKDRPSI GVI TM AEAKRRKS - - - - - VCGVAGDLP FRNSPVNGLI TAQVI SLD
 PXYL-2 943 YVQEMLLVLQ ALLCTTKLPKDRPSM GVI SM LGEAKRRKSNSNEESTSR - - - - - SLAEKHGKVFSTTTPDSDYANGNEVKLD
 OsPXY 959 HVREEMLLVLR AVLCTARLPDRPSM GVI TM LGEAKRRKSSTGSASAKAPTAPPAAVAAVVDDKQPVFTTTPDSDYANGNEVKLD
 pt t PXY 796 RVAEGVVISI K IAFACLLANPGSPRTM GVI VASELI ARWVPLPKS - - - - - FSAI TVEDLMPQTTVTGT - - - - - AGLVDRLR
 consensus 991
 consensus 1 v r A C R P r r v L I

CLV-1 77 VSFTPLFGTI SPEI GMLTHLVNLTALANFTGELPLEMKSLTSLKVLNI SNNGNLTGTTFPGEI LKAMVDLEVLDTYNNNNFCKLPPEMSE
 PXY 88 LSHRNLSGRI PI QI RYLSLLYLNLSGN LSEGGFPTSI FDLTKLTLTDI SRN - SFDSFFP - PGI SKLKFLKLVNFASNNFEG LPSDVS
 PXYL-1 84 LSNMNLGNSVDQI QSFPSLQALDLSNI AFESSLPKSLNLTSLKVI DSVSN - SFFGTGP - YGLGMATGLTHVNASNNFCS LTPEDLGN
 PXYL-2 78 LAGMNLTKGI SDSI SQLSSLVFSNI SCNGFATLLPKSI P - - - - - PLKSI DI SQN - SFSGSLF - L FSNESLGLVHLNANSGNNLS LTPEDLAN
 OsPXY 79 LSGKNLSGKAVDVRFLPALAVNI SNNAFATLLPKSLPSLKVFDVSN - SFFEGGP - AGLGGCADLVNANSGNNFAC LTPEDLAN
 pt t PXY 35 - - - - - SSATSF GAAKYEAAGN EEA EALLKWRASLDDSHSQSVLSS - - - - - VVGSSPCKVLG L TCDNSGS
 consensus 91

CLV-1 167 LKKLKYSFGGNFSGEIPESYGGI QSLLEYLNGAGLSGKSPAFSLRNLKREMYI GYVNSYTCGV PEFGGTLKLEI LDMASCTLTCE
 PXY 176 LRLFELNFGYFSGEIPESYGGI QRLKFI H AGNVLGGKLPRLGLLTQLHMEI G - YNHNENI PSEFALLSNLYKFDVSNCSLSGS
 PXYL-1 172 ATLEVLDFRGYFSGVPSFKNLKQLKFLG SGNNTGELPSVLGQLPSLETAI L G - YNFGMCEI PEEFGKLTQLQYLDLAVGNLTQ
 PXYL-2 163 LVSLLEVLDFRGYFSGVPSFKNLKFLG SGNNTGELPSVLGQLPSLETAI L G - YNFGMCEI PEEFGKLTQLQYLDLAVGNLTQ
 OsPXY 167 ATLETI DMRCGSFGGAI PAAYRRLTKLFLG SGNNTGKI PPEI GEMESLESII L G - YNELEGGI PPELGNLANQLYLDLAVGNLTQ
 pt t PXY 95 VANFSLPHFG - - - - - LRGTLSHFNFSSFPNLTPNLRN - - - - - N SLYETI PSHI NLTKI TLNLTCHNHFNCS
 consensus 181

CLV-1 257 IPTSLSNKHIHTLFIHI NNLTCIHI PELSGLVSI KSLDLSI NQLTCEI QCSFI NLGNI TLNI NFRNNLYCQIPEAI GELPKIEVFVME
 PXY 265 LQCELGINSNLTFLI FQAGFTCEI PESYNGKI KSLDLSI NQLTCEI QCSFI NLGNI TLNI NFRNNLYCQIPEAI GELPKIEVFVME
 PXYL-1 261 IPTSLSGKISNLTFLI FQAGFTCEI PESYNGKI KSLDLSI NQLTCEI QCSFI NLGNI TLNI NFRNNLYCQIPEAI GELPKIEVFVME
 PXYL-2 252 IPTSLSGKISNLTFLI FQAGFTCEI PESYNGKI KSLDLSI NQLTCEI QCSFI NLGNI TLNI NFRNNLYCQIPEAI GELPKIEVFVME
 OsPXY 256 IPTSLSGKISNLTFLI FQAGFTCEI PESYNGKI KSLDLSI NQLTCEI QCSFI NLGNI TLNI NFRNNLYCQIPEAI GELPKIEVFVME
 pt t PXY 158 LPEMNNITHTLMLVHIFSNFTCHLRDLCLGGLLVNFTASYNHFSGPI KSLRNLCTSLFRVRIDWNLGTNI SEDFGLYPNI NYVDLSH
 consensus 271

CLV-1 347 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 PXY 355 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 PXYL-1 351 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 PXYL-2 342 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 OsPXY 346 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 pt t PXY 248 NNFTLQIPANLGRNGNI KLVDVSNHLTCLIKDLGRGEKEMLI LSNHFFFCPI PEEGLKCKSI TKI RI VKNLLNLTIVAGLFI PLVT
 consensus 361

CLV-1 437 IIEITDFFSGELPVTMSG DVLDDI YLSNNVSGEIPPAI GNFPNLQTLFLDRNRFCNI PREI FELKHLRSINTSANNI TCGI PDSI S
 PXY 445 FVDISNRRFTDQI PADFATAPYQYLNLSITFFHRLKPENI WKAPNLQI FSAFSLNI CEI PNYVG - CKSFYRI ELQGLSNLNTI PDI
 PXYL-1 441 HLEIAGRLNTGKI PDI ALSTLSFI DFNHLS - SLSSSI FSSPNLQTFI ASHNNFACI PQI QDRPSLVLDLSNHFSGS PERI A
 PXYL-2 432 RLEIAGRLSGGI PDI SDSVLSFI DFRNRI RSLPSTI LSI HNLQALVADNFI SCEVDDQDQDCPSLNLDLSSNTLTETI PSSIA
 OsPXY 436 RLEIAGRLSGGI PDI SDSVLSFI DFRNRI RSLPSTI LSI HNLQALVADNFI SCEVDDQDQDCPSLNLDLSSNTLTETI PSSIA
 pt t PXY 338 ALNLSNHLG - - - - - GSI PKQLGECNNLLQLNLNFKI GSI PSEI G
 consensus 451

CLV-1 526 RCTSI SVDISNRI NCEI KGI NNKVLGTLNI SGNLTCEI PTGI GNMTSLTTLDSFNDLSRVLGGGFLVFNETSFAGITYLPL
 PXY 534 HCEKILCLNLISQNLNLNI PCEI STLSPI ADVDSL NLTCTI PSDFGSSKTI TTFNVSYNDI GPI P - SGSAHLNPSFFSSNEGLCD
 PXYL-1 530 SFEKIVSLNLISQNLNLNI PCEI STLSPI ADVDSL NLTCTI PSDFGSSKTI TTFNVSYNDI GPI P - SGSAHLNPSFFSSNEGLCD
 PXYL-2 522 SCORVSLNLNLNLNLNI PCEI STLSPI ADVDSL NLTCTI PSDFGSSKTI TTFNVSYNDI GPI P - SGSAHLNPSFFSSNEGLCD
 OsPXY 526 SCORVSLNLNLNLNLNI PCEI STLSPI ADVDSL NLTCTI PSDFGSSKTI TTFNVSYNDI GPI P - SGSAHLNPSFFSSNEGLCD
 pt t PXY 380 FLHFLGDLISGILLAGEISEIGQLKQLETMNLNHNKLSGELITAFVDLVSLTVDI SYNELEGP KGI KGFI EAPLEAFNMNLSGCGN
 consensus 541

CLV-1 616 HRVSCPTRPGQSDHNHTALFSPRSI VITI AAI TGLI - - - - - LI SVAI - - - - - RQMKNKKKQKSLAWKLTAFQKLDKF
 PXY 623 LVGKPCNSDRFNAGNADI DGHKKEERPKATAGI VWI L - AAAGI GVGFVLVAATRCFQKSYGNRVDDGGGRNGDI GPWKLTAQRLNLT
 PXYL-1 612 VLPCCSKSLASAKGRNPRI HYNHVAHGI VGTSVI V - AMGMFF - LAGRWI YTRWDLYSNFAREYI FCKPREEVPRLVAFQRLCFT
 PXYL-2 620 VLPCCSKSRATRS - - - - - SHSLHGRI VAGALI GI ASVL - ALGI LT - I VTRTLKWKYSN - - - - - GFCGDDETAS - - - - - KGEVMPRLMAHRLGFT
 OsPXY 616 VLPCCSKSRATRSAGPRSGARLRI AVGLVGMVAVV - AFAAAL - FGHYAYRRWYVDGAGCGDDEANLGGESGAPWRLTAQRLGFT
 pt t PXY 470 ANGLKPTCLTISRKSNKI VI LI LFLFLGSLLLLI NVGCLYFH - - - - - HGTSRERI SCLGERGSPLSFVVMGHEEII
 consensus 631

CLV-1 685 SEDVLECLKEE - - - - - NITCAGGAGI VVRGSPN - - - - - NVDVNI KRLVG - - - - - RGTGRSDHG - - - - - FTAHI QTLGRI RHNI VRLLCYVA
 PXY 711 ADDVLECLSKTD - - - - - NITCAGGAGI VVRGSPN - - - - - NVDVNI KRLVG - - - - - RGTGRSDHG - - - - - FTAHI QTLGRI RHNI VRLLCYVA
 PXYL-1 707 AGDI LSI KES - - - - - NITCAGGAGI VVRGSPN - - - - - NVDVNI KRLVG - - - - - RGTGRSDHG - - - - - FTAHI QTLGRI RHNI VRLLCYVA
 PXYL-2 692 ASVI LACI KES - - - - - NITCAGGAGI VVRGSPN - - - - - NVDVNI KRLVG - - - - - RGTGRSDHG - - - - - FTAHI QTLGRI RHNI VRLLCYVA
 OsPXY 703 CADELACI KEA - - - - - NITCAGGAGI VVRGSPN - - - - - NVDVNI KRLVG - - - - - RGTGRSDHG - - - - - FTAHI QTLGRI RHNI VRLLCYVA
 pt t PXY 543 LHETI QAANNFNFNICIGEGYI VWRMLPT - - - - - GGVVAKKFFP - - - - - SRDGLMNLR - - - - - TFRNEI RMI DI RHNI VRLLCYVA
 consensus 721

CLV-1 758 NKDTNLLLYEYMPNCS GELLHGS - - - - - KGGHLQETRRHVAVEAAKGLV LHHNLSPLI LHHVKSNNI LLDSDFEARVAHTEAKFLVD
 PXY 790 NRDCITMLLYEYMPNCS DDLHGGDKMTAAEATLYALQI GVAQGI CYLHDCDPPVI IHRD KPSNI LLDADFEARVAHTEAKFLVD
 PXYL-1 793 NDERVMVVEYMPNCS GELLHGS - - - - - KGGHLQETRRHVAVEAAKGLV LHHNLSPLI LHHVKSNNI LLDSDFEARVAHTEAKFLVD
 PXYL-2 796 NRKNNI VEFMLNENI GDAL HGKNAAGRLVLDV LSRVNI ALGVAHGLV LHHNLSPLI LHHVKSNNI LLDSDFEARVAHTEAKFLVD
 OsPXY 785 NEADAMMLVEYMPNCS MEALHGP - PERRTLVDV LSRVNYAVAGVAGGLV LHHNLSPLI LHHVKSNNI LLDSDFEARVAHTEAKFLVD
 pt t PXY 622 LI EHSFLVVEI ERCS KMLLSSE - - - - - EQVMOLDV NRRNLNVKGVASALSYLHDCDPPVI IHRD SSSVNLDDSEYAHVSDGFTARLLMP
 consensus 811

CLV-1 845 GAASECMSSI ACSYGYI APEYAVILKVDEKSI SVSYFGVLLSLI AKCKPVG - FEGGEDI VRVWRNTEEEI TQPSDAAI VVAI VDPRLTG
 PXY 880 - - - - - DESMSVYAGSYGYI APEYAVILKVDEKSI SVSYFGVLLSLI AKCKPVG - FEGGEDI VRVWRNTEEEI TQPSDAAI VVAI VDPRLTG
 PXYL-1 882 K - - - - - NETVSMVYAGSYGYI APEYAVILKVDEKSI SVSYFGVLLSLI AKCKPVG - FEGGEDI VRVWRNTEEEI TQPSDAAI VVAI VDPRLTG
 PXYL-2 859 K

Fig. 18(cont.)

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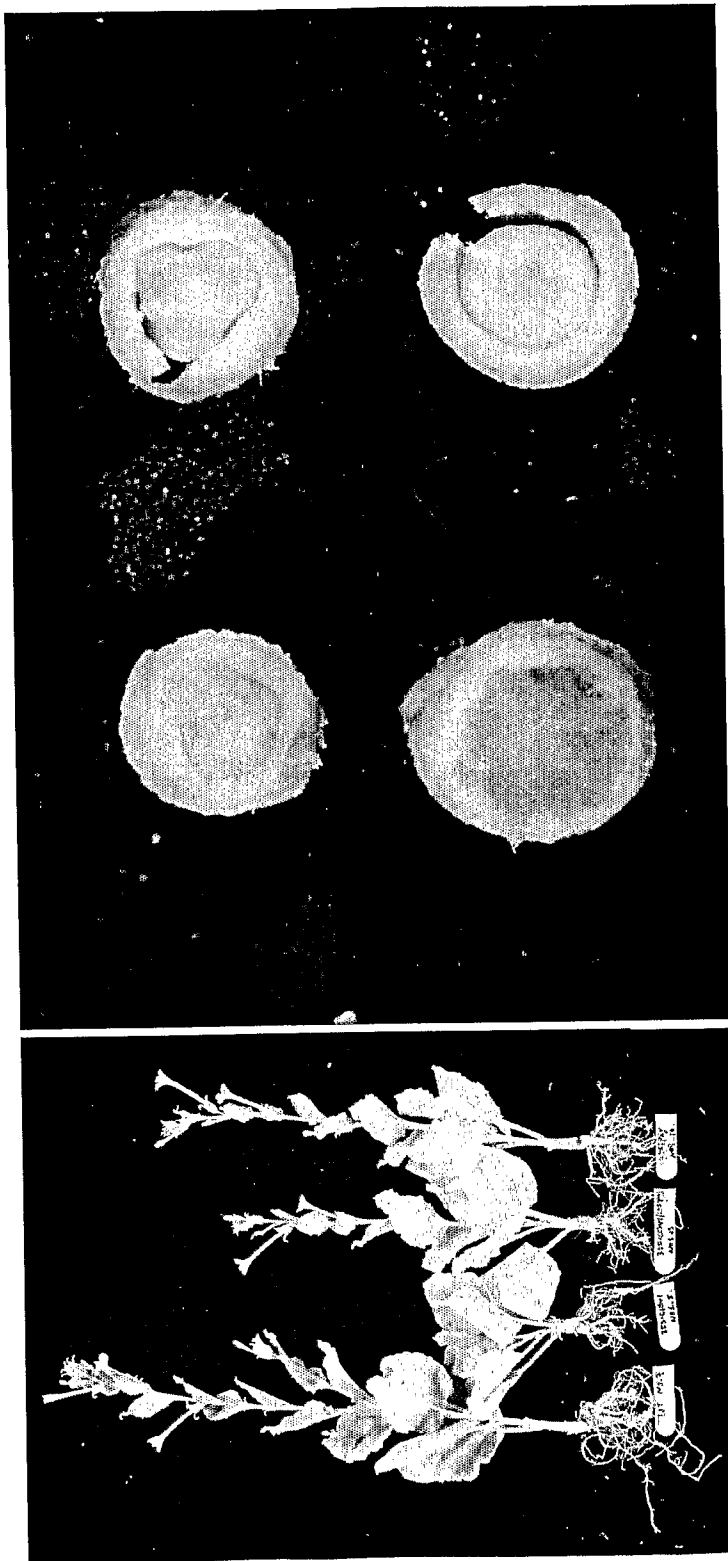


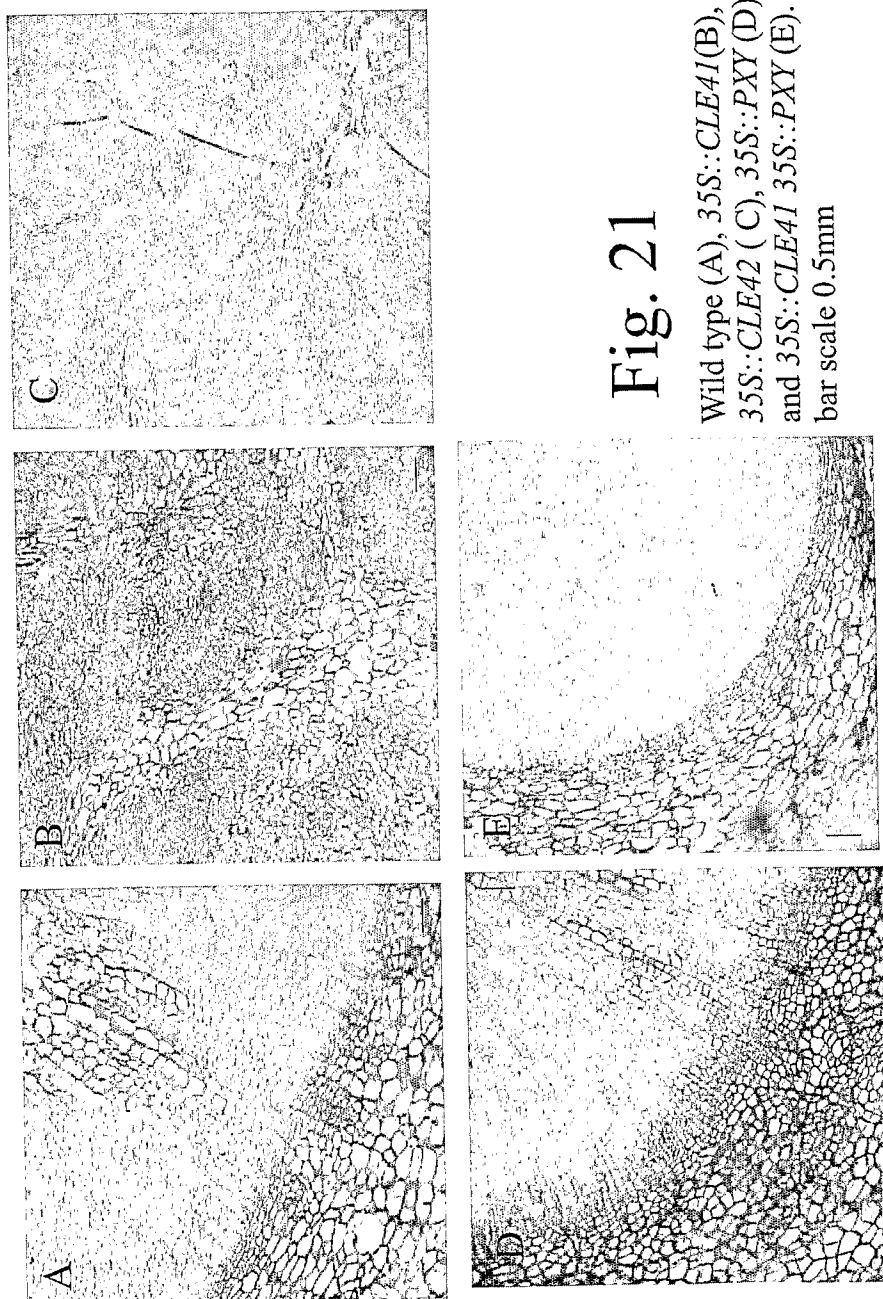
Fig. 19

Wild type, 35S::CLE41, 35S::CLE41 35S::PXY, 35S::PXY plant (LHS-RHS) at 50 days

Fig. 20

Hypocotyls cross section 35S::CLE41 35S::PXY (A), 35S::CLE41 (B), 35S::PXY (C), Wild type (D) section

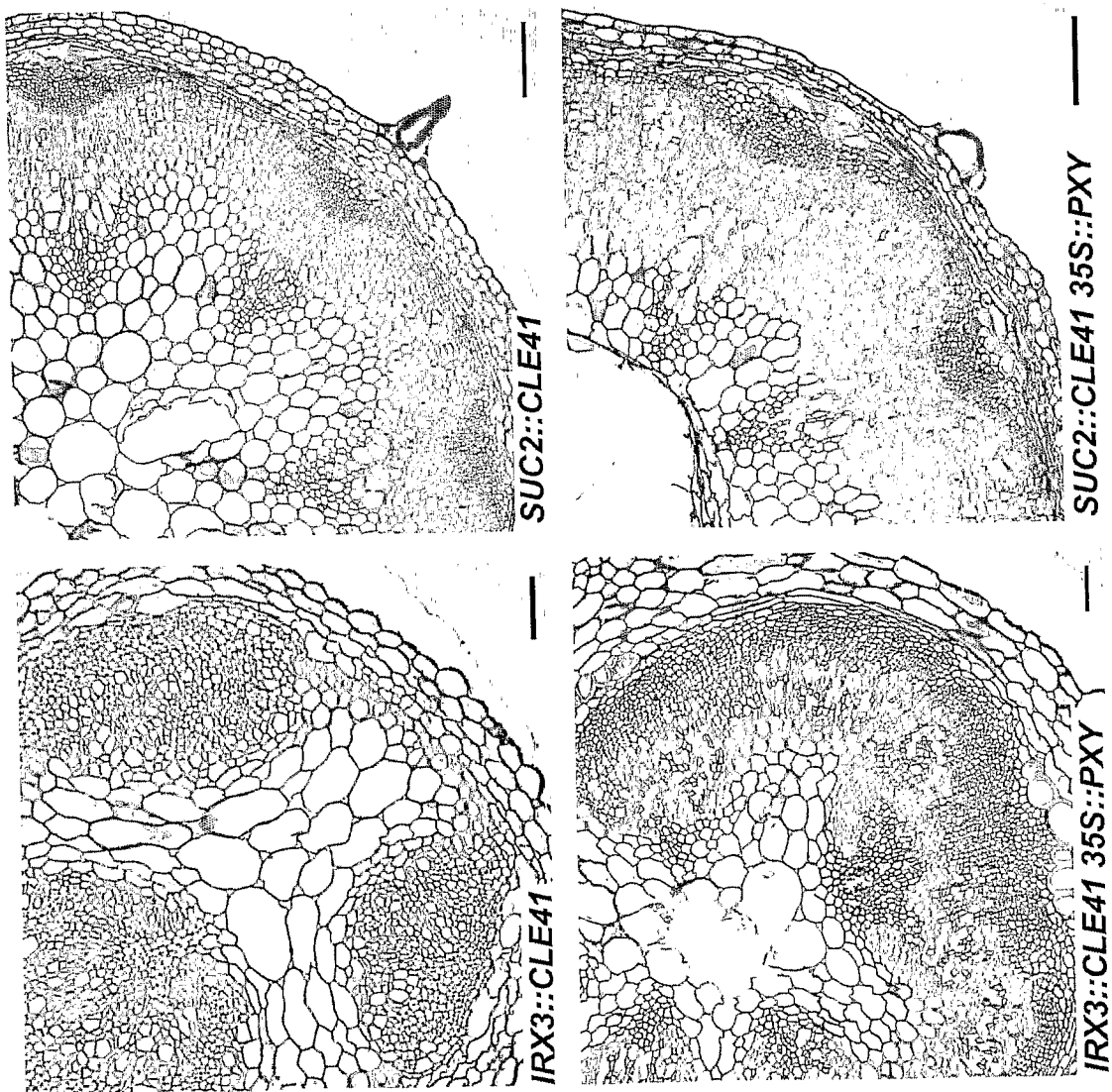
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**Fig. 21**

Wild type (A), 35S::CLE41(B),
35S::CLE42 (C), 35S::PXY (D)
and 35S::CLE41 35S::PXY (E).
bar scale 0.5mm

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Fig. 22



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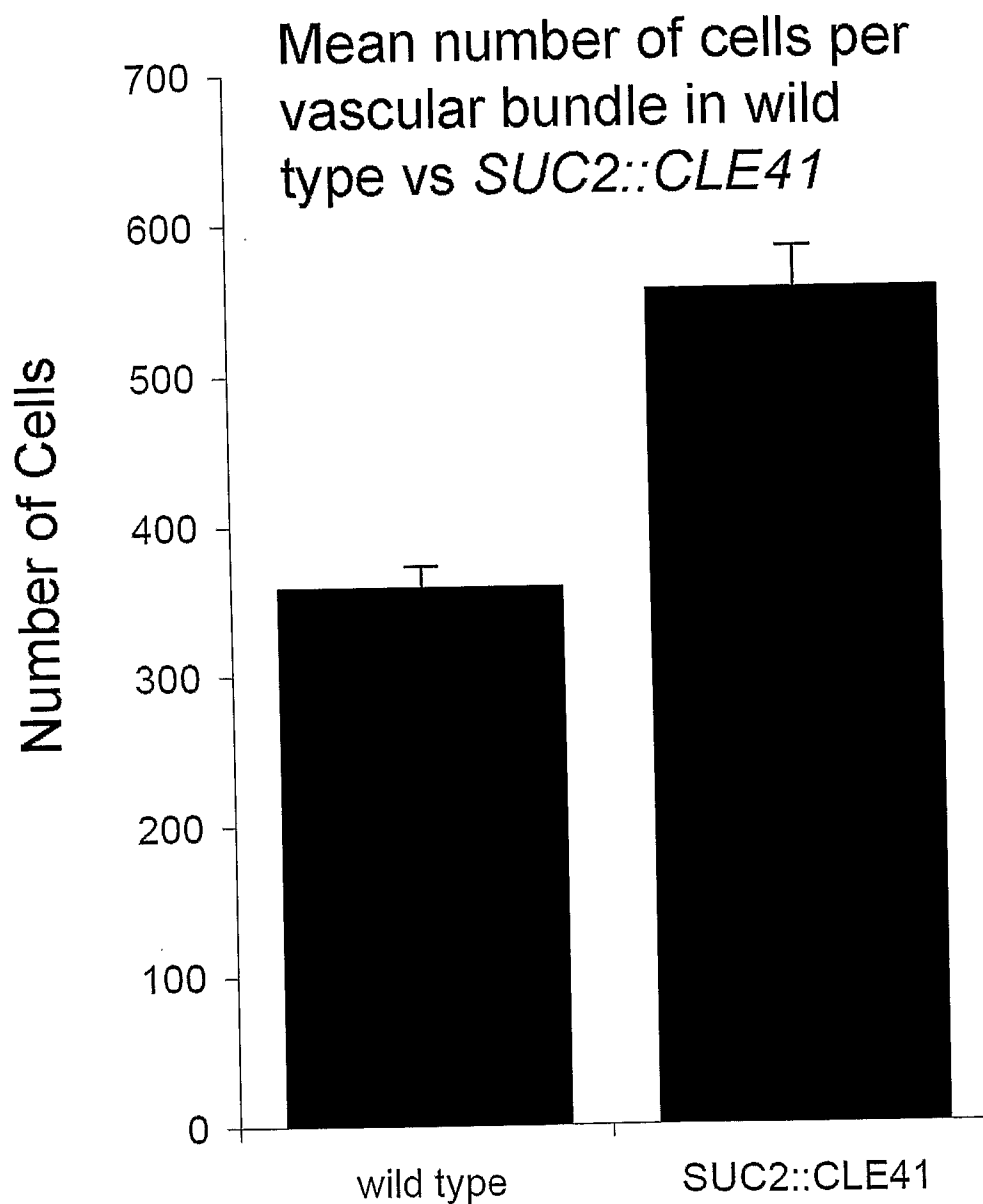


Fig. 23

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Hypocotyl Diameter at Senescence

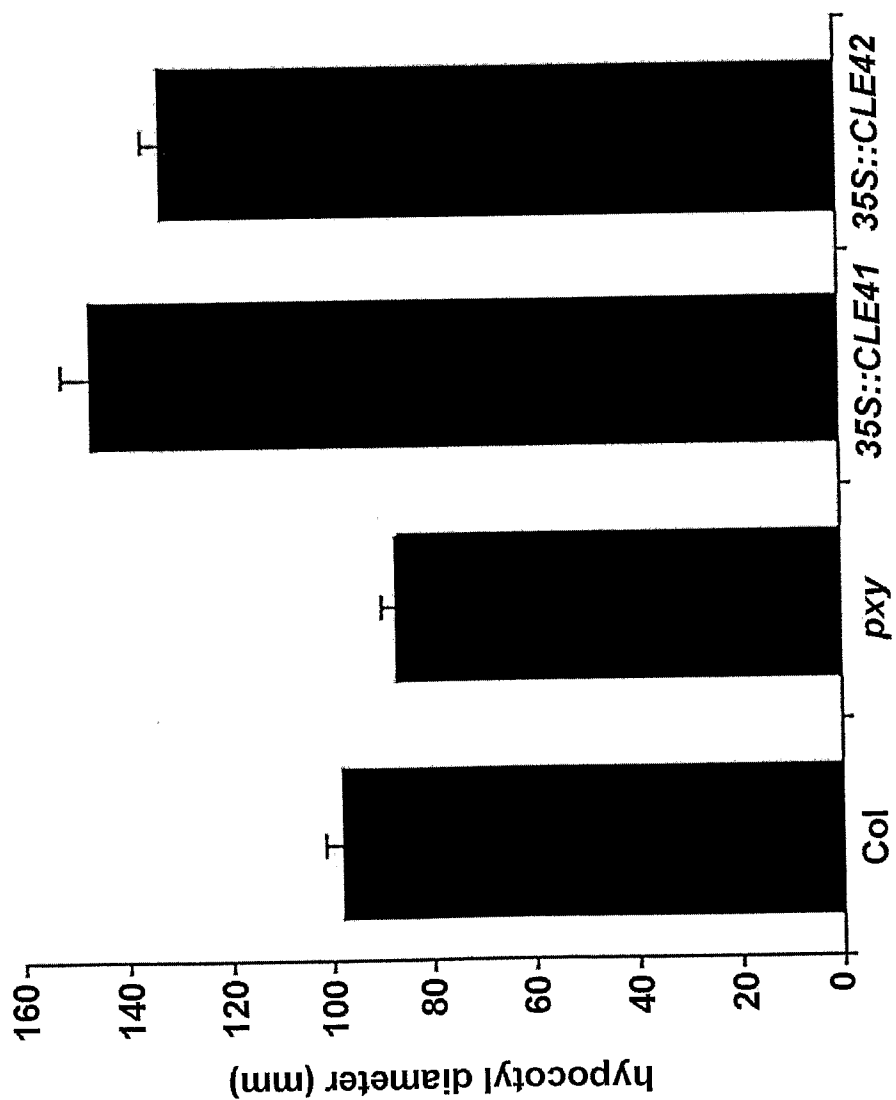


Fig. 24

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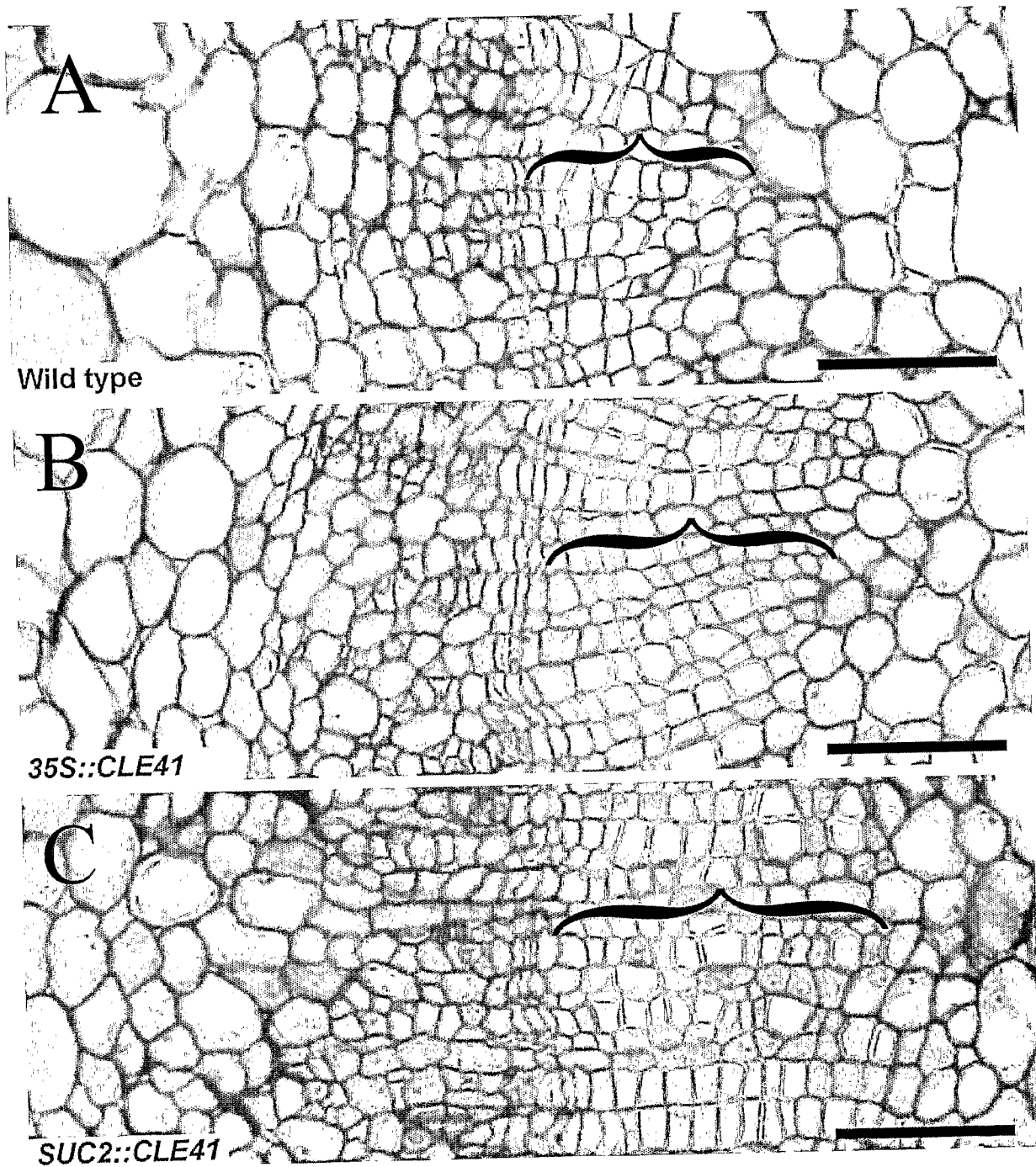


Fig. 25