(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
**12 September 2024 (12.09.2024)**

(10) **International Publication Number**
**WO 2024/184443 A1**

(51) **International Patent Classification:**
*C07K 14/415* (2006.01) *C12N 15/82* (2006.01)

(21) **International Application Number:** PCT/EP2024/055957

(22) **International Filing Date:** 07 March 2024 (07.03.2024)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
23160362.2 07 March 2023 (07.03.2023) EP
23160385.3 07 March 2023 (07.03.2023) EP

(71) **Applicants: VIB VZW** [BE/BE]; Suzanne Tassierstraat 1, 9052 Gent (BE). **UNIVERSITEIT GENT** [BE/BE]; Sint-Pietersnieuwstraat 25, 9000 Gent (BE).

(72) **Inventors: DE RYBEL, Bert**; c/o VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Gent (BE). **GOOSSENS, Alain**; c/o VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Gent (BE). **YANG, Baojun**; c/o VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Gent (BE). **SUN, Yanbiao**; c/o VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Gent (BE). **NTELKIS, Nikolaos**; c/o VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Gent (BE).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** MEANS AND METHODS FOR INCREASING THE ROOT SIZE OF CROPS

(57) **Abstract:** The present invention relates to the field of agriculture, even more particularly to the field of increasing the root length and root biomass of plants including also hairy root biomass. The present invention provides chimeric genes and constructs which can be used to increase the root size of crops, to increase the yield of crops and to increase the biomass of hairy roots.

WO 2024/184443 A1

# MEANS AND METHODS FOR INCREASING THE ROOT SIZE OF CROPS

Abstract

The present invention relates to the field of plant molecular biology, more particularly to the field of agriculture, even more particularly to the field of increasing the root length and root biomass of plants including also hairy root biomass. The present invention provides chimeric genes and constructs which can be used to increase the root size of crops, to increase the yield of crops and to increase the biomass of hairy roots.

## Introduction to the invention

In agriculture, soil compaction is a complex problem in which soil, crops, weather, and machinery interact. External pressure due to the use of heavy machinery and inappropriate soil management can lead to the compaction of subsoil, creating impermeable layers within the soil that restrict water and nutrient cycles. This process causes on-site effects such as reduced crop growth, yield and quality. There are no known plant traits to remediate soil compaction.

A chimeric gene consisting of the *Arabidopsis* meristematic promoter (for example the RPS5A promoter) or a chimeric gene consisting of a root specific promoter operably linked to a gene encoding an SPL transcription factor, introduced in *Arabidopsis* or corn surprisingly leads to thicker and longer roots (>100%) – such roots are better adopted for penetrating compacted soils.

Downregulation of SPL transcription factors (via overexpression of miR156 (Aung B *et al* (2015) *Plant Biotech. J.* 13, 779-790) leads to abnormal phenotypes in plants. Transgenic plants overexpressing SPL transcription factors show delays in the production of vegetative leaves (Martin RD *et al* (2010) *Seed Sc. Res.* 20, 79-87).

The squamosa promoter binding protein-like (SPL) gene family are transcription factors that affect the expression of downstream genes and result in the regulation of a large plant growth and development network. Indeed, a number of traits, including emergence of vegetative leaves, shoot branching, floral transition, fertility, biomass production and grain yield are modulated. Our chimeric gene of the invention, upon expression, leads to a spectacular root phenotype which has not been documented in the prior art.

Figures

Figure 1: Overexpression of AtSPL13 induced primary root growth. **A**: Primary root length of Col-0 and p*AtRPS5A*::SPL13-GFP (8 Independent line) grown on 1/2MS medium for 11 days. **B**: Primary root length was quantified (n=12,7,11,11,9,8,8,8,9). **C**: Transcript level of *SPL13* transcripts in Col-0 and 8 p*AtRPS5A*::SPL13-GFP lines were detected by qRT-PCR in Primary root (n=3).

Figure 2**:** **A**: Cross section of Col-0 and p*RPS5A*:AtSPL13-GFP (two independent lines, Line 6 with weaker expression of AtSPL13, Line 12 with stronger expression AtSPL13) in Day12 after germination. Both overexpression lines have two endodermis layers were marked by bule line. **B**: Cell numbers were quantified in different cell types. (n=3,3,3). **C**: Root meristem size of Col-0 and RPS5A:AtSPL13-GFP were marked by red arrow.

Figure 3: Phenotype of the mature overexpression lines using the p*At*RPS5A promoter. **A**: Phenotype of Col-0 and p*At*RPS5A::*At*SPL13-GFP grown in soil for 25 days. Overexpression lines with fewer rosette leaves **B**: long florescent stem of p*At*RPS5A::*At*SPL13-GFP due to flowering period being extended by about one month.

Figure 4: Phenotypes of chimeric genes comprising several orthologous *At*SPL proteins under control of the p*At*RPS5A promoter. Overexpression (in a chimeric gene construct with the pAtRPS5A promoter) of AtSPL2, AtSPL3 leads to slightly thicker roots, overexpression of AtSPL6, SPL11 and SPL15 have an intermediate effect, while specifically overexpression of SPL9 and SPL10 have a strong effect.

Figure 5: Motif search based on the SPL full-length protein sequences of several plants (Arabidopsis, maize, rice, wheat, poplar, tomato, potato, soybean and tobacco). Conserved motifs are indicated by numbered coloured boxes. Conserved motif study of the *Arabidopsis* SPL proteins was identified using online MEME (Multiple Expectation Maximization for Motif Elicitation)5.4.1 with default settings; the minimum width is 6, the maximum width is 50, and the maximum number of motifs to find is 8.

Figure 6: The phenotype of three independent transformed corn lines harboring the chimeric gene (SEQ ID NO: 24 operably coupled to SEQ ID NO: 13) as compared to the B104 non-transformed line is shown in Figure 6. Figure 6A shows the plants at day 6 after transformation. Root length, root width and the number of vascular cell lines are compared between the non-transformed line and the transformed lines. Figure 6B shows the plants at day 60. There is a striking formation of strong crown roots in the transformed corn lines. Figure 6C shows the plants at day 114. A reduced plant height of the transformed corn lines is observed but with a strong abundance of crown roots as compared to the non-transformed B104 line.

Figure 7: A significant (p-value 4.8E-15; n>50) increase in the root meristem width was observed in tomato hairy root meristems comprising the chimeric gene of the invention.

Figure 8. Effect of SPL13 overexpression on tomato hairy root biomass. Independent transformed lines (n=5) of tomato hairy roots with Empty Vector (control), pRPS5A:SPL13 and pRolD:SPL13 were grown for 10 days in three independent experiments. The biomass (fresh weight) was determined for each T-DNA line (n=18).

Figure 9. Transcript expression of SPL13 on tomato hairy roots. Independent transformed lines (n=5) of tomato hairy roots with Empty Vector (control), pRPS5A:SPL13 and pRolD:SPL13 were grown for 10 days. Transcripts levels of SPL13 were detected by RT-qPCR (n=3).

## Detailed description of the invention

Throughout the specification and claims, the following terms take the meanings explicitly associated herein, unless the context clearly dictates otherwise. The phrase "in one embodiment" as used herein does not necessarily refer to the same embodiment, though it may. Furthermore, the phrase "in another embodiment" as used herein does not necessarily refer to a different embodiment, although it may. Thus, as described below, various embodiments of the invention may be readily combined, without departing from the scope or spirit of the invention. To facilitate the understanding of this invention a number of terms are defined below. Terms defined herein (unless otherwise specified) have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. As used in this specification and its appended claims, terms such as "a", "an" and "the" are not intended to refer to only a singular entity but include the general class of which a specific example may be used for illustration, unless the context dictates otherwise. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

To facilitate an understanding of the present technology, a number of terms and phrases are defined below. Additional definitions are set forth throughout the detailed description.

The present invention provides a chimeric gene comprising a plant specific promoter selected from a plant meristem specific promoter or a plant root specific promoter operably coupled to the nucleotide sequence encoding for a plant SPL protein wherein said plant SPL protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1),

SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), and a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.

In another embodiment the invention provides a chimeric gene construct comprising the following operably linked DNA elements: a) a plant specificpromoter selected from a plant meristem specific promoter or a plant root specific promoter, b) a DNA region encoding a plant SPL protein wherein said plant protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1), SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.

In a specific embodiment the plant meristem specific promoter is the *Arabidopsis* ribosomal meristem specific promoter pAtRPS5A (depicted in SEQ ID NO: 4). The AtRPS5A promoter is strongly active in the division zone of the primary root tip, and the shoot apical meristem (SAM) and in leaf primordia. In another specific embodiment the root specific promoter is the *Oryza sativa* RCc3 promoter (depicted in SEQ ID NO: 24). The RCc3 promoter is described in Ramireddy E. *et al* (2021), *Plant Molecular Biology*, Volume 106, pp. 555-567.

In yet another embodiment the invention provides a recombinant vector comprising the chimeric genes as defined herein.

In yet another embodiment the invention provides a plant cell comprising the chimeric genes as defined herein or a recombinant vector as defined herein.

In yet another embodiment the invention provides the use of a chimeric gene as defined herein or a recombinant vector as defined herein to increase the root length of plants.

In yet another embodiment the invention provides the use of a chimeric gene as defined herein or a recombinant vector as defined herein to increase the yield of plants.

For the identification of functionally equivalent plant meristem specific promoters (for example in other plant genera or other plant species), the promoter strength and/or expression pattern of a candidate meristem specific promoter may be analysed for example by operably linking the promoter to a reporter gene and assaying the expression level and pattern of the reporter gene in the plant. Suitable well-known reporter genes include for example beta-glucuronidase; beta-galactosidase or any fluorescent protein. The promoter activity is assayed by measuring the enzymatic activity of the beta-glucuronidase or beta-galactosidase. Alternatively, promoter strength may also be assayed by quantifying mRNA levels or by comparing mRNA levels of the nucleic acid, with mRNA levels of housekeeping genes such as 18S rRNA,

using methods known in the art, such as Northern blotting with densitometric analysis of autoradiograms, quantitative real-time PCR or RT- PCR (Heid et al., 1996 Genome Methods 6: 986-994).

The term "operably linked" as used herein refers to a functional linkage between the promoter sequence (here the meristem specific promoter) and the gene of interest (here the SPL gene or an orthologous homologue thereof as defined herein above), such that the meristem promoter sequence is able to initiate transcription of the SPL gene (or an orthologous homologue thereof) of interest.

A “chimeric gene” or "chimeric construct" is a recombinant nucleic acid sequence in which a promoter or regulatory nucleic acid sequence is operatively linked to, or associated with, a nucleic acid sequence that codes for an mRNA, such that the promoter or regulatory nucleic acid sequence is able to regulate transcription or expression of the associated nucleic acid coding sequence. The promoter or regulatory nucleic acid sequence of the chimeric gene is not normally operatively linked to the associated nucleic acid sequence as found in nature.

The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. The terminator can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The terminator to be added may be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

The term "hairy root yield" as used herein generally refers to a measurable product from a plant, particularly a hairy root from a plant. The terms "improved hairy root yield" or "increased hairy root yield" can be used interchangeable. For example, the improvement in hairy root yield can comprise a 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater increase in any measured parameter.

"Selectable marker", "selectable marker gene" or "reporter gene" includes any gene that confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells that are transfected or transformed with a nucleic acid construct of the invention. These marker genes enable the identification of a successful transfer of the nucleic acid molecules via a series of different principles. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as nptll that phosphorylates neomycin and kanamycin, or hpt, phosphorylating hygromycin, or genes conferring resistance to, for example, bleomycin, streptomycin, tetracyclin, chloramphenicol, ampicillin, gentamycin, geneticin (G418),

spectinomycin or blasticidin), to herbicides (for example bar which provides resistance to Basta®; aroA or gox providing resistance against glyphosate, or the genes conferring resistance to, for example, imidazolinone, phosphinothricin or sulfonylurea), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source or xylose isomerase for the utilisation of xylose,
5 or antinutritive markers such as the resistance to 2-deoxyglucose). Expression of visual marker genes results in the formation of colour (for example β-glucuronidase, GUS or β- galactosidase with its coloured substrates, for example X-Gal), luminescence (such as the luciferin/luciferase system) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). This list represents only a small number of possible markers. The skilled worker is familiar with such markers. Different markers are preferred,
10 depending on the plant and the selection method.

It is known that upon stable or transient integration of nucleic acids into plant cells, only a minority of the cells takes up the foreign DNA and, if desired, integrates it into its genome, depending on the expression vector used and the transfection technique used. To identify and select these integrants, a gene coding for a selectable marker (such as the ones described above) is usually introduced into the
15 host cells together with the gene of interest. These markers can for example be used in mutants in which these genes are not functional by, for example, deletion by conventional methods. Furthermore, nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector that comprises the sequence encoding the polypeptides of the invention or used in the methods of the invention, or else in a separate vector. Cells which have been stably transfected with the introduced
20 nucleic acid can be identified for example by selection (for example, cells which have integrated the selectable marker survive whereas the other cells die).

For the purposes of the invention, "transgenic", "transgene" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes
25 or vectors according to the invention.

A transgenic plant or a transgenic hairy root for the purposes of the invention is thus understood as meaning, as above, that the nucleic acids used in the method of the invention are not present in, or originating from, the genome of said plant, or are present in the genome of said plant but not at their natural locus in the genome of said plant, it being possible for the nucleic acids to be expressed
30 homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acids according to the invention or used in the inventive method are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that

the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, heterologous expression of the nucleic acids takes place. Preferred transgenic plants or transgenic hairy roots are mentioned herein.

5 For the purpose of this invention related or orthologous genes of the SPL gene as described herein before can be isolated from the (publicly) available sequence databases. The "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e., a position in an alignment where a residue is present in one sequence but not in 10 the other is regarded as a position with non-identical residues. The alignment of the two sequences is performed by the Needleman and Wunsch algorithm (Needleman and Wunsch (1970) *J Mol Biol.* 48: 443-453) The computer-assisted sequence alignment above, can be conveniently performed using standard software program such as GAP which is part of the Wisconsin Package Version 10.1 (Genetics Computer Group, Madison, Wisconsin, USA) using the default scoring matrix with a gap creation penalty 15 of 50 and a gap extension penalty of 3. Sequences are indicated as “essentially similar” when such sequence have a sequence identity of at least about 75%, particularly at least about 80 %, more particularly at least about 85%, quite particularly about 90%, especially about 95%, more especially about 100%, quite especially are identical.

Alternatively,the skilled person can isolate orthologous plant SPL genes through methods of genetic 20 hybridization. Such methods are well known to the skilled (plant) molecular biologist.

The term "expression" or "gene expression" means the transcription of a specific gene or specific genes or specific genetic construct. The term "expression" or "gene expression" in particular means the transcription of a gene or genes or genetic construct into structural RNA (rRNA, tRNA) or mRNA with or without subsequent translation of the latter into a protein. The process includes transcription of DNA 25 and processing of the resulting mRNA product.

The term "introduction" or "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. 30 The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue

(e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell may then be used to regenerate a
5 transformed plant in a manner known to a person skilled in the art.

The transfer of foreign genes into the genome of a plant is called transformation. Transformation of plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for
10 transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., (1982) Nature 296, 72-74; Negrutiu I et al. (1987) Plant Mol Biol 8: 363- 373); electroporation of protoplasts
15 (Shillito R.D. et al. (1985) Bio/Technol 3, 1099-1 102); microinjection into plant material (Crossway A et al., (1986) Mol. Gen Genet 202: 179-185); DNA or RNA-coated particle bombardment (Klein TM et al., (1987) Nature 327: 70) infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via Agrobacterium-mediated transformation. An advantageous transformation method is the transformation in planta. To this end, it is possible, for
20 example, to allow the agrobacteria to act on plant seeds or to inoculate the plant meristem with agrobacteria. It has proved particularly expedient in accordance with the invention to allow a suspension of transformed agrobacteria to act on the intact plant or at least on the flower primordia. The plant is subsequently grown on until the seeds of the treated plant are obtained (Clough and Bent, Plant J. (1998) 16, 735-743). Methods for Agrobacterium-mediated transformation of rice include well known methods
25 for rice transformation, such as those described in any of the following: European patent application EP1198985, Aldemita and Hodges (Planta 199: 612-617, 1996); Chan et al. (Plant Mol Biol 22 (3): 491 - 506, 1993), Hiei et al. (Plant J 6 (2): 271 -282, 1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol 14(6): 745-50, 1996) or Frame et al. (Plant Physiol 129(1): 13-22,
30 2002), which disclosures are incorporated by reference herein as if fully set forth. Said methods are further described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1 , Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The nucleic acids or the construct to be expressed is preferably cloned into a vector, which is suitable for transforming

Agrobacterium tumefaciens, for example pBin19 (Bevan et al (1984) *Nucl. Acids Res.* 12-8711). Agrobacteria transformed by such a vector can then be used in known manner for the transformation of plants, such as plants used as a model, like Arabidopsis (Arabidopsis thaliana is within the scope of the present invention not considered as a crop plant), or crop plants such as, by way of example, tobacco plants, for example by immersing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. The transformation of plants by means of *Agrobacterium tumefaciens* is described, for example, by Hofgen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1 , Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

In addition to the transformation of somatic cells, which then have to be regenerated into intact plants, it is also possible to transform the cells of plant meristems and in particular those cells which develop into gametes. In this case, the transformed gametes follow the natural plant development, giving rise to transgenic plants. Thus, for example, seeds of Arabidopsis are treated with agrobacteria and seeds are obtained from the developing plants of which a certain proportion is transformed and thus transgenic [Feldman, KA and Marks MD (1987). Mol Gen Genet 208:1 -9; Feldmann K (1992). In: C Koncz, N-H Chua and J Shell, eds, Methods in Arabidopsis Research. Word Scientific, Singapore, pp. 274-289]. Alternative methods are based on the repeated removal of the inflorescences and incubation of the excision site in the center of the rosette with transformed agrobacteria, whereby transformed seeds can likewise be obtained at a later point in time (Chang (1994). Plant J. 5: 551 -558; Katavic (1994). Mol Gen Genet, 245: 363-370). However, an especially effective method is the vacuum infiltration method with its modifications such as the "floral dip" method. In the case of vacuum infiltration of *Arabidopsis*, intact plants under reduced pressure are treated with an agrobacterial suspension [Bechthold, N (1993). CR Acad Sci Paris Life Sci, 316: 1 194-1 199], while in the case of the "floral dip" method the developing floral tissue is incubated briefly with a surfactant-treated agrobacterial suspension [Clough, SJ and Bent AF (1998) The Plant J. 16, 735-743]. A certain proportion of transgenic seeds are harvested in both cases, and these seeds can be distinguished from non-transgenic seeds by growing under the above-described selective conditions. In addition, the stable transformation of plastids is of advantages because plastids are inherited maternally is most crops reducing or eliminating the risk of transgene flow through pollen. The transformation of the chloroplast genome is generally achieved by a process which has been schematically displayed in Klaus et al., 2004 [Nature Biotechnology 22 (2), 225-229]. Briefly the sequences to be transformed are cloned together with a selectable marker gene between flanking sequences homologous to the chloroplast genome. These homologous flanking sequences direct site-specific integration into the plastome. Plastidal transformation has been described for many different plant species and an overview is given in Bock (2001) Transgenic plastids in basic research and plant

biotechnology. J Mol Biol. 2001 Sep 21; 312 (3):425-38 or Maliga, P (2003) Progress towards commercialization of plastid transformation technology. Trends Biotechnol. 21 , 20-28. Further biotechnological progress has recently been reported in form of marker free plastid transformants, which can be produced by a transient co-integrated maker gene (Klaus et al., 2004, Nature Biotechnology 5 22(2), 225-229).

The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Hofgen and Willmitzer.

Generally, after transformation, plant cells or cell groupings are selected for the presence of one or more 10 markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant. To select transformed plants, the plant material obtained in the transformation is, as a rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected 15 to a suitable selection by spraying. A further possibility consists in growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker such as the ones described above.

Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for 20 instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively, or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal 25 propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2) transformants selected, and the T2 plants may then further be propagated through classical breeding techniques. The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); 30 grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The terms "increase", "improve" or "enhance" are interchangeable and shall mean in the sense of the application at least a 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%, preferably at least 15% or 20%, more preferably 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more yield and/or growth in comparison to control plants as defined herein.

5 The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, leaves, roots (including tubers), flowers, and tissues and organs, wherein each of the aforementioned comprise the gene/nucleic acid of interest. The term "plant" also encompasses plant cells, suspension cultures, callus tissue, hairy root culture, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the 10 aforementioned comprises the gene/nucleic acid of interest.

"Plant" as used herein refers to vascular plants (e.g. gymnosperms and angiosperms). A "transgenic plant" refers to a plant comprising a recombinant polynucleotide and/or a recombinant polypeptide according to the specification. A transgenic plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, and progeny thereof. A 15 transgenic plant can be obtained by transforming a plant cell with an expression cassette of the present specification and regenerating such plant cell into a transgenic plant. Such plants can be propagated vegetatively or reproductively. The transforming step may be carried out by any suitable means, including by *Agrobacterium*-mediated transformation and non-*Agrobacterium*-mediated transformation, as discussed in detail below. Plants can be regenerated from the transformed cell (or 20 cells) by techniques known to those skilled in the art. Where chimeric plants are produced by the process, plants in which all cells are transformed may be regenerated from chimeric plants having transformed germ cells, as is known in the art. Methods that can be used to transform plant cells or tissue with expression vectors of the present specification include both *Agrobacterium* and non-*Agrobacterium* vectors. *Agrobacterium*-mediated gene transfer exploits the natural ability of 25 *Agrobacterium tumefaciens* to transfer DNA into plant chromosomes and is described in detail in Gheysen, G., Angenon, G. and Van Montagu, M. 1998. *Agrobacterium*-mediated plant transformation: a scientifically intriguing story with significant applications. In K. Lindsey (Ed.), Transgenic Plant Research. Harwood Academic Publishers, Amsterdam, pp. 1-33 and in Stafford, H.A. (2000) Botanical Review 66: 99-118. A second group of transformation methods is the non-Agrobacterium mediated transformation 30 and these methods are known as direct gene transfer methods. An overview is brought by Barcelo, P. and Lazzeri, P.A. (1998) Direct gene transfer: chemical, electrical and physical methods. In K. Lindsey (Ed.), Transgenic Plant Research, Harwood Academic Publishers, Amsterdam, pp.35-55.

Methods include particle gun delivery, microinjection, electroporation of intact cells, polyethyleneglycol mediated protoplast transformation, electroporation of protoplasts, liposome-mediated transformation, silicon-whiskers mediated transformation etc.

Any plant tissue or plant cells capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a construct of the present specification. The term 'organogenesis' means a process by which shoots and roots are developed sequentially from meristematic centers; the term 'embryogenesis' means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include protoplasts, leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g. apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyls meristem).

A "control plant" as used herein refers to a plant cell, seed, plant component, plant tissue, plant organ or whole plant used to compare against transgenic or genetically modified plant for the purpose of identifying a difference in production of secondary metabolite (as described further herein) in the transgenic or genetically modified plant. A control plant may in some cases be a transgenic plant line that comprises an empty vector or marker gene, but does not contain the recombinant polynucleotide of the present specification that is expressed in the transgenic or genetically modified plant being evaluated. In general, a control plant is a plant of the same line or variety as the transgenic or genetically modified plant being tested. A suitable control plant would include a genetically unaltered or non-transgenic plant of the parental line (wild type) used to generate a transgenic plant herein.

Plants of the present specification may include, but not limited to, plants or plant cells of agronomically important crops which are or are not intended for animal or human nutrition, such as maize or corn, wheat, barley, oat, Brassica spp. plants such as Brassica napus or Brassica juncea, soybean, bean, alfalfa, pea, rice, sugarcane, beetroot, tobacco, sunflower, quinoa, cotton, Arabidopsis, vegetable plants such as cucumber, leek, carrot, tomato, lettuce, peppers, melon, watermelon, diverse herbs such as oregano, basil and mint.

The choice of suitable control plants is a routine part of an experimental setup and may include corresponding wild type plants or corresponding plants without the gene of interest. The control plant is typically of the same plant species or even of the same variety as the plant to be assessed. The control plant may also be a nullizygote of the plant to be assessed. Nullizygotes are individuals missing the

transgene by segregation. A "control plant" as used herein refers not only to whole plants, but also to plant parts, including seeds and seed parts.

The term "expression cassette" refers to any recombinant expression system for the purpose of expressing a nucleic acid sequence of the invention in vitro or in vivo, constitutively or inducibly, in any cell, including, in addition to plant cells, prokaryotic, yeast, fungal, insect or mammalian cells. The term includes linear and circular expression systems. The term includes all vectors. The cassettes can remain episomal or integrate into the host cell genome. The expression cassettes can have the ability to self-replicate or not (i.e., drive only transient expression in a cell). The term includes recombinant expression cassettes that contain only the minimum elements needed for transcription of the recombinant nucleic acid.

Means and methods for increasing the growth of plant hairy roots

Plant cell cultivations are being considered as an alternative to agricultural processes for producing valuable phytochemicals. Since many of these products (secondary metabolites) are obtained by direct extraction from plants grown in natural habitat, several factors can alter their yield. The use of plant cell cultures has overcome several inconveniences for the production of these secondary metabolites. Organized cultures, and especially root cultures, can make a significant contribution in the production of secondary metabolites. Most of the research efforts that use differentiated cultures instead of cell suspension cultures have focused on transformed (hairy) roots. *Agrobacterium rhizogenes* causes hairy root disease in plants. The neoplastic (cancerous) roots produced by *A. rhizogenes* infection are characterized by high growth rate, genetic stability and growth in hormone free media. These genetically transformed root cultures can produce levels of secondary metabolites comparable to that of intact plants. Hairy root cultures offer promise for high production and productivity of valuable secondary metabolites (used as pharmaceuticals, pigments and flavors) in many plants. The main constraint for commercial exploitation of hairy root cultivations is the development and scaling up of appropriate reactor vessels (bioreactors) containing hair roots. The present invention shows that the introduction of a chimeric gene – as defined below – leads to an increase of the biomass of the hairy roots. It is also observed that the increased production of secondary metabolites correlates with the increase in biomass.

Accordingly the present invention provides a chimeric gene comprising a plant promoter (id est a promoter active in plants and plant cells) operably coupled to the nucleotide sequence encoding for a plant SPL protein wherein said plant SPL protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1),

SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), and a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.

In another embodiment the invention provides a chimeric gene construct comprising the following operably linked DNA elements: a) the promoter region of a plant meristem specific promoter, b) a DNA
5 region encoding a plant SPL protein wherein said plant protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1), SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.

In a specific embodiment the plant meristem specific promoter is the *Arabidopsis* ribosomal meristem
10 specific promoter pAtRPS5A (depicted in SEQ ID NO: 4). The AtRPS5A promoter is strongly active in the division zone of the primary root tip, and the shoot apical meristem (SAM) and in leaf primordia.

In another specific embodiment the plant specific promoter is the RolD promoter which is the promoter of the gene RolD from *Agrobacterium rhizogenes* TL-DNA and is a strong promoter comparable to the plant 35S promoter (see Altamura M.M. (2004) *Plant Cell, tissue and organ culture* Vol. 77, pp. 89-101).

15 In yet another embodiment the invention provides a recombinant vector comprising the chimeric gene as defined herein.

In yet another embodiment the invention provides a plant cell comprising the chimeric gene as defined herein or a recombinant vector as defined herein.

In yet another embodiment the invention provides the use of a chimeric gene as defined herein or a
20 recombinant vector as defined herein to increase the yield of plant hairy root cultures.

In yet another embodiment the invention provides the use of a chimeric gene as defined herein or a recombinant vector as defined herein to increase the yield of secondary metabolites of a plant hairy root culture.

In yet another embodiment the invention provides a method for producing a plant secondary metabolite
25 wherein the method comprises introducing or transforming a plant hairy root culture with a chimeric gene as defined herein or a recombinant vector as defined herein, cultivating the obtained hairy root culture and extracting the secondary metabolite from said hairy root culture.

The term "hairy root yield" as used herein generally refers to a measurable product from a plant, particularly a hairy root from a plant. The terms "improved hairy root yield" or "increased hairy root
30 yield" can be used interchangeable. For example, the improvement in hairy root yield can comprise a

10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300% or greater increase in any measured parameter.

The following non-limiting Examples describe methods and means according to the invention. Unless stated otherwise in the Examples, all techniques are carried out according to protocols standard in the art. The following examples are included to illustrate embodiments of the invention. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Examples

1. Recombinant *Arabidopsis thaliana* plants comprising the chimeric gene of the invention

The phenotype at seedling level of recombinant *Arabidopsis* plants comprising the chimeric gene of the invention show that overexpression of *At*SPL13 promotes primary root growth and root system architecture (see Figure 1A and 1B) at a dose-dependent level (Figure 1C). T2-1 to T2-12 are 8 independent lines coming from independent transformation events. Weaker expression levels of the transgene (e.g. T2-6) show a moderate phenotype, while strong expression of the transgene (e.g. T2-12) show a strong effect on root system architecture.

The overexpression of AtSPL13 also generates a root with larger meristem size and thicker cross sections, indicating a promoting effect on cell divisions in both the longitudinal and radial dimensions (see Figure 2).

2. Phenotypes of mature recombinant *A. thaliana* plants

In the prior art the function of SPL10 in root development has been investigated using the endogenous promoter with removal of the miRNA binding sites (resistant versions – rSPL10 - PMID: 31642910). These lines show modest effects on meristem size, root length and root system architecture. The RPS5A promoter we use in the chimeric gene of the invention, when introduced into plants results results in a drastic enlargement of the root system architecture, the meristem size and areal organ sizes. As such, this combination of the AtRPS5A ribosomal meristematic promoter and AtSPL13 CDS in the chimeric gene results in a unique and unexpected phenotype.

The phenotype of the mature overexpression lines using the p*At*RPS5A promoter grown in soil conditions are shown below. Minor effect at seedling level, strong effect in mature plants (see Figure 3).

3. Chimeric genes with orthologous SPL transcription factors

In order to understand the specificity of AtSPL13 in *Arabidopsis*, we generated overexpression lines driven by the same p*At*RPS5A promoter. Several other orthologous AtSPL proteins from the same subclade have the same effect. These results suggest the root promoting effect is not limited to SPL13 but is also present in the more closely related SPL proteins in the phylogenetic tree (see Figure 4).

4. Alignment of orthologous SPL transcription factors of several plants

Alignment of multiple plants (Arabidopsis, maize, rice, wheat, poplar, tomato, potato, soybean and tobacco) is shown in Figure 5. SPL CDS and protein sequences were created using CLC Main workbench (version 22.0.1) with default setting.

5. Root specific expression of SPL6 in corn

A chimeric gene comprising the *Oryza sativa* root-specific promoter RCc3 (SEQ ID NO: 24) was operably coupled to the *Zea mays* SPL6 gene (SEQ ID NO: 13). This chimeric gene was introduced into *Zea mays* (B104 line) via transformation. The phenotype of three independent transformed lines as compared to the B104 wild type (non-transformed line) is shown in Figure 6. Figure 6A shows the plants at day 6. Root length, root width and the number of vascular cell lines are compared between the non-transformed line and the transformed lines. It is clear that the thickness of the root is increased in the transgenic lines which is due to the increased number of vascular cells in the roots. Figure 6B shows the plants at day 60. What is striking is the formation of early crown roots in the transformant lines. Figure 6C shows the plants at day 114. A reduced plant height of the transformed plants is observed together with a strong abundance of crown roots.

6. Plant orthologous SPL transcription factors which can be used to construct the chimeric gene of the invention, the Arabidopsis RPS5A promoter sequence and the rice root specific promoter

6.1 Nucleotide sequence of the *Arabidopsis thaliana* RPS5A promoter (SEQ ID NO: 4):

ct cgaggaactg taggagctcc ccataatcgt gagtagatat attactcaac ttttgattcg
ctatttgcag tgcacctgtg gcgttcatca catcttttgt gacactgttt gcactggtca
ttgctattac aaaggacctt cctgatgttg aaggagatcg aaagtaagta actgcacgca
taaccatttt ctttccgctc tttggctcaa tccatttgac agtcaaagac aatgtttaac
cagctccgtt tgatatattg tctttatgtg tttgttcaag catgtttagt taatcatgcc
tttgattgat cttgaatagg ttccaaatat caaccctggc aacaaaactt ggagtgagaa

acattgcatt cctcggttct ggacttctgc tagtaaatta tgtttcagcc atatcactag
ctttctacat gcctcaggtg aattcatcta tttccgtctt aactatttcg gttaattaaa
gcacgaacac cattactgca tgtagaagct tgataaacta tcgccaccaa tttatttttg
ttgcgatatt gttactttcc tcagtatgca gctttgaaaa gaccaaccct cttatccttt
5 aacaatgaac aggtttttag aggtagcttg atgattcctg cacatgtgat cttggcttca
ggcttaattt tccaggtaaa gcattatgag atactcttat atctcttaca tacttttgag
ataatgcaca agaacttcat aactatatgc tttagtttct gcatttgaca ctgccaaatt
cattaatctc taatatcttt gttgttgatc tttggtagac atgggtacta gaaaaagcaa
actacaccaa ggtaaaatac ttttgtacaa acataaactc gttatcacgg aacatcaatg
10 gagtgtatat ctaacggagt gtagaaacat ttgattattg caggaagcta tctcaggata
ttatcggttt atatggaatc tcttctacgc agagtatctg ttattcccct tcctctagct
ttcaatttca tggtgaggat atgcagtttt ctttgtatat cattcttctt cttctttgta
gcttggagtc aaaatcggtt ccttcatgta catacatcaa ggatatgtcc ttctgaattt
ttatatcttg caataaaaat gcttgtacca attgaaacac cagctttttg agttctatga
15 tcactgactt ggttctaacc aaaaaaaaaa aaatgtttaa tttacatatc taaaagtagg
tttagggaaa cctaaacagt aaaatatttg tatattattc gaatttcact catcataaaa
acttaaattg caccataaaa ttttgtttta ctattaatga tgtaatttgt gtaacttaag
ataaaaataa tattccgtaa gttaaccggc taaaaccacg tataaaccag ggaacctgtt
aaaccggttc tttactggat aaagaaatga aagcccatgt agacagctcc attagagccc
20 aaaccctaaa tttctcatct atataaaagg agtgacatta gggtttttgt tcgtcctctt
aaagcttctc gttttctctg ccgtctctct cattcgcgcg acgcaaacga tcttcaggtg
atcttctttc tccaaatcct ctctcataac tctgatttcg tacttgtgta tttgagctca
cgctctgttt ctctcaccac agcc

25 <u>6.2 Orthologous sequences of SPL proteins</u>

*Triticum aestivum* (TaSBP15A) (SEQ ID NO: 5)

MDWDLKMPPGAWDLAELEGDAPAAGGQASAGGIANAAGRQECSVDLKLGGLGECGAAPDSRGLGKAPAEAPSSA
PSAAKRPRASSGGGGGSGSGAGQQQCPSCAVDGCRADLSRCRDYHRRHKVCEAHSKTPVVTVAGREMRFCQQCSR
FHLLTEFDETKRSCRKRLDGHNRRRRKPQPDVMNSASFMTSQQGTRFSSFPTPRPEQNWQGIIKTEENPYYAHQLPL
30 GISSRQHFGGSASTYAKEGRRFPFLQEGEINFATGVALEPSVCQPLLKTVAPPESSSSSSKMFSDGLTPVLDSDCALSLLS
APANSSGIDVGQMVQQTEHIPIAQPLFSNLQFSSSSWFSRTQASTGTVSATGFSCPAVENEQLNNVLSSDNNDLNYN
GIFHVGGEGSSDGAPPSLPFPWQ

*Triticum aestivum* (TaSBP15B) (SEQ ID NO: 6)

MDWDLKMPPGAWDLTELENDAAAAPAAAQASAGGIANAAGRPECSVDLKLGGLGECGAAPDSRGLGKAPAEAAS
SASAPSAAKRPRASSGGGGWSGAGQQQCPSCAVDGCRADLSKCRDYHRRHKVCEAHSKTPVVTVAGREMRFCQQ
CSRFHLLTEFDEAKRSCRKRLDGHNRRRRKPQPDVMNSASFMTSQQGTRFSSFPTPRPEQNWPGIIKTEENPYYAHQ
5 LPLGISNRQHFGGSASTYAKEGRRFPFLQEGEINFATGVALEPSVCQPLLKTVAPPESSSSSSKMFSDGLTPVLDSDCAL
SLLSAPANSSGIDVGPMVQQTEHIPIAQPLFSNLQFSSSSWFSRTQASTGTVSATGFSCPVGENEQLNNVLSSDNNDL
NYNGIFHVGGEGSSDGAPPSLPFPWQ

*Triticum aestivum* (TaSBP15D) (SEQ ID NO: 7)

MDWDLKMPPGAWDLAELESDAAAAPAAGGQASAGGIANAAGRQECSVDLKLGGLGECGAAPGSRGLGKAPAEA
10 ASSASASAPSAAKRPRASSGGGGGSGSGAGQQQCPSCAVDGCKADLSRCRDYHRRHKVCEAHSKTPVVTVAGREM
RFCQQCSRFHLLTEFDEAKRSCRKRLDGHNRRRRKPQPDVMNSASFMTSQQGTRFSSFPTPRPEQNWPGIIKTEENP
YYAHQLPLGISNRQHFGRSASTYAKEGRRFPFLQEGEINFATGVALEPSVCQPLLKTVAPPDSSSSSSKMFSDGLTPVLD
SDCALSLLSAPANSSGIDVGQMVQQTEHIPIAQPLFSNLQFSSSSWFSRTQASTGTVSATGFSCPAVENEQLNNVLSS
DNNDLNYNGIFHVGGEGSSDGAPPSLPFPWQ

15 *Triticum aestivum* (TaSBP7A) (SEQ ID NO: 8)

MDWDLKMPGSWDLAELEHDGVPAMAAPAAAGIAAAAARGPPGRPECSVDLKLGGLGEFGPAADGAMKQQQPP
VATAAANGPSASLSASASNAAAVVPSASPLKRPRPGAGGGGGAGAGHCPSCAVDGCKADLSKCRDYHRRHKVCEA
HSKTPLVVVAGREMRFCQQCSRFHLLAEFDEAKRSCRKRLDGHNRRRRKPQVDSMTSGSFMTTQQGLYSNAGTRFA
SFSAPRPESSWSGIIKSEDSNPYYTTTHQINFAGSSSSYSKEGRRFPFLHEGDQMSFSTGAAALEIPPVCQPLLKAVAPP
20 PPPPESSSSNKMFSDGQLTHMLDSDCALSLLSSPANSSSVDVSRMVRPSEHIPSVPNLQQFGSSSWFACSQASSAATG
FAAFAGGMDGEQQLNAGGALVPSSNDNEMNCHGIFHVGADGSSEGTSPSLPFSWQ

*Triticum aestivum* (TaSBP7B) (SEQ ID NO: 9)

MDWDLKMPGSWDLAELEHDGVPAMAAPAAAGIAAAAARGPPGRPECSVDLKLGGLGEFGPAADGAMKQPPVAT
AAAAANGPAASLSASASNAAAVVPSASPLKRPRPGAGGAGAGHCPSCAVDGCKADLSKCRDYHRRHKVCEAHSKTP
25 LVVVAGREMRFCQQCSRFHLLAEFDEAKRSCRKRLDGHNRRRRKPQVDSMNSGSFMTTQQGLYSNSGTRFASFSAP
RPEPSWSGIIKSEDSNPYYTTTHQINFAGSSSSYSKEGRRFPFLHEGDQMSFSTGAPALEIPPVCQPLLKAVAPPPPPPP
ESSSSNKMFSDGQLTHVLDSDCALSLLSSPANSSSVDVSRMVRPSEHIPSVPNLQQFGSSSWFACSQASSAATGFAAF
PGGMDGEQLNAGGALVPSSNDNEMNCHGIFHVGADGSSEGTSPSLPFSWQ

*Triticum aestivum (*TaSBP7D) (SEQ ID NO: 10)

30 MDWDLKMPGSWDLAELEHDGVPAMVAPAAAGITAAAARGPPGRPECSVDLKLGGLGEFGPAADGAMKQQPPVA
TAAANGPAASLSASASNAAAVVPSASPLKRPRPGAGGGAGAGHCPSCAVDGCKADLSKCRDYHRRHKVCEAHSKTP

LVVVAGREMRFCQQCSRFHLLAEFDEAKRSCRKRLDGHNRRRRKPQVDSMNSGSFMTTQQGTRFASFSAPRPEPS
WSGIIKSEDSNPYYTSTHQINFAGSSSSYSKEGRRFPFLHEGDQMSFSTGATALEIPPVCQPLLKAVAPPPPPPESSSSN
KMFSDGQLTHVLDSDCALSLLSSPANSSSVDVSRMVRPSEHIPSVPNLQQFGSSSWFACSQASSAATGFAAFAGGM
DGEQLNACGALVPSSNDNEMNCHGIFHVGADGSSEGTSPSLPFSWQ

5 *Oryza sativa* (OsSPL16) (SEQ ID NO: 11)

MEWDLKMPPAASWELADELENSGGGGVPAAVSSSSAAVGGGVNAGGGGRQECSVDLKLGGLGEFGGGGAQPRV
AVAGEPAKGKGPAAAATGAAAAASSAPAKRPRGAAAAGQQQCPSCAVDGCKEDLSKCRDYHRRHKVCEAHSKTPL
VVVSGREMRFCQQCSRFHLLQEFDEAKRSCRKRLDGHNRRRRKPQPDPMNSASYLASQQGARFSPFATPRPEASWT
GMIKTEESPYYTHHQIPLGISSRQQHFVGSTSDGGRRFPFLQEGEISFGTGAGAGGVPMDQAAAAAAASVCQPLLKT
10 VAPPPPPHGGGGSGGGKMFSDGGLTQVLDSDCALSLLSAPANSTAIDVGGGRVVVQPTEHIPMAQPLISGLQFGGG
GGSSAWFAARPHHQAATGAAATAVVVSTAGFSCPVVESEQLNTVLSSNDNEMNYNGMFHVGGEGSSDGTSSSLPF
SWQ

*Oryza sativa* (OsSPL18) ((SEQ ID NO: 12)

MDWDLKMPVSWDLAELEHNAVPNMAAAASAAEPGIAAVAASRGAPGRPECSVDLKLGGLGEFGAADALKEPAAA
15 AKAPVSSAAAAASVAKVPPSTSTLKRPRGGGGGGGGQCPSCAVDGCKADLSKHRDYHRRHKVCEPHSKTPVVVVSG
REMRFCQQCSRFHLLGEFDEAKRSCRKRLDGHNRRRRKPQADSMSSGSFMTSQQGTRFASFTPPRPEPSWPGIIKSE
ETPYYSHHHHPHPVMTSRQPHFVGSPSSATTAAFSPKEGRRFPFLHEGDQISFGGGGGAAAAATLEISVCQTTVVAPP
PPESSSSNKMFSSDGLTTATTTTTTAHHHHHHHQVLDSDCALSLLSSPANSSSVDVSRMVQPSPAAAAGAEHHHHH
QIPMAQPLVPNLQQQFGGSSPWFASSPAAAAVAGGGFACPSMDSEQQQQQQLNAVLVPGSNENEMNYHGMFH
20 VGGEGSSDGTSPSLPFSWQ

*Zea mays* (ZmSPL6) (SEQ ID NO: 13)

MDWDLKMPVSWDLPDLEHDAMPPPPPVSAASTAAASGIAAAAAAPSSATAPSRAECSVDLKLGGLGEFGAADGTA
TKEPAAATAAPSASPMKRPRLGPGGAGGAQCPSCAVDGCKADLSKCRDYHRRHKVCEAHSKTPVVVVAGREMRFC
QQCSRFHLLLEFDEAKRSCRKRLDGHNRRRRKPQPDTMNSGSFMTSQQGTRFSSFPAPRPEPSWSGVIKSEDSSYYT
25 HHPVLSNRPHVAGTSTSPAYSKEGRRFPFLQDGDQVSFSASGAGTLEVSTVCQPLLKTTAAVAPPPPESSKMLAPVLD
SDCALSLLSSPANSSSVDVSRMVQPAERIPMAQPLVPGLQHHQFGGSPVPDWFAGSGAVPAAGTGGFACPHSVESE
QFNTVLVPSSDGGHEMNYHGIFHVGGEGSSDGTSPSLPFSWQ

*Zea mays* (ZmSPL11) (SEQ ID NO: 14)

MDWDLNAAGAWDLAELERDHAAAAPSSGGHAANAAAAGTGTESRPPAPGAAGAPAECSVDLKLGGMGECEPGA
30 ARREREAAAGAAKRPRPAGPGGQQQQQQCPSCAVDGCRADLGKCRDYHRRHKVCEAHSKTPVVVVAGREMRFC
QQCSRFHLLAEFDADKRSCRKRLDGHNRRRRKPQPDTMASASFIASQQGTRFSPFAHPRLEASWPPGVMKTEESPY

HITHQIPLGSSSSSRQQHFVALGAATPAYAKEGRRFPFLQEGEISFATGVVLEPPAAAPACQPLLRTGAPSESSGAGGS
KMFSDQGLARVLDSDCALSLLSAPANSSGIDVSRMVRPTEHVPMAQQPVVPGLQFGSASWFPRPQASTGGSFVPSC
PAAVEGEQQLNAVLGPNDSEVSMNYGGMFHVGGGSGGGEGSSDGGTSSSMPFSWQ

*Zea mays* (ZmSPL12) (SEQ ID NO: 15)

5 MDWDLKAVGAWDLAELEQDHAAATAAAGPSEGHATDTAAAGTGTGTGTESRPPGAGAPAECSVDLKLGGMGEC
ELGAGAATACREREAAAGATKRPRPAGQQQQQCPSCAVDGCRADLSKCRDYHRRHKVCEAHSKTPVVVVAGREM
RFCQQCSRFHLLAEFDADKRSCRKRLDGHNRRRRKPQPDTMASASSITSQQGTRFSPFAPPRLEASWPGVMKTEESP
YRITHQIHLGSSSSSRQQHFVGAATSAYAKEGPRFPFLQEGEISFATGVVLEPPAAAPACQPLLKSGAPPPESSSAGGGK
MFSDQGLTRVLDSDCALSLLSAPANYSGIDVSRMVRPTEHVPMAQQLVVSGLQFGSASWFPRPQASTGGSFVSSCP
10 AVQVEGEQQLNAVLGPNDSEVSMNYGGMFHVGGGSGGGEGSSDGGTSSSMPFSWQ

*Glycine max* (SPL13a) (SEQ ID NO: 16)

MQLIRGPSWCGIQKGEQRKTEKVQVFMDWNLKAPSWDLSEVDQANLPNMETMEGSSRYGMYRTKGEFSVDLKLG
QVGNSGRESSVLTKSKDGGAVGVSKMISSSSSGSAKRARSLSNGTQTVSCLVDGCHSDLSNCRDYHRRHKVCEVHSK
TAQVTIGGQKQRFCQQCSRFHSLEEFDEGKRSCRKRLDGHNRRRRKPQPEVVLTRSASFLSNYHQGTQLLSFSSSHVY
15 PSTTVVNPTLGGVVTSGDVRLHGQNHHHHQMHLVDKQDLFLGSSPTGYKEGKHLAFMQGDHTLNNQSPHLPGAS
VGPAQMFLRTSPYSESGGLRCKMFCDSLTGSAHGNTSRALSLLSSPPQPHSPGNGLNQMVNPHSPLMQPLGLSLHD
NSLGSVDPVLGPNGSDHSSSMHHIGSNGSQVNEAPPLFPFQWE

*Glycine max* (SPL13b) (SEQ ID NO: 17)

MDWDGKEFAWDPRGLELLANGEGQKSEAASVDLRLGGEKIAPDVAKDTKESKTVSSPSGSSKRSRLQNGSQNMCCS
20 VDGCNSDLSDCRDYHRRHRVCEKHSKTPVVLVGGKQQRFCQQCSRFHSLGEFDEVKRSCRKRLDGHNRRRRKPQPP
SLFMAAEKFMYNYKGPRILHFGSPEAYANPIMRNMWPAAAKTGAESGYDPPRLLYRIDKHKQDKGHPLWQENDPK
VGSGDEAMPGTPICQPIHGTIAPSTGGKSTRKLSTDAKPGSFDSGCALYLLSTLQSQSPELSMAQSSITCPMQSPSGSV
HFDAVNEYSCSETEKDKPSGQVLVFDANTTNLHYNGMLQMGLDGLVENEDPLTLPFLWE

*Glycine max* (SPL13c) (SEQ ID NO: 18)

25 MDWNLKAPSWDLVDVDKATLQQHRGAMEEQNRFGVYRMKGEFSVDLKLGHHVGNSGTESALANKSKDAAAAGV
SKMASSPSGSSKRARAINSTSLTVACLVDGCNSDLSNCRDYHRRHKVCELHSKTPEVTIGGFKQRFCQQCSRFHSLEQF
DERKRSCRKRLDGHNRRRRKPQPEPLSRPGSFLSNYQGTQLLPFSSPHVYPSTAIVSPAWSASLVTSSADARLHNHNQ
QQHHHHQQDLFLGSSHKEGKQLQHSAAKPRHLFHPLPFATPFSGPTLYQKLAAELEAKCSVKA

*Glycine max* (SPL13d) (SEQ ID NO: 19)

DTKDSKTVSSPSGSSKRSRLQNGLQNMCCSVDGCNSDLSDCREYHRRHRVCEKHSKTPVVMVGGKQQRFCQQCSR
FHSLGEFDEVKRSCRKRLDGHNRRRRKPQPPSLFMAAEX

*Poplar trichocarpa* (Potri.015G098900) (SEQ ID NO: 20)

5 MDWNLKATSWDLTEFEQGAVPSISIDAFDRSTNFGVNRSGGGFSIDLKLGRVGDSSDESIINWKQPGVSKLQPLPSGS
TKRARGANSGTQVAMCLVDGCNSDLSTCRDYHRRHKVCELHSKTPQVTVGGQKQRFCQQCSRFHSLEEFDEGKRSC
RKRLDGHNRRRRKPQPDPHSRPPSFLSNYQGTQLFPFSSSHVYPSSTVLNPTWSGVASTEADGRHHNLHQLPDKQNL
FFGSSSSSYHGVKQFPFLHWHSPGLNNQTSPEASVCQPLLRTIALPGSSGASSHSMFCDRLTQIQDSDCALSLLSSTQT
HASGNLMVQHNSVPLSHPIGPTVHDHGLGPIDSVLVFNNRDANVHFPGTFQPQSGGSSGNKAPQTLPFNWE

10 *Poplar trichocarpa* (Potri.012G100700) (SEQ ID NO: 21)

MDWNLKATSWDLTEFEQGAVPSICIDAFGRPTNFGANRTGGGFSIDLKLGRVGNSSDESMVNWKQPGVSKLESSPS
GSTKRARGANNGTQVAMCLVDGCNSDLSTCRDYHRRHKVCELHSKTPQVTIGGQKQRFCQQCSRFHSLEEFDEGKR
SCRKRLDGHNRRRRKPQPDPFSHPPSFLPNHQGTQIFPFSSSHVYHSTAVVNSTWSGVANTEADGGHYNLHQLPDK
QNLFLGSSSNSYKGGKLFPFLQCDNPCLNNQTSEASVCLPLPRAIAFPGSSGASSHSMFCDRLATQVQDSDCALSLFCH
15 QHRRMHGETPCCNDITQSPFRIP

*Arabidopsis thaliana* (AtSPL13A) (SEQ ID NO: 22)

MDWNFKLSSGYLSGFDQEPDLSPMDGSISFGGSSQSKADFSFDLKLGRNIGNSSSVFGDTEQVISLSKWKDSALAKPE
GSRSSSSKRTRGNGVGTNQMPICLVDGCDSDFSNCREYHKRHKVCDVHSKTPVVTINGHKQRFCQQCSRFHALEEFD
EGKRSCRKRLDGHNRRRRKPQPEHIGRPANFFTGFQGSKLLEFSGGSHVFPTTSVLNPSWGNSLVSVAVAANGSSYG
20 QSQSYVVGSSPAKTGIMFPISSSPNSTRSIAKQFPFLQEEESSRTASLCERMTSCIHDSDCALSLLSSSSSSVPHLLQPPLS
LSQEAVETVFYGSGLFENASAVSDGSVISGNEAVRLPQTFPFHWE

*Arabidopsis thaliana* (AtSPL13B) (SEQ ID NO: 23)

MDWNFKLSSGYLSGFDQEPDLSPMDGSISFGGSSQSKADFSFDLKLGRNIGNSSSVFGDTEQVISLSKWKDSALAKPE
GSRSSSSKRTRGNGVGTNQMPICLVDGCDSDFSNCREYHKRHKVCDVHSKTPVVTINGHKQRFCQQCSRFHALEEFD
25 EGKRSCRKRLDGHNRRRRKPQPEHIGRPANFFTGFQGSKLLEFSGGSHVFPTTSVLNPSWGNSLVSVAVAANGSSYG
QSQSYVVGSSPAKTGIMFPISSSPNSTRSIAKQFPFLQEEESSRTASLCERMTSCIHDSDCALSLLSSSSSSVPHLLQPPLS
LSQEAVETVFYGSGLFENASAVSDGSVISGNEAVRLPQTFPFHWE

6.3 *Oryza sativa* root-specific promoter

RCc3-promoter derived from Os02g44310.1 (SEQ ID NO: 24)

TTAGAAGCAGTACGATCTTATTTGGTGGAGTTGAAAATTATAAGAAACAACTGACAAGCAATCAACCAACATATA
CTGAATATGGGAAAGTTTCTTTTAGCTTTTCTAAATTAAGTACTGATTCTTAAACTTAAGTGAGAATCTAGCCTGTT
CAGGGGCGACGGCTAAAGGACATAGCACCACTAGTCTACGCGATTGCAAAAAAGAAGAATGCAAGCCTGCAAC
AAGTATCGCTTTCCCGACCAATGGTTGGTTGACCTCGGTTTGCCGGTAACCTCAGGCTGGACGACAGAACTAATT
AGCCAACTTGTCAATGTCTAGGGTGCTGTTCATAGCCTGCAGTTGACAGAGTACGAAAAGGACAAGATCACATG
GAAGCTAACTAGTCACGGCGAATACATGACGACATCGGCCTACAACGCACAACTTCTTGGCATAAAAGCTTCAAT
TTCAATGCCCCTATCTGGAAGCCCTAGGCGCCGCGCAAATGTAAAACATTCGCTTCGCTTGGCTTGTTATCCAAAA
TAGAGTATGGACCTCCGACAGATTGGCAACCCGTGGGTAATCGAAAATGGCTCCATCTGCCCCTTTGTCGAAGG
AATCAGGAAACGGCCCTCACCTCCTGGCGGAGTGTAGATATGTGAAAGAATCTAGGCGACACTTGCAGACTGGA
CAACATGTGAACAAATAAGACCAACGTTATGGCAACAAGCCTCGACGCTACTCAAGTGGTGGGAGGCCACCGCA
TGTTCCAACGAAGCGCCAAAGAAAGCCTTGCAGACTCTAATGCTATTAGTCGCCTAGGATATTTGGAATGAAAG
GAACCGCAGAGTTTTTCAGCACCAAGAGCTTCCGGTGGCTAGTCTGATAGCCAAAATTAAGGAGGATGCCAAAA
CATGGGTCTTGGCGGGCGCGAAACACCTTGATAGGTGGCTTACCTTTTAACATGTTCGGGCCAAAGGCCTTGAG
ACGGTAAAGTTTTCTATTTGCGCTTGCGCATGTACAATTTTATTCCTCTATTCAATGAAATTGGTGGCTCACTGGTT
CATTAAAAAAAAAAGAATCTAGCCTGTTCGGGAAGAAGAGGATTTTGTTCGTGAGAGAGAGAGAGAGAGAGAG
AGAGAGAGAGAGAGAAGGAGGAGGAGGATTTTCAGGCTTCGCATTGCCCAACCTCTGCTTCTGTTGGCCCA
AGAAGAATCCCAGGCGCCCATGGGCTGGCAGTTTACCACGGACCTACCTAGCCTACCTTAGCTATCTAAGCGGG
CCGACCTAGTAGCCACGTGCCTAGTGTAGATTAAAGTTGCCGGGCCAGCAGGAAGCCACGCTGCAATGGCATCT
TCCCCTGTCCTTCGCGTACGTGAAAACAAACCCAGGTAAGCTTAGAATCTTCTTGCCCGTTGGACTGGGACACCC
ACCAATCCCACCATGCCCCGATATTCCTCCGGTCTCGGTTCATGTGATGTCCTCTCTTGTGTGATCACGGAGCAAG
CATTCTTAAACGGCAAAAGAAAATCACCAACTTGCTCACGCAGTCACGCTGCACCGCGCGAAGCGACGCCCGAT
AGGCCAAGATCGCGAGATAAAATAACAACCAATGATCATAAGGAAACAAGCCCGCGATGTGTCGTGTGCAGCA
ATCTTGGTCATTTGCGGGATCGAGTGCTTCACAGCTAACCAAATATTCGGCCGATGATTTAACACATTATCAGCGT
AGATGTACGTACGATTTGTTAATTAATCTACGAGCCTTGCTAGGGCAGGTGTTCTGCCAGCCAATCCAGATCGCC
CTCGTATGCACGCTCACATGATGGCAGGGCAGGGTTCACATGAGCTCTAACGGTCGATTAATTAATCCCGGGGCT
CGACTATAAATACCTCCCTAATCCCATGATCAAAACCATCTCAAGCAGCCTAATCATCTCCAGCTGATCAAGAGCT
CTTAATTAGCTAGCTAGTGATTAGCTGCGCTTGTGATCGATCGATCTCGGGTACGTAGCA

7. Overexpression of an SPL transcription factor increases hairy root growth

We transformed tomato hairy root cultures with a chimeric gene comprising the *Arabidopsis* RPS15A promoter operably linked to the SPL13 transcription factor. An increase in biomass production was obtained which corresponds with an enhanced production of secondary metabolites. Hairy root tips (1-2 cm long) were inoculated in 6-well plates containing 5 mL MS medium (4.4g/L MS+vit, 0.5 g/L MES, 30 g/L sucrose) and incubated for 12 days at 25C / 130 rpm. There was a significant (p-value 4.8E-15; n>50)

increase in the root meristem width for tomato hairy root meristems carrying SPL13 overexpression constructs (see Figure 7).

We assessed the total root biomass by measuring the wet weight of multiple independent transformation events. For these biomass measurement, excess liquid was removed with clean paper towel and each well was measured separately. There was a significant (p-value 4.7E-6; n>4) increase in the hairy root biomass for tomato hairy root biomass carrying SPL13 overexpression constructs compared to the control lines (see Figure 8). Figure 9 depicts the transcript expression levels of SPL13 in the tomato hairy roots of the corresponding lines depicted in Figure 8.

These results show that the chimeric gene of the invention is capable of increasing plant hairy root biomass and this can be specifically used to produce more biomass and accordingly also more secondary metabolites in both *in vivo* and *in vitro* culture systems.

Claims

1. A chimeric gene construct comprising the following operably linked DNA elements: a) the promoter region of a plant meristem specific promoter or a plant root specific promoter, b) a DNA region encoding a plant SPL protein wherein said plant protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1), SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.
2. A recombinant vector comprising the chimeric gene of claim 1.
3. A plant, plant cell or plant seed comprising a chimeric gene according to claim 1 or a recombinant vector according to claim 2.
4. Use of a chimeric gene according to claim 1 or a recombinant vector according to claim 2 to increase the root length of plants.
5. Use of a chimeric gene according to claim 1 or a recombinant vector according to claim 2 to increase the yield of plants.
6. Use according to claims 4 or 5 wherein the plants are crops.
7. Use according to claim 6 wherein the crops are cereals.
8. A method for producing a plant with increased yield as compared to a corresponding wild type plant, whereby the method comprises introducing or transforming a plant with a chimeric gene according to claim 1 or a recombinant vector according to claim 2 and selecting a plant with a stable expression of said chimeric gene.
9. A chimeric gene construct comprising the following operably linked DNA elements: a) the promoter region of a plant specific promoter, b) a DNA region encoding a plant SPL protein wherein said plant protein comprises the sequences YHRRHKVCEVHSKAPKVTVSGLEQRFCQQCSRFHLLSEFDEGKRSCRRRL (SEQ ID NO: 1), SSQVPRCQVDGCEADLSSAKD (SEQ ID NO: 2) and AGHNRRRRKPQ (SEQ ID NO: 3), c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of a plant.
10. A recombinant vector comprising the chimeric gene of claim 9.
11. A plant cell comprising a chimeric gene according to claim 9 or a recombinant vector according to claim 10.
12. Use of a chimeric gene according to claim 9 or a recombinant vector according to claim 10 to increase the yield of plant hairy root cultures.
13. Use of a chimeric gene according to claim 9 or a recombinant vector according to claim 10 to increase the yield of secondary metabolites of a plant hairy root culture.

14. A method for producing a plant secondary metabolite wherein the method comprises introducing or transforming a plant hairy root culture with a chimeric gene according to claim 9 or a recombinant vector according to claim 10, cultivated the obtained hairy root culture and extracting the secondary metabolite from said hairy root culture.

5

**Figure 1**

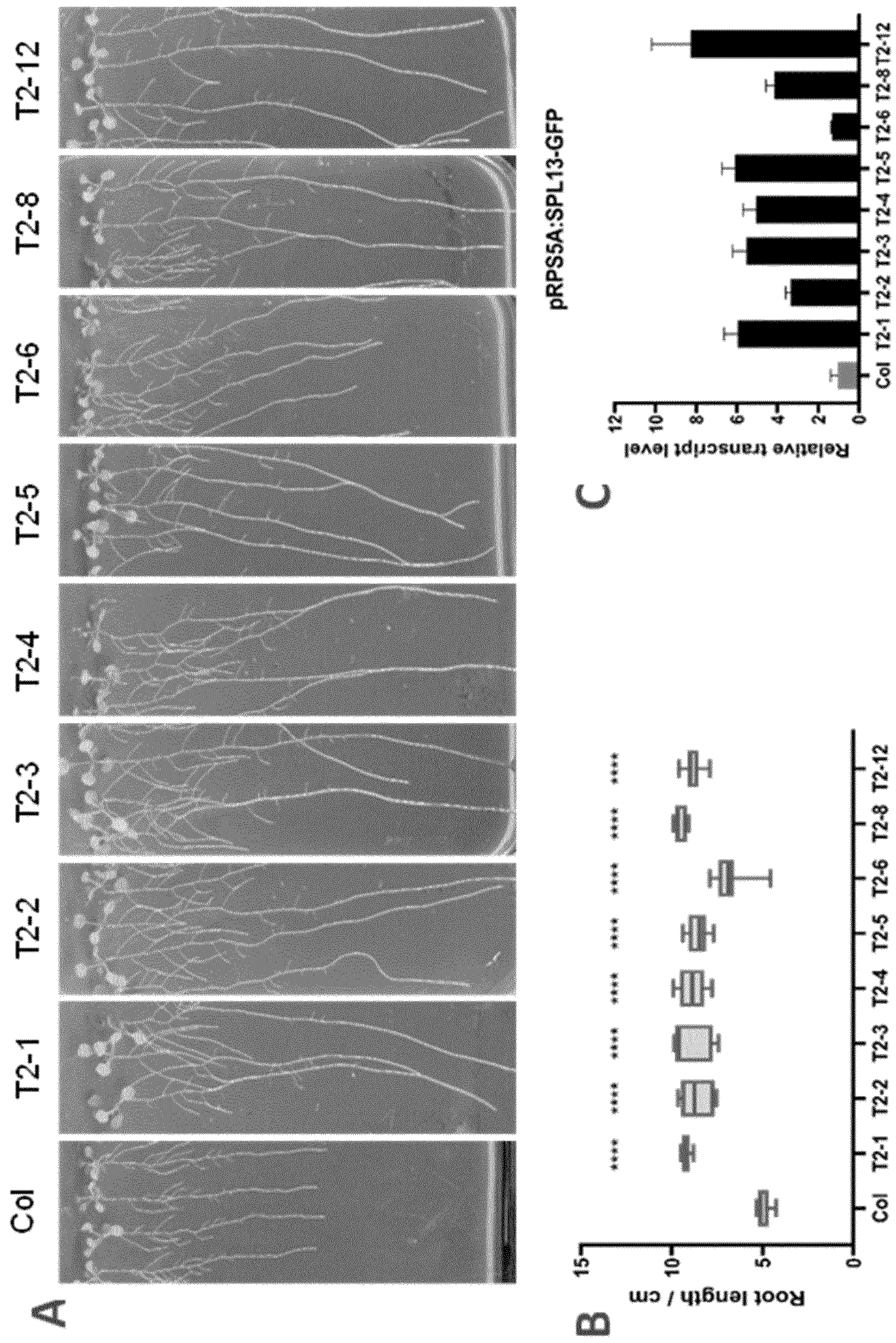


Figure 2

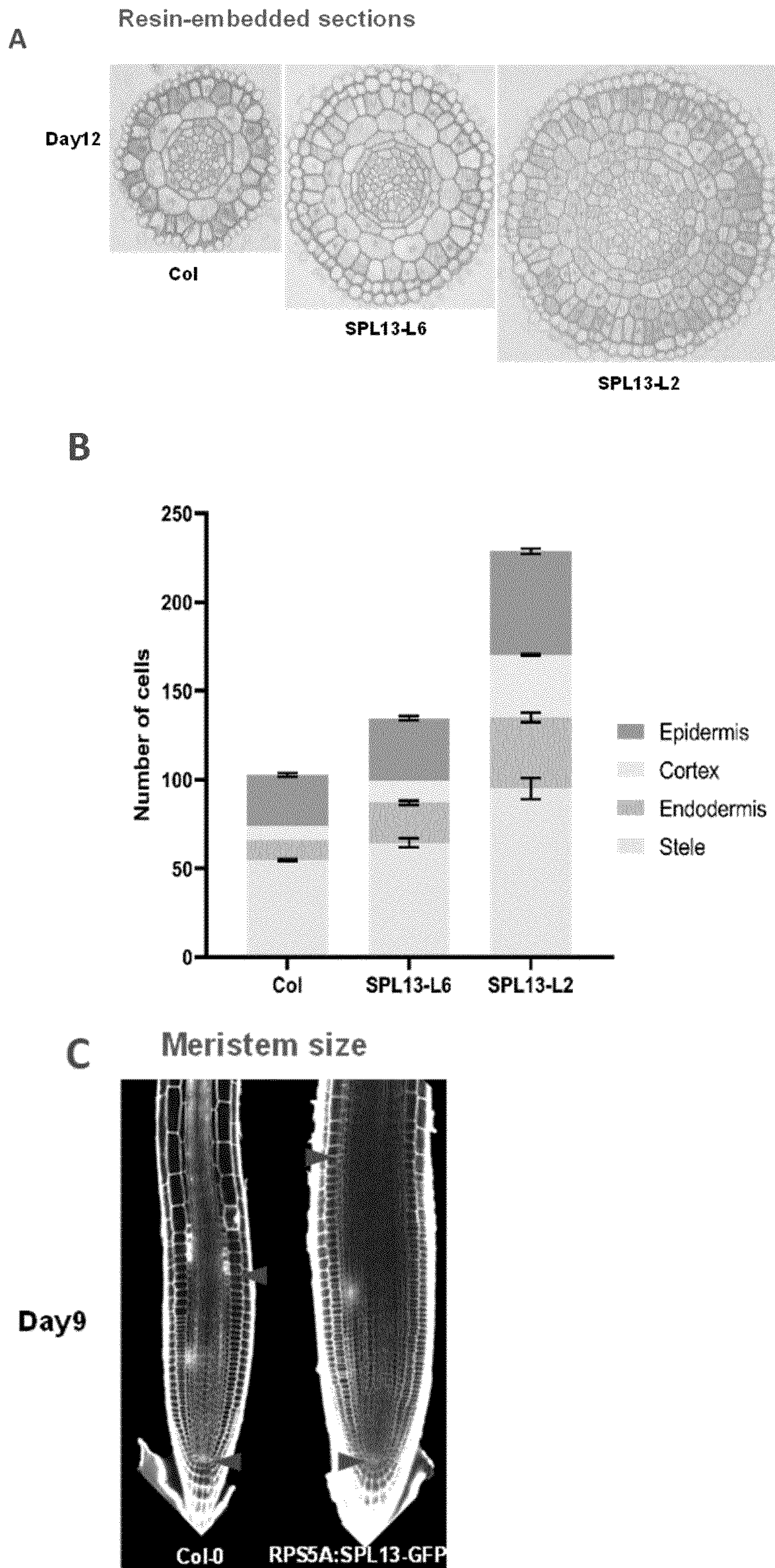


Figure 3

**Figure 4**

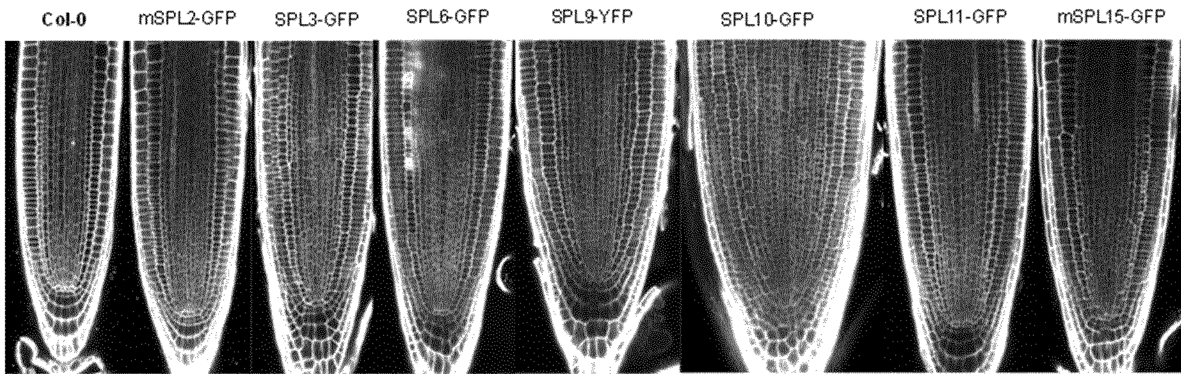


Figure 5

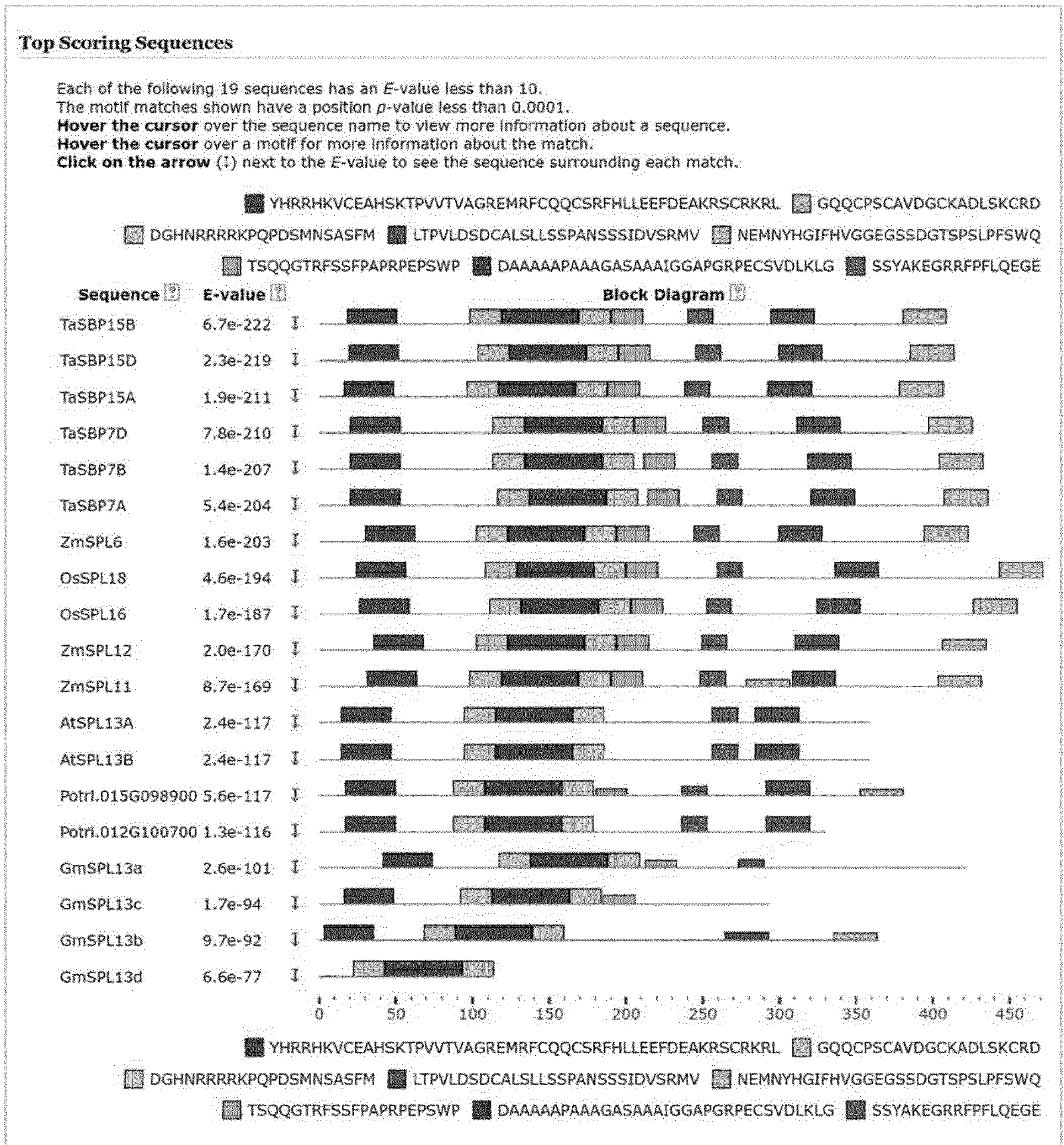


**Figure 6**

**A**

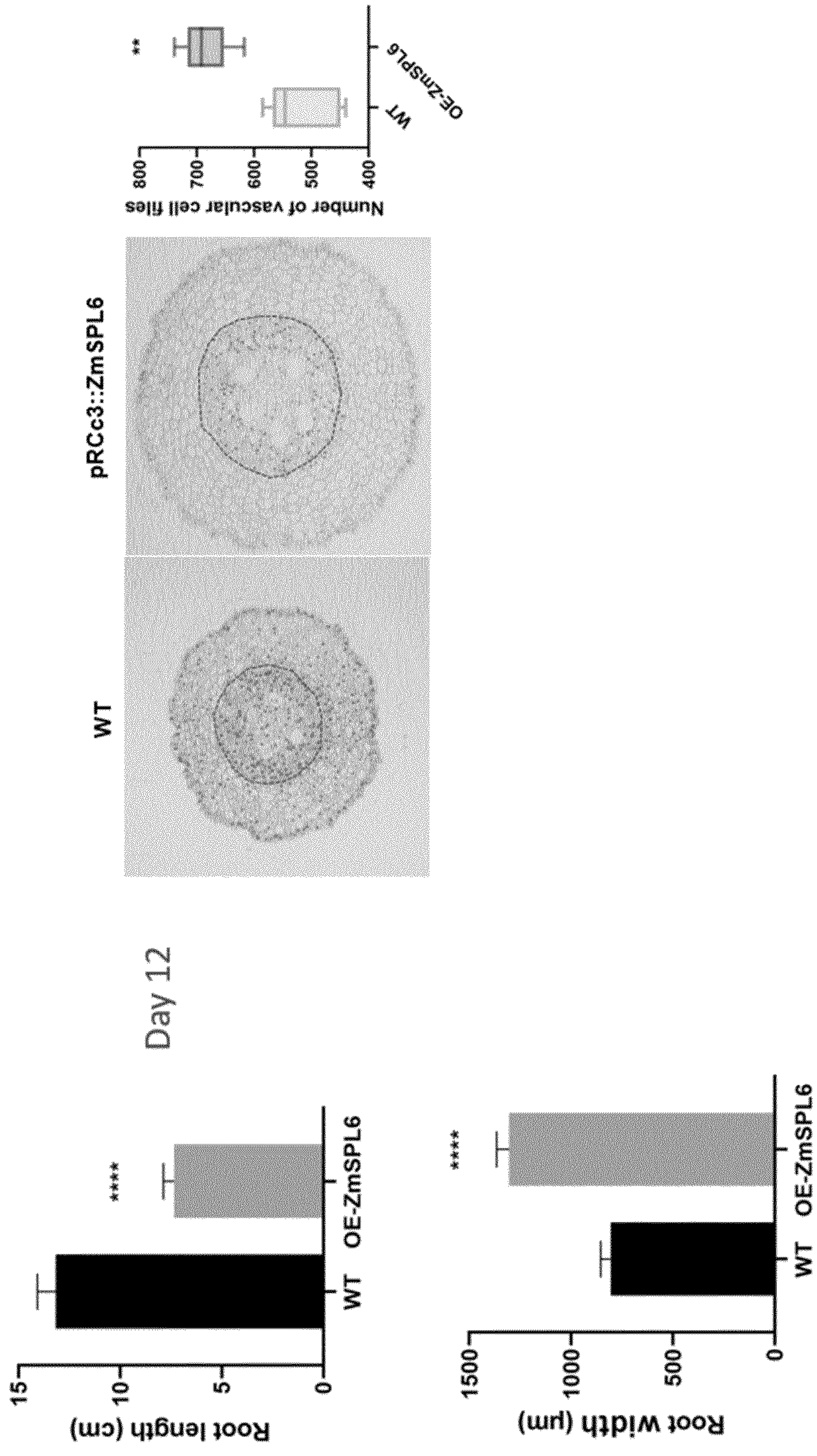
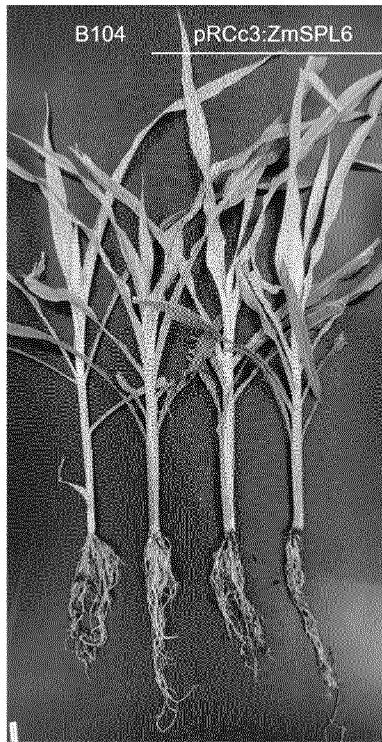


**Figure 6 continued**

**B**




Day 60

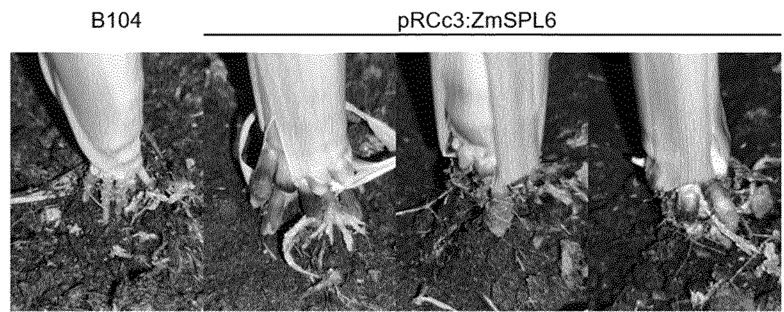


**Figure 6 continued**

**C**

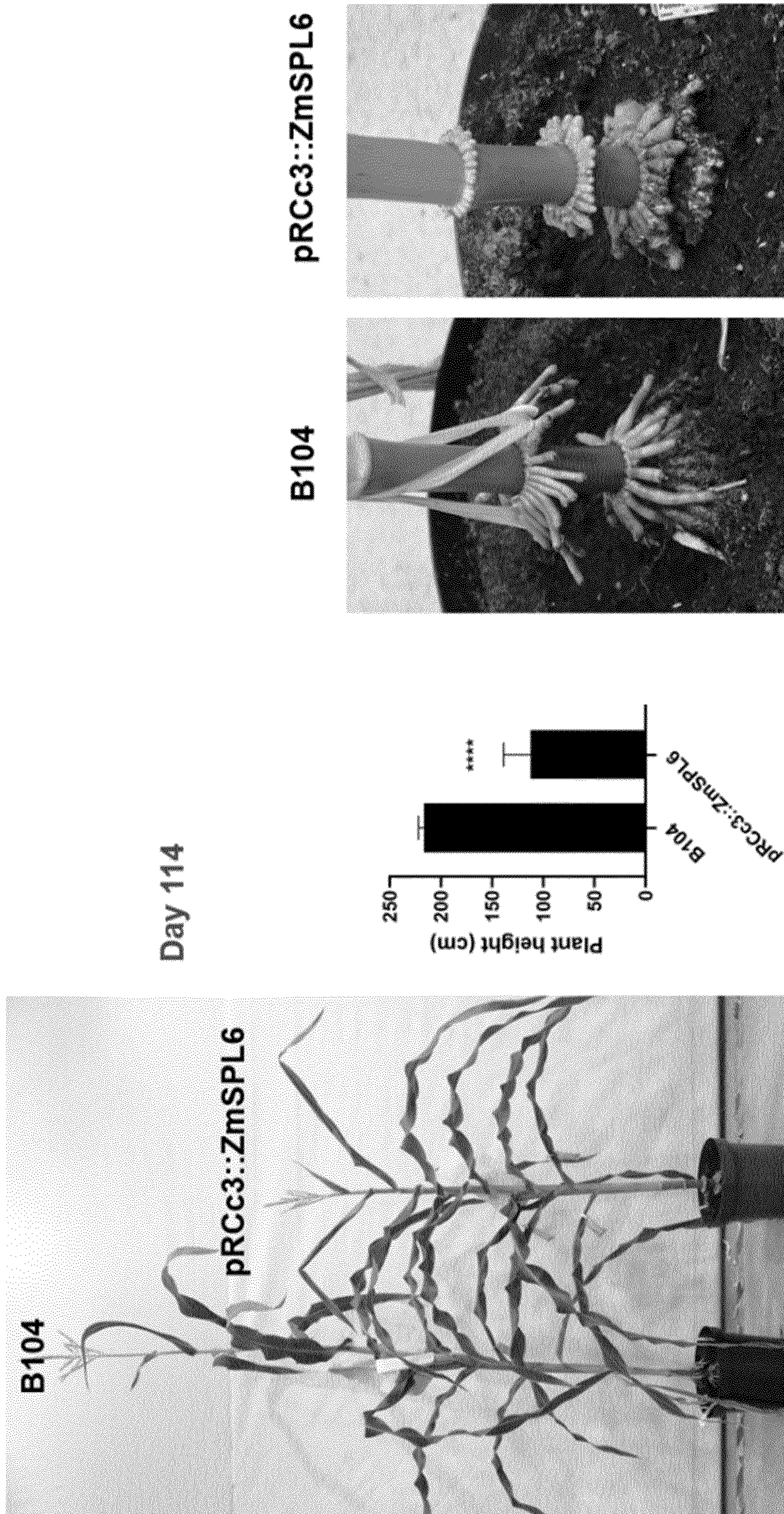


**Figure 7**

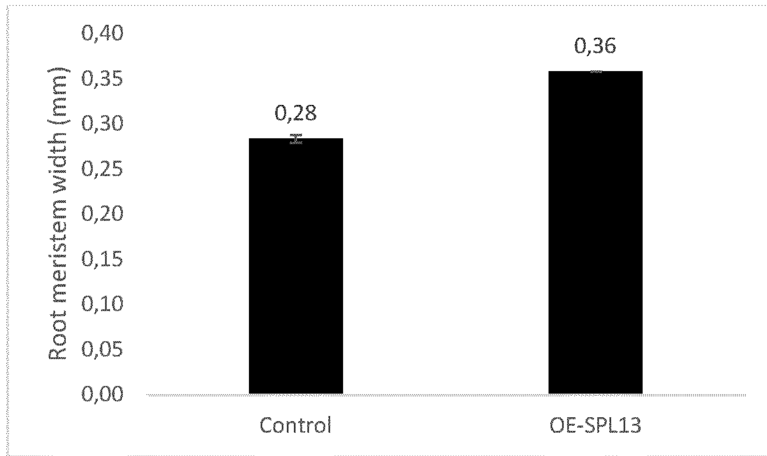


Figure 8

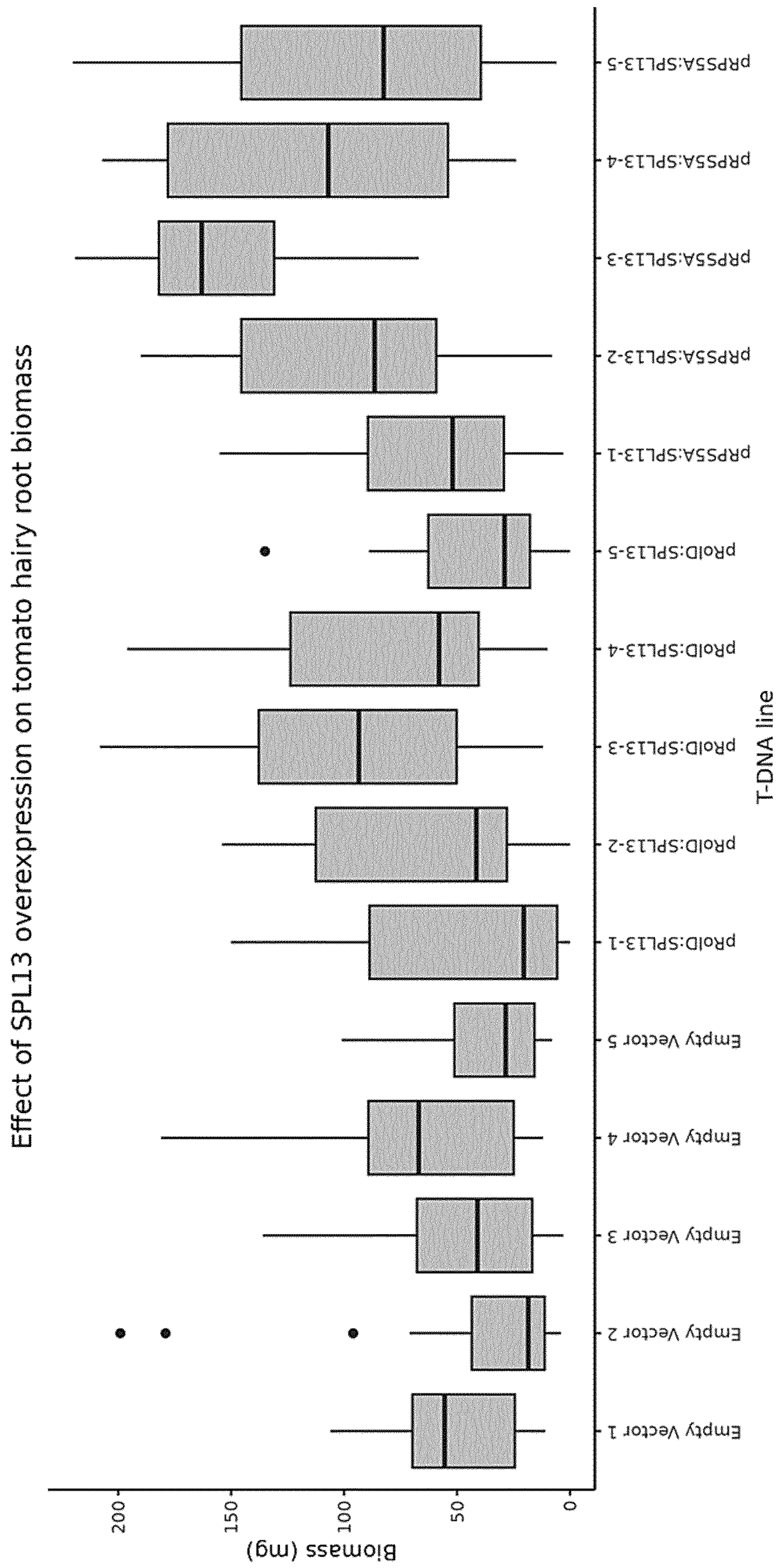
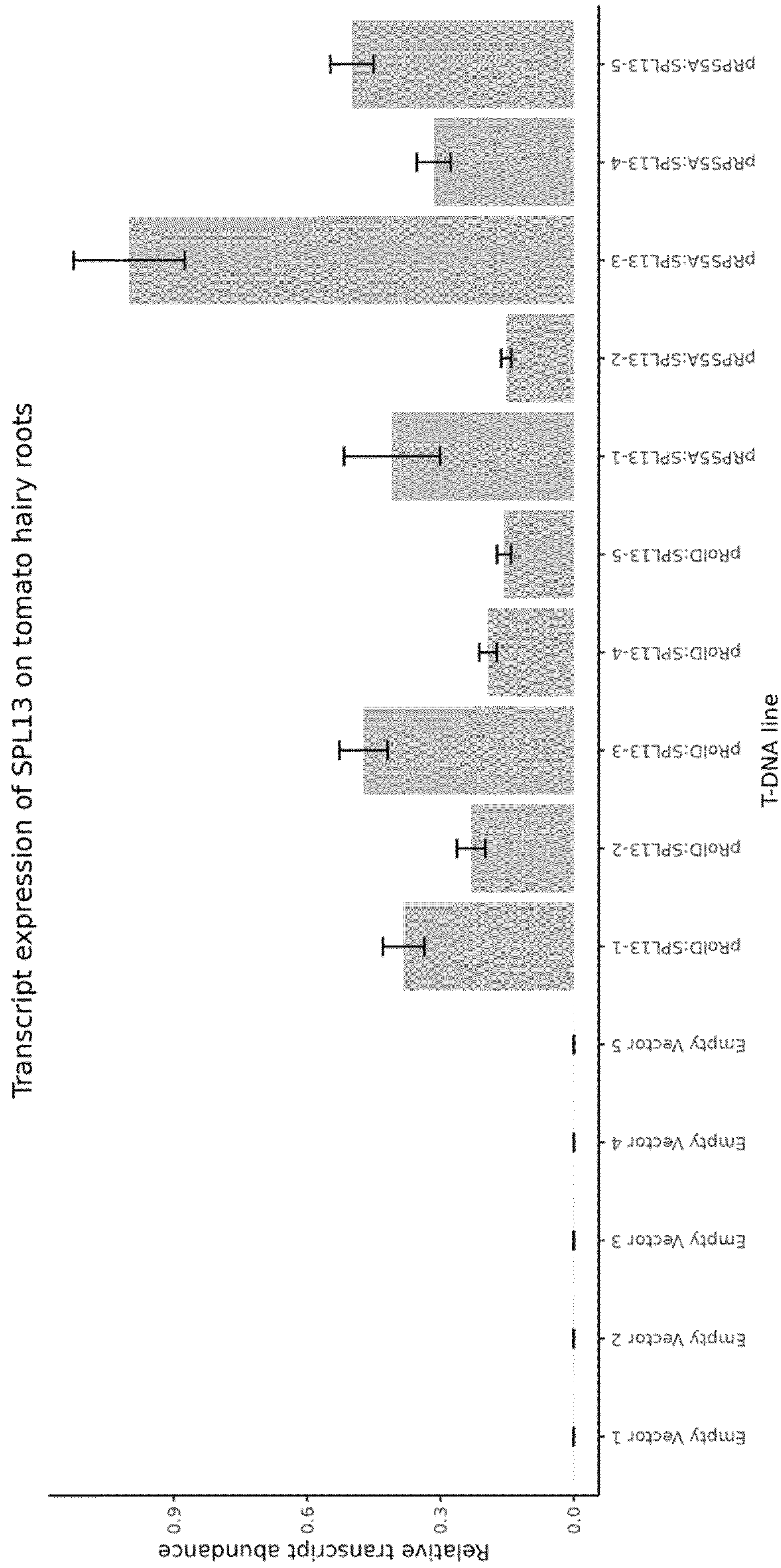


Figure 9



INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/055957

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/055957

**A. CLASSIFICATION OF SUBJECT MATTER**
INV. C07K14/415 C12N15/82
ADD.

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | CN 105 624 188 A (UNIV ZHEJIANG) 1 June 2016 (2016-06-01) SEQ ID NO: 1 fully comprises present SEQ ID NO:3; the whole document ----- | 1-14 |
| A | CN 114 807 212 A (UNIV SHANGHAI JIAOTONG) 29 July 2022 (2022-07-29) the whole document ----- | 1-14 |
| | -/-- | |

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

* Special categories of cited documents :

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

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

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 2 May 2024 | 21/05/2024 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | Kania, Thomas |

1

## INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/055957

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | JING YANG: "Genome-Wide Characterization of SPL Gene Family in Codonopsis pilosula Reveals the Functions of CpSPL2 and CpSPL10 in Promoting the Accumulation of Secondary Metabolites and Growth of C. pilosula Hairy Root", GENES, vol. 12, no. 10, 9 October 2021 (2021-10-09), page 1588, XP93158492, US ISSN: 2073-4425, DOI: 10.3390/genes12101588 the whole document | 1-14 |

-----

1

# INTERNATIONAL SEARCH REPORT

**Information on patent family members**

International application No
PCT/EP2024/055957

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| CN 105624188 A | 01-06-2016 | NONE | |
| CN 114807212 A | 29-07-2022 | NONE | |