



(51) International Patent Classification:

A01N 37/10 (2006.01)

(21) International Application Number:

PCT/EP2017/081258

(22) International Filing Date:

01 December 2017 (01.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1620383.8 01 December 2016 (01.12.2016) GB

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(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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))

(54) Title: AGROCHEMICAL COMPOSITIONS COMPRISING PHENYL-PROPANOIC ACID DERIVATIVES FOR CROP YIELD INCREASE

(57) Abstract: The present invention relates to agrochemical formulations and uses thereof for improving the yield increase in plants. More specifically the invention provides agrochemical compositions comprising compounds with formula (I) and (II) which are useful to increase vegetative yield increase in crops.



AGROCHEMICAL COMPOSITIONS COMPRISING PHENYL-PROPANOIC ACID DERIVATIVES FOR CROP YIELD INCREASEField of the invention

The present invention relates to agrochemical formulations and uses thereof for improving the yield increase in plants. More specifically the invention provides agrochemical compositions comprising compounds with formula (I) and (II) which are useful to increase vegetative yield increase in crops.

Introduction to the invention

By the year 2050, nearly 80% of the earth's population will reside in urban centers. Applying the most conservative estimates to current demographic trends, the human population will increase by about 3 billion people during the interim. An estimated 10^9 hectares of new land (about 20% more land than is represented by the country of Brazil) will be needed to grow enough food to feed them, if traditional farming practices continue as they are practiced today. At present, throughout the world, over 80% of the land that is suitable for raising crops is in use (sources: FAO and NASA). Historically, some 15% of that has been laid waste by poor management practices. Thus, there exists a continuing pressure to develop new farming technologies and deploying of them on a global basis in order to cope with the demand for feed, fuel, and food without the commitment of large land areas to new production. One technology to overcome this urgent problem is to develop efficient systems for indoor farming practices. Hydroponic culture conditions are very often applied in indoor farming practices. There is a need for the identification of new molecules which can be applied to increase the yield of crops in hydroponic culture conditions. The present invention satisfies this need and provides *cis*-cinnamic acid and derivatives thereof as molecules which can be used to enhance the biomass increase in plants. Cinnamic acid (CA) is a common constituent in the plant kingdom (Yin, 2003; Wong et al., 2005). Having a double-bound in its propanoid side-chain CA, the structures comes in two different forms. The *trans*-isoform of CA (*t*-CA) is the most abundant form. *t*-CA is produced by the deamination of the aromatic amino acid phenylalanine catalyzed by PHENYLALANINE AMMONIA LYASE (PAL) and further converted by CINNAMIC ACID-4-HYDROXYLASE (C4H) to *p*-coumaric acid (Baucher et al., 2003). These reactions are the initial steps of the general phenylpropanoid pathway leading towards a varied array of secondary metabolites with diverse biological functions (Vogt, 2010). *t*-CA, for instance, is a precursor of salicylic acid, an important stress-related signaling molecule, and flavonoids, which are considered important ultraviolet light (UV)-protectants derived from *p*-coumaric acid (Vogt, 2010). Not at least, this pathway provides the building blocks for lignin, an aromatic, heteropolymer composed of three *p*-hydroxycinnamyl alcohol monomers (monolignols) that differ in their degree of methoxylation (Boerjan et al.,

2003). The *cis*-isoform of cinnamic acid (*c*-CA) is a photo-isomerization product of *t*-CA and the relative abundance of both isoforms of CA inside plants spans orders of magnitudes (Yin, 2003; Wong et al., 2005). *c*-CA is not channeled in the phenylpropanoid pathway and is found in trace amounts *in planta* (Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943). The role of *cis*-CA in plants is confusing and contradictory in the art. Nishikawa K. *et al* (2013) *Phytochemistry* 96, 132-147, Nishikawa K. *et al* (2013) *Phytochemistry* 96, 223-234 and Abe M. *et al* (2012) *Phytochemistry* 84, 56-67) teach derivatives of cinnamic acid and disclose that *cis*-CA and derivatives thereof act as plant growth inhibitors.

In the present invention we tested the effect of *cis*-CA on the growth of the rosette of *Arabidopsis thaliana* and surprisingly found that *cis*-CA positively affects leaf growth, by promoting cell proliferation, and not expansion, and this by affecting the spatiotemporal distribution of auxin within the plant. We also confirmed the increase in plant biomass in other plants such as the plant *Nicotiana benthamiana*, lettuce and several herbs. By treating *Nicotiana* with *cis*-CA we were able to increase the overall plant biomass of *in vitro* grown plants with 54.08%. Furthermore, *cis*-CA-treated *Nicotiana* plants had a different cell wall composition in comparison to mock-treated plants, containing lignin with a different composition and higher levels of matrix polysaccharides. The combined effect on plant yield and secondary cell wall composition resulted in a 67.54% surge in cellulose-to-glucose conversion of *cis*-CA-treated plants compared to mock-treated plants. This positive effect of *cis*-CA on both plant productivity and composition make *cis*-CA and derivatives thereof desirable agrochemical molecules for the use in increasing biomass in plants.

Figures of the invention

Figure 1: Effect of CA on the growth of the rosette of *Arabidopsis*.

(A) Rosette area of seedlings grown on 0.5xMS-medium supplemented with different concentrations of CA (n=10). Every hour from stratification onwards a picture was taken up to 21 days after stratification (DAS). (B) Leaf series were performed on the seedlings of (A) 21 DAS (n=10). Labels on the X-axis indicate the leaf position. (C) Comparison between data obtained in-between the IGIS and the leaf series. (D-E) Fresh and dry weight of the rosettes of seedlings grown on normal 0.5xMS-medium supplemented with different concentrations of CA 21 DAS (n=10). Error bars represent standard deviations in-between the mock-treatment and the CA-treatment. Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

Figure 2: Effect of CA on the growth of the rosette of *Arabidopsis* at the cellular level.

(A) Analysis of cell drawings done at the abaxial side at the basis of the 3rd leaf for seedlings grown on 0.5xMS-medium supplemented with different concentrations of CA (n=18). (B) Circularity and cell area of the pavement cells. (C) Cell number of the pavement cells and guard cells. Error bars represent standard deviations in-between the mock-treatment and the CA-treatment. Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

Figure 3: CA affects leaf growth in an auxin-dependent manner.

(A) The concentration of IAA-glutamate and IAA-aspartate in 12 DAG *Arabidopsis* seedlings grown on 0.5xMS-medium supplemented with 2.5, 5 or 10 μ M CA. (B) The concentration of free IAA in 12 DAG *Arabidopsis* seedlings grown on 0.5xMS-medium supplemented with 2.5, 5 or 10 μ M CA. Ten seedlings were pooled for each technical repeat, and four repeats were analyzed for each treatment. Error bars represent standard deviations and asterisks statistically significant differences between compound-treated and mock-treated plants as determined by Dunnett's test. P-values: *P < 0.05, **P < 0.001, *** P < 0.0001. (C-D) Leaf series were performed on the seedlings of (A) 21 DAS (n=10). Labels on the X-axis indicate the leaf position.

Figure 4: Effect of *c/t*-CA on growth and development of *Nicotiana benthamiana*.

(A) Phenotype of representative seedlings 21 DAG grown on 0.5xMS-medium supplemented with *c/t*-CA (n=28 for each concentration) (scale bar: 1 cm). (B) *c/t*-CA dose response curve for primary root growth (Exponential rise to maximum, single, 3 parameters) and the length of the first lateral root (Exponential decay, single, 3 parameters) under the shoot/root junction (n=28). (C) Length root hairs measured at the main root tip of seedlings 21 DAG, grown on 0.5xMS-medium supplemented with *c/t*-CA (n=10). (D) Binocular pictures of the main root tip of representative seedlings 21 DAG grown on 0.5xMS-medium supplemented with *c/t*-CA (n=28 for each concentration) (scale bar: 0.1 cm). (E-F) Lateral root density and lateral root number of seedlings 21 DAG, grown on 0.5xMS-medium supplemented with *c/t*-CA (n=28). (G) Adventitious root number on top of the shoot of seedlings 21 DAG, grown on 0.5xMS-medium supplemented with *c/t*-CA (n=28). (H) Number of adventitious roots of seedlings 12 DAG grown on 0.5xMS-medium supplemented with *c/t*-CA. Plants were grown for 7 days in darkness (after a short light-pulse of 4h with light to induce germination) and subsequently transferred to light to stimulate adventitious rooting. Adventitious root numbers are represented in grey-scale (n>60). Average values and standard deviations are mentioned above. (H) Histogram showing the *c/t*-CA-induced gravitropic response in the main root. Seeds were germinated on 0.5xMS-medium and 6 DAG plates were rotated 90 degrees and each root was assigned to one of 12 30° sectors after 48h incubation (n>60). Error bars represent standard deviations in-between the

mock-treatment and the CA-treatment. Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

Figure 5: Effect of CA on the growth of the rosette of *Nicotiana*.

- 5 (A) Rosette area of seedlings grown on 0.5xMS-medium supplemented with different concentrations of CA 21 DAS (n=10). (B-C) Fresh and dry weight of the rosettes of seedlings grown on normal 0.5xMS-medium supplemented with different concentrations of CA 21 DAS (n=10). Error bars represent standard deviations in-between the mock-treatment and the CA-treatment. Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

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Figure 6: Effect of CA on plant biomass productivity in *Nicotiana*.

- (A-B) The belowground and vegetative phenotype of 3-months old *Nicotiana benthamiana* plants grown on 0.5xMS-medium supplemented with 2.5 μ M CA (n=12) (scale bar: 1.5 cm). (C) Height of the main stem. (D) Diameter of the main stem measured 1 cm on top of the root-shoot junction.
- 15 (E) Fresh weight of the whole plant. (F) Fresh and dry weight of the main stem. Error bars represent standard deviations and asterisks were used to indicate statistically significant differences compared to the corresponding mock-treated control sample as determined by Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

20 Figure 7: CA affects the stem morphology of *Nicotiana*.

- (A-B) Representative light microscopic images of a stem-segment at the basis of the plant of 3-months old *Nicotiana* plants grown on culture-medium supplemented with 2.5 μ M CA (n=9; 3 plants and 3 transverse sections each) (A; scale bar: 50 μ m and B; scale bar: 10 μ m). Toluidine blue was used as counterstain to visualize the secondary cell wall. (C) Software analysis of cell
- 25 drawings performed on earlier mentioned transverse sections. (D-E) Cell area and cell number. (F) Circularity of the cells, with an absolute value 1 representing a perfect circle. (G) Cell size distribution, starting at 0.000002667 with an interval-length of 0.00004. (H) Cell wall thickness of cells. Cells were randomly picked. (n>175). Error bars represent standard deviations. Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

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Figure 8: Shift in IAA-related metabolites upon treatment with 2.5, 5 or 10 μ M CA.

- The concentration of free IAA, IAA-precursors, IAA-amino acid conjugates and IAA-degradation products in 12 DAG *Arabidopsis* seedlings grown on 0.5xMS-medium supplemented with 2.5, 5 or 10 μ M CA. Ten seedlings were pooled for each technical repeat, and four repeats were
- 35 analyzed for each treatment. Error bars represent standard deviations and asterisks statistically significant differences between compound-treated and mock-treated plants as determined by Dunnett's test. P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

Figure 9: Saccharification data of CA-treated *Nicotiana* plants.

Samples were saccharified with either (A) no pre-treatment, (B) acid pre-treatment (1M HCl) or alkaline pretreatment (6.25 mM NaOH). The cellulose-to-glucose conversion was measured at 4, 8, 24, and 48 hours (n = 4) for the mock treated (black dot) and *t*-CA treated (white dot) plants. The cellulose-to-glucose conversion is expressed on a dry weight basis. Error bars represent standard deviations and asterisks are used to indicate statistically significant differences compared to the corresponding control as determined by Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

Figure 10: Bioassay to recognize variants of cis-CA which can stimulate vegetative growth in plants. Several variants of cis-CA have been tested for their effects on the primary root inhibition and on lateral root induction. Compounds 6, 7 and cis-2-phenylcyclopropane-1-carboxylic acid also showed a clear growth promoting effect on vegetative biomass of *Arabidopsis thaliana*. Blank spaces in the table indicate that the compounds were not tested for growth promoting potential.

Figure 11: Measurement of root growth biomass of green lettuce plants grown in soil (white cupboard boxes were used; each box contains 28 plants). Several concentrations of *c/t*-CA were used. Treatment with *c/t*-CA was done when plantlets were 2 weeks old (*id est* at week 2) and Week3. Mock (0 mg), 10 mg, 25 mg and 50 mg *c/t*-CA was added to each box (dissolved in 2.0 liter H₂O). Root biomass (fresh weight (FW)/dry weight (DW) was harvested at Week 4 and Week 5. 10 mg and 25 mg of *c/t*-CA show a very clear increase in root biomass.

Figure 12: *Arabidopsis thaliana* and *Nicotiana benthamiana* plants grown in hydroponic conditions. The picture shows *N. benthamiana* plants which have been treated with 1 μ M *c/t*-CA. There is a clear shoot increase for the two plant species when treated with *c/t*-CA.

Figure 13: Hydroponic cultures of basil, parsley, green lettuce and red lettuce. Different plant species are shown in the figure which have been treated with *c/t*-CA. A significant increase in shoot biomass can be observed for all four plant species.

Tables

	mock	CA	Change (%)	Significance
CWR	67.08 ± 3.82	62.26 ± 2.32	-7.19	*
AcBr	7.34 ± 0.78	6.61 ± 0.94	-9.95	-
H+G+S	7285.47 ± 4191.776	8559.50 ± 3147.39	17.49	-
%H	0.17 ± 0.01	0.22 ± 0.02	31.44	***
%G	29.62 ± 1.88	36.63 ± 1.76	23.65	***
%S	70.21 ± 1.89	63.15 ± 1.75	-10.05	***
S/G	2.38 ± 0.22	1.73 ± 0.13	-27.41	***

Table 1: Lignin content and composition.

- 5 The cell wall residue (CWR) (expressed as %dry weight) was determined gravimetrically after a sequential extraction (n=12). Lignin content and composition for 3 months old CA-treated and mock-treated *Nicotiana* plants (n = 12) ($\pm$ standard deviation). Lignin content was determined with the acetyl bromide (AcBr) method and expressed as percentage cell wall residue (CWR). Lignin composition was determined with the thioacidolysis method. The sum of H, G, and S is
- 10 expressed in $\mu\text{mol g}^{-1}$ AcBr lignin. The relative proportions of the different lignin units were calculated based on the total thioacidolysis yield. S/G was calculated based on the absolute values for S and G (expressed in $\mu\text{mol g}^{-1}$ AcBr lignin). Absolute values represent averages and standard deviations. Asterisks (*) indicate statistically significant differences in between CA-treated and mock-treated *Nicotiana* plants as determined by Dunnett's test P-values: *P < 0.05,
- 15 **P < 0.001, *** P < 0.0001.

	mock	t-CA	Change (%)	Significance
Cellulose	40.20 ± 1.27	35.48 ± 2.91	-11.74	***
Matrix polysaccharides	40.65 ± 3.69	43.82 ± 2.72	7.80	*
Rhamnose	3.46 ± 0.29	4.08 ± 0.47	17.61	**
Fucose	0.14 ± 0.01	0.18 ± 0.03	27.27	**
Arabinose	6.17 ± 2.09	8.85 ± 0.71	43.38	**
Xylose	69.81 ± 7.05	58.10 ± 5.31	-16.77	***
Mannose	1.98 ± 0.36	1.90 ± 0.23	-3.78	-
Glucose	4.64 ± 1.48	6.60 ± 1.39	42.04	**
Galactose	14.08 ± 3.72	20.52 ± 2.96	45.75	***

Table 2: Polysaccharide content and composition.

Cellulose and matrix polysaccharide contents for 3 months old CA-treated and mock-treated *Nicotiana* plants (n = 12) ($\pm$ standard deviation) were expressed as percentage cell wall residue (CWR). Matrix polysaccharide content was determined gravimetrically based on a trifluoroacetic acid (TFA) extraction and its composition was expressed as mol%. Absolute values represent averages and standard deviations. Asterisks (*) indicate statistically significant differences in between CA- and mock-treated *Nicotiana* plants as determined by Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

	mock	CA	Significance
<u>Saccharification (no pretreatment)</u>			
Cellulose-to-glucose conversion (%)	23.48 $\pm$ 3.64	39.34 $\pm$ 9.39	*
Glucose release (per %DW)	6.50 $\pm$ 0.87	7.48 $\pm$ 1.30	-
Glucose release (per stem)	487.84 $\pm$ 65.50	790.72 $\pm$ 136.87	*
<u>Saccharification (acid pretreatment) (1M HCl)</u>			
Cellulose-to-glucose conversion (%)	28.20 $\pm$ 3.37	38.73 $\pm$ 0.79	**
Glucose release (per %DW)	7.82 $\pm$ 0.81	7.48 $\pm$ 0.80	-
Glucose release (per stem)	586.76 $\pm$ 60.91	790.67 $\pm$ 84.15	*
<u>Saccharification (alkaline pretreatment) (62.5 mM NaOH)</u>			
Cellulose-to-glucose conversion (%)	27.76 $\pm$ 5.24	29.79 $\pm$ 4.72	-
Glucose release (per %DW)	7.69 $\pm$ 1.41	5.69 $\pm$ 0.50	*
Glucose release (per stem)	577.20 $\pm$ 105.56	601.35 $\pm$ 53.16	-

Table 3: Cellulose conversions and pre-treatment effects - 48 hours

Cellulose-to-glucose conversions for 3 months old CA- and mock-treated *Nicotiana* plants (n = 12) ($\pm$ standard deviation) without and with acid or alkaline pretreatment were calculated based on the cellulose content and the saccharification yield (or glucose release) (both on a CWR basis) and are expressed as percentage cellulose. The glucose release is also stated on a dry weight basis (%DW) and a dry weight stem basis (%stem). Absolute values represent averages and standard deviations. Asterisks (*) indicate statistically significant differences in between CA- and mock-treated *Nicotiana* plants as determined by Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P < 0.0001.

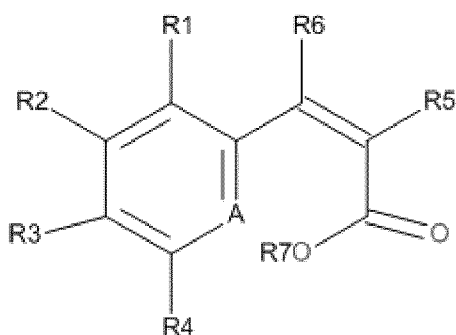
Detailed description of the invention

The present invention will be described with respect to particular embodiments but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

The following terms or definitions are provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook *et al*, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Press, Plainsview, New York (2012); and Ausubel *et al.*, Current Protocols in Molecular Biology (Supplement 100), John Wiley & Sons, New York (2012), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

Cinnamic acid (CA) is a plant-endogenous compound that exists in two different isoforms and is detected in different plant species (Yin, 2003; Wong *et al.*, 2005). Recently, we re-evaluated the role of both isoforms and were able to confirm that *trans*-(*t*)-CA is an inactive, intermediate of the core phenylpropanoid pathway. Contrary to its *t*-form, *c*-CA is the biologically active form that affects the root architecture of an evolutionary diverse set of plant species, while being added to the tissue culture medium. We previously showed that *c*-CA belongs to a group of compounds that inhibits cellular auxin efflux and alters the auxin distribution within the root (Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943). In the present invention we surprisingly showed that *cis*-CA and variants thereof have a strong positive effect on the vegetative growth of plants. More particularly we found that *cis*-CA and its functional variants have a positive effect on the yield biomass of plants, such as inducing increased root biomass, increased shoot biomass and increased leaf biomass of plants. A functional variant of *cis*-CA is herein recognized a chemical variant which has an effect on the primary root inhibition and on lateral root induction as is shown in Figure 10.

Accordingly the present invention provides in a first embodiment the use of a compound of formula (I) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof



(I)

wherein in (I)

R1 is hydrogen, halogen, C1-C4 alkyl, or a C1-C4-alkoxy group, and

5 R2 is hydrogen, halogen, CF₃, C1-C4-alkyl or a C1-C4-alkoxy group, and

R3 is hydrogen, halogen, C1-C4 alkyl or a C1-C4-alkoxy group, and

R4 is hydrogen, CX₃ (where X is any halogen), or a C1-C4-alkoxy group, and

R5 is hydrogen or alkyl, and

R6 is hydrogen or alkyl, and

10 R7 is hydrogen or alkyl

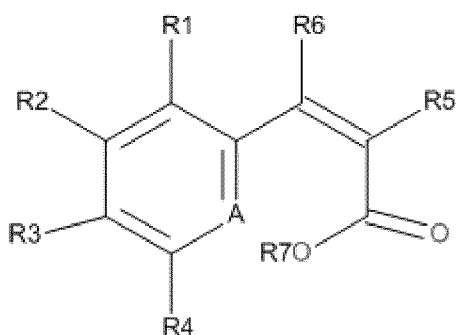
R5 and R6 can form a closed ring structure to form a cyclopropyl group or R1 and R6 can form a closed ring structure selected from the list consisting of cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, and

A can be C or N

15 O can be S

for the stimulation of biomass in plants.

In another embodiment the invention provides the use of a compound of formula (II) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof



(II)

wherein in (II)

R1 is hydrogen, halogen, C1-C4 alkyl, or a C1-C4-alkoxy group, and

5 R2 is hydrogen, halogen, CF₃, C1-C4-alkyl or a C1-C4-alkoxy group, and

R3 is hydrogen, halogen, C1-C4 alkyl or a C1-C4-alkoxy group, and

R4 is hydrogen, CF₃ or a C1-C4-alkoxy group, and

R5 is hydrogen or methyl, and

R6 is hydrogen or methyl, and

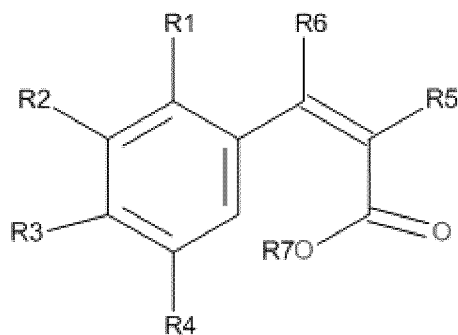
10 R7 is hydrogen

R5 and R6 can form a closed ring structure to form a cyclopropyl group or R1 and R6 can form a closed ring structure selected from the list consisting of cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, and

A can be C or N.

15 for the stimulation of biomass in plants.

In yet another embodiment the invention provides the use of a compound of formula (II) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof



(III)

wherein in (III)

R1 is hydrogen, halogen, a methyl group or a methoxy group, and

5 R2 is hydrogen, halogen, CF₃, a methyl group or a methoxy group, and

R3 is hydrogen, halogen, a methyl group or a methoxy group, and

R4 is hydrogen, CF₃ or a methoxy group, and

R5 is hydrogen or methyl, and

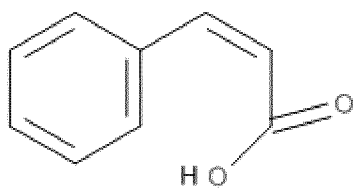
R6 is hydrogen or methyl, and

10 R7 is hydrogen, and

R5 and R6 can form a closed ring structure to form a cyclopropyl group or R1 and R6 can form a closed ring structure selected from the list consisting of cyclopentyl, and cyclohexyl,

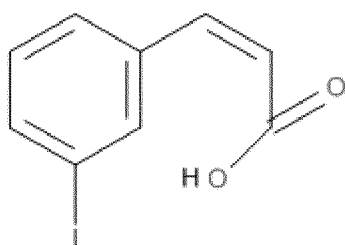
for the stimulation of biomass in plants.

15 In yet another embodiment the invention provides the use of a compound selected from the list consisting of:

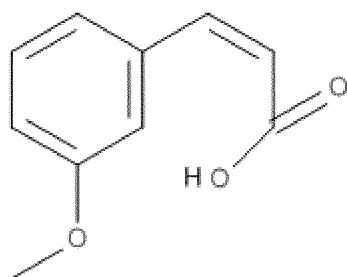


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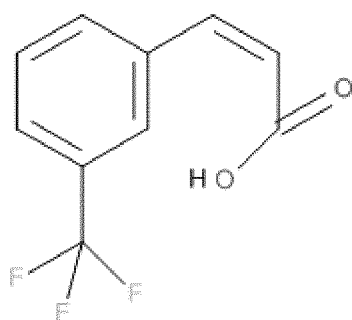
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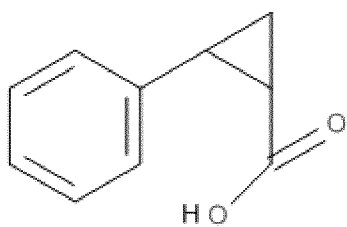


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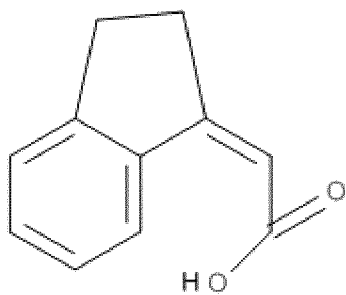


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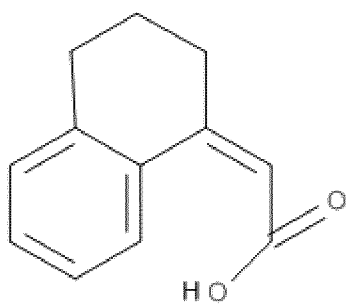
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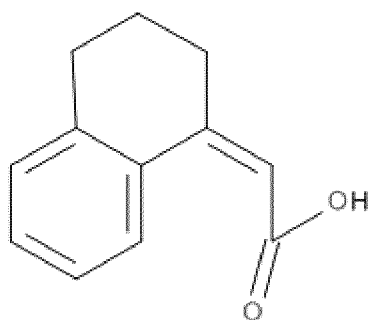
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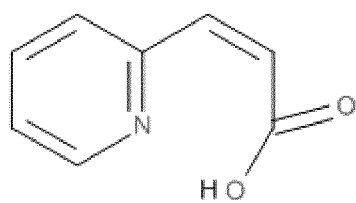
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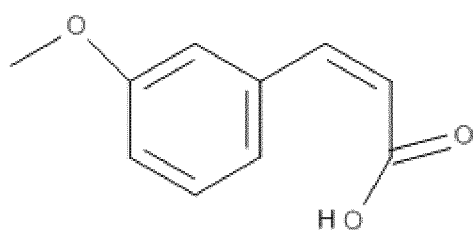
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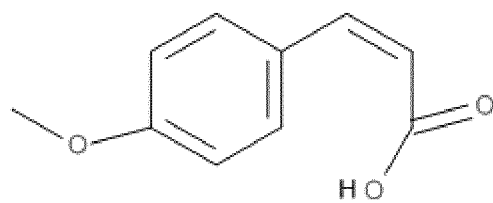
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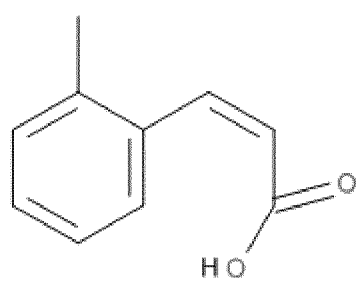
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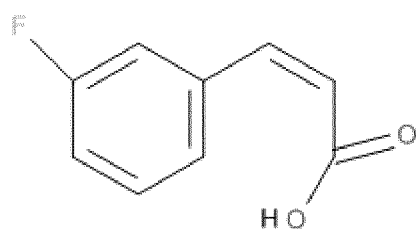


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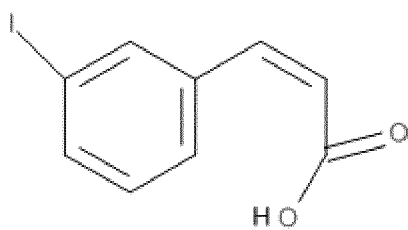
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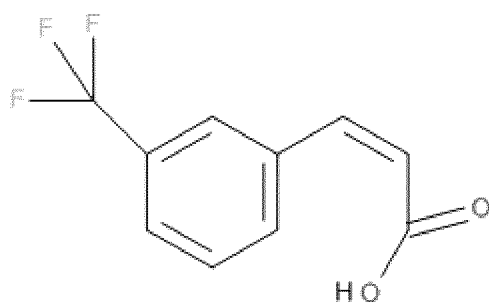
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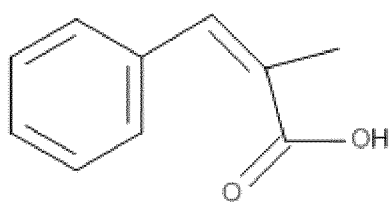
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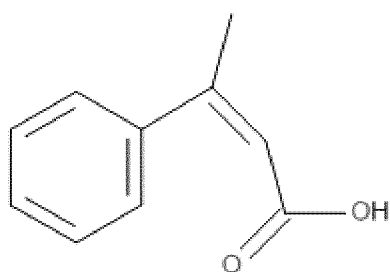


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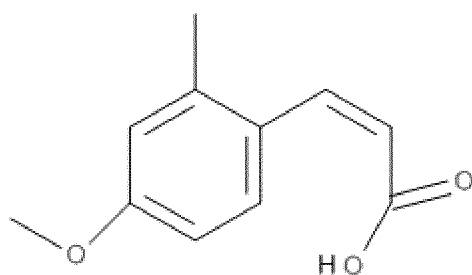


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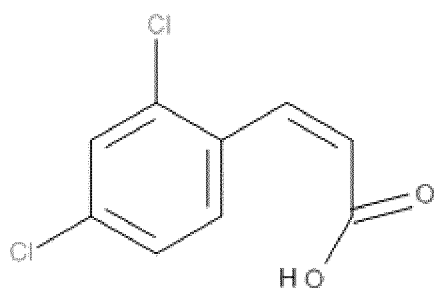


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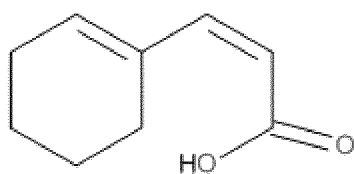


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for stimulating the biomass of plants.

In yet another specific embodiment the invention provides a compound with the structure



for use to stimulate biomass in plants.

In yet another embodiment the compounds of the invention (as specified herein before – *id est* compounds of Formulae I and II and the specific compounds cited herein before) can be used to stimulate the vegetative growth of plants.

- 5 In yet another embodiment the compounds of the invention (as specified herein before) can be used to enhance the yield of plants.

In yet another embodiment the compounds of the invention (as specified herein before) can be used to stimulate the shoot and leaf growth of plants.

- 10 In yet another embodiment the compounds of the invention (as specified herein before) can be used to stimulate the root growth of plants.

In yet another embodiment the compounds of the invention (as specified herein before) can be used to coat plant seeds.

- 15 In yet another embodiment the compounds of the invention (as specified herein before) can be used to coat plant seeds wherein said coated plant seeds when planted and germinate have an increased vigor.

In yet another embodiment the invention provides a coated plant seed comprising the compounds of the invention (as specified herein before).

According to the invention, the following generic terms are generally used with the following meanings:

- 20 The term "alkoxy" refers to an alkyl linked to an oxygen, which may also be represented as: -O-R, wherein the R represents the alkyl group. Examples of alkoxy include methoxy, ethoxy, propoxy and butoxy. These latter groups are herein designated as C1-C4-alkoxy groups.

The term "halogen" includes chloro, fluoro, bromo and iodo. Preferred halogens are chloro, iodo and fluoro.

- 25 The term "C1-C4-alkyl" refers to a linear or branched-chain saturated, mono- unsaturated and poly-unsaturated hydrocarbyl substituent (i.e., a substituent obtained from a hydrocarbon by removal of a hydrogen) containing one to four carbon atoms. Mono- and poly-unsaturated substituents, also called alkenyl, have 2 to 4 carbon atoms. The alkenyl group may exist as the pure E (*entgegen*) form, the pure Z (*zusammen*) form, or any mixture thereof. Poly-unsaturated
30 includes multiple double bonds and one or more triple bonds. Such triple bond containing alkyl

groups, a so called alkynyl group, has 2 to 4 carbon atoms. Examples of such saturated substituents include methyl, ethyl, propyl (including n-propyl and isopropyl), butyl (including n-butyl, isobutyl, sec-butyl and tert-butyl) and the like. Examples of unsaturated alkyl include ethenyl, 1-propenyl, 2-propenyl (allyl), iso-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, and the like. Examples of alkynyl include ethynyl, propynyl, butynyl, 3,3-dimethylbutynyl and the like. Particularly preferred C1-C4-alkyl groups are methyl, ethyl, propyl and isopropyl groups.

Any of the compounds according to the invention can exist as one or more stereoisomers depending on the number of stereogenic centres (as defined by the IUPAC rules) in the compound. The invention thus relates equally to all the stereoisomers, and to the mixtures of all the possible stereoisomers, in all proportions. Typically one of the stereoisomers has enhanced biological activity compared to the other possibilities and in the present invention we show that the cis-isomer of cinnamic acid and the cis-isomers of the cis-cinnamic acid derivatives as specified in compounds of formulae I and II are biologically active. Furthermore it should be appreciated that all tautomeric forms (single tautomer or mixtures thereof), racemic mixtures and single isomers of compounds of formula (I) and (II) are included within the scope of the present invention.

Suitable pharmaceutically acceptable acid addition salts of the compounds of the present invention when possible include those derived from inorganic acids, such as hydrochloric, hydrobromic, hydrofluoric, boric, fluoroboric, phosphoric, metaphosphoric, nitric, carbonic, sulfonic, and sulfuric acids, and organic acids such as acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glycolic, isothionic, lactic, lactobionic, maleic, malic, methanesulfonic, trifluoromethanesulfonic, succinic, toluenesulfonic, tartaric, and trifluoroacetic acids. Suitable organic acids generally include, for example, aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic, and sulfonic classes of organic acids.

Furthermore, since the compounds of the invention carry an acidic moiety, suitable agrochemical acceptable salts thereof may include alkali metal salts, e.g. sodium or potassium salts; alkaline earth metal salts, e.g. calcium or magnesium salts; and salts formed with suitable organic ligands, e.g. quaternary ammonium salts. In another embodiment, base salts are formed from bases which form non-toxic salts, including aluminum, arginine, benzathine, choline, diethylamine, diolamine, glycine, lysine, meglumine, olamine, tromethamine and zinc salts. In one embodiment, hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

The present invention also includes isotopically labelled compounds, which are identical to those recited in formula I and (II), but for the fact that one or more atoms are replaced by an atom

having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that may be incorporated into compounds of the present invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chlorine, such as ^2H , ^3H , ^{13}C , ^{11}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively.

5 Compounds of the present invention and pharmaceutically acceptable salts of said compounds or which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ^3H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^3H , and carbon-14, i.e., ^{14}C ,
10 isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of formula (I) and (II) of this invention may generally be prepared by carrying
15 out the procedures disclosed in Nishikawa K. *et al* (2013) *Phytochemistry* 96, 132-147, Nishikawa K. *et al* (2013) *Phytochemistry* 96, 223-234 and Abe M. *et al* (2012) *Phytochemistry* 84, 56-67), by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

The term "plants" generally comprises all plants of economic importance and/or men-grown
20 plants. They are preferably selected from agricultural, silvicultural and ornamental plants, more preferably agricultural plants and silvicultural plants, most preferably agricultural plants. The term "plant (or plants)" is a synonym of the term "crop" which is to be understood as a plant of economic importance and/or a men-grown plant. The term "plant" as used herein includes all parts of a plant such as germinating seeds, emerging seedlings, herbaceous vegetation as well
25 as established woody plants including all belowground portions (such as the roots) and aboveground portions.

The plants to be treated according to the invention are selected from the group consisting of agricultural, silvicultural, ornamental and horticultural plants, each in its natural or genetically modified form, preferably from agricultural plants.

30 In a preferred embodiment, the plant to be treated according to the method of the invention is an agricultural plant. "Agricultural plants" are plants of which a part or all is harvested or cultivated on a commercial scale or which serve as an important source of feed, food, fibres (e.g. cotton, linen), combustibles (e.g. wood, bioethanol, biodiesel, biomass) or other chemical compounds. Agricultural plants also include vegetables. Thus, the term agricultural plants
35 include cereals, e.g. wheat, rye, barley, triticale, oats, sorghum or rice; beet, e.g. sugar beet or

fodder beet; leguminous plants, such as lentils, peas, alfalfa or soybeans; oil plants, such as rape, oil-seed rape, canola, juncea (*Brassica juncea*), linseed, mustard, olives, sunflowers, cocoa beans, castor oil plants, oil palms, ground nuts or soybeans; cucurbits, such as squashes, cucumber or melons; fiber plants, such as cotton, flax, hemp or jute; vegetables, such as cucumbers, spinach, lettuce, asparagus, cabbages, carrots, onions, tomatoes, potatoes, cucurbits or paprika; lauraceous plants, such as avocados, cinnamon or camphor; energy and raw material plants, such as corn, soybean, rape, canola, sugar cane or oil palm; corn; tobacco; nuts; coffee; tea; vines (table grapes and grape juice grape vines); hop; turf and natural rubber plants.

- 10 In one embodiment, the plant to be treated according to the method of the invention is a horticultural plant. The term "horticultural plants" are to be understood as plants which are commonly used in horticulture - e.g. the cultivation of ornamentals, vegetables and/or fruits. Examples for ornamentals are turf, geranium, pelargonium, petunia, begonia and fuchsia. Examples for vegetables are potatoes, tomatoes, peppers, cucurbits, cucumbers, melons, watermelons, garlic, onions, carrots, cabbage, beans, peas and lettuce and more preferably from tomatoes, onions, peas and lettuce. Examples for fruits are apples, pears, cherries, strawberry, citrus, peaches, apricots and blueberries.

- 20 In one embodiment, the plant to be treated according to the method of the invention is an ornamental plant. Ornamental plants" are plants which are commonly used in gardening, e.g. in parks, gardens and on balconies. Examples are turf, geranium, pelargonium, petunia, begonia and fuchsia.

- 25 In one embodiment, the plant to be treated according to the method of the invention is a silvicultural plant. The term "silvicultural plant" is to be understood as trees, more specifically trees used in reforestation or industrial plantations. Industrial plantations generally serve for the commercial production of forest products, such as wood, pulp, paper, rubber tree, Christmas trees, or young trees for gardening purposes. Examples for silvicultural plants are conifers, like pines, in particular *Pinus spec*, fir and spruce, eucalyptus, tropical trees like teak, rubber tree, oil palm, willow (*Salix*), in particular *Salix spec*, poplar (cottonwood), in particular *Populus spec*, beech, in particular *Fagus spec*, birch, oil palm and oak.

- 30 The term "plants" also includes plants which have been modified by breeding, mutagenesis or genetic engineering (transgenic and non-transgenic plants). Genetically modified plants are plants, which genetic material has been modified by the use of recombinant DNA techniques in a way that it cannot readily be obtained by cross breeding under natural circumstances, mutations or natural recombination. Typically, one or more genes have been integrated into the

genetic material of a genetically modified plant in order to improve certain properties of the plant. Such genetic modifications also include but are not limited to targeted post-translational modification of protein(s), oligo- or polypeptides e.g. by glycosylation or polymer additions such as prenylated, acetylated or farnesylated moieties.

- 5 Plants as well as the propagation material of said plants, which can be treated with the agrochemical formulations comprising the compounds of formula (I) and (II) include all modified non-transgenic plants or transgenic plants, e.g. crops which tolerate the action of herbicides or fungicides or insecticides owing to breeding, including genetic engineering methods, or plants which have modified characteristics in comparison with existing plants, which can be generated
10 for example by traditional breeding methods and/or the generation of mutants, or by recombinant procedures.

For example, agrochemical formulations comprising the compounds of formula (I) and (II) according to the present invention can be applied (as seed treatment, foliar spray treatment, in-furrow application or by any other means) also to plants which have been modified by breeding,
15 mutagenesis or genetic engineering including but not limiting to agricultural biotech products on the market or in development (cf. http://www.bio.org/speeches/pubs/er/agri_products.asp).

Plants that have been modified by breeding, mutagenesis or genetic engineering, e.g. have been rendered tolerant to applications of specific classes of herbicides.

Furthermore, plants are also covered that are generated by the use of recombinant DNA
20 techniques capable to synthesize one or more insecticidal proteins, especially those known from the bacterial genus *Bacillus*, particularly from *Bacillus thuringiensis*.

Furthermore, plants are also covered that are generated by the use of recombinant DNA techniques capable to synthesize one or more proteins to increase the resistance or tolerance of those plants to bacterial, viral or fungal pathogens. Furthermore, plants are also covered that
25 are by the use of recombinant DNA techniques capable to synthesize one or more proteins to increase the productivity (e.g. biomass production, grain yield, starch content, oil content or protein content), tolerance to drought, salinity or other growth-limiting environmental factors or tolerance to pests and fungal, bacterial or viral pathogens of those plants.

Furthermore, plants are also covered that contain, by the use of recombinant DNA techniques,
30 a modified amount of substances of content or new substances of content, specifically to improve human or animal nutrition, e.g. oil crops that produce health-promoting long-chain omega-3 fatty acids or unsaturated omega-9 fatty acids.

Furthermore, plants are also covered that contain, by the use of recombinant DNA techniques, a modified amount of substances of content or new substances of content, specifically to improve raw material production, e.g. potatoes that produce increased amounts of amylopectin.

The term "plant propagation material" is to be understood to denote all the generative parts of the plant such as seeds and vegetative plant material such as cuttings and tubers (e.g. potatoes), which can be used for the multiplication of the plant. This includes seeds, grains, roots, fruits, tubers, bulbs, rhizomes, cuttings, spores, offshoots, shoots, sprouts and other parts of plants, including seedlings and young plants, which are to be transplanted after germination or after emergence from soil, meristem tissues, single and multiple plant cells and any other plant tissue from which a complete plant can be obtained. The term "propagules" or "plant propagules" is to be understood to denote any structure with the capacity to give rise to a new plant, e.g. a seed, a spore, or a part of the vegetative body capable of independent growth if detached from the parent. In a preferred embodiment, the term "propagules" or "plant propagules" denotes for seed.

In a particular embodiment the compounds of formula (I) and (II) or an agrochemical formulation comprising a compound of formula (I) and (II) are used for increasing the yield of plants.

According to the present invention, "increased yield" of a plant, in particular of an agricultural, silvicultural and/or horticultural plant means that the yield of a product of the respective plant is increased by a measurable amount over the yield of the same product of the plant produced under the same conditions, but without the application of the agrochemical formulation of the invention.

Increased yield can be characterized, among others, by the following improved properties of the plant: increased plant weight, increased biomass such as higher overall fresh weight (FW) or higher total dry matter (TDM), increased number of flowers per plant, higher grain and/or fruit yield, more tillers or side shoots (branches), larger leaves, increased shoot growth, increased protein content, increased oil content, increased starch content, increased pigment content, increased chlorophyll content (chlorophyll content has a positive correlation with the plant's photosynthesis rate and accordingly, the higher the chlorophyll content the higher the yield of a plant), In a preferred embodiment, the term "yield" refers to fruits in the proper sense, vegetables, nuts, grains and seeds. "Grain" and "fruit" are to be understood as any plant product which is further utilized after harvesting, e.g. fruits in the proper sense, vegetables, nuts, grains, seeds, wood (e.g. in the case of silviculture plants), flowers (e.g. in the case of gardening plants, ornamentals) etc., that is anything of economic value that is produced by the plant.

According to the present invention, the yield is increased by at least 5 %, preferable by 5 to 10 %, more preferable by 10 to 20 %, or even 20 to 30 %, or even 30 to 40%, or even 40 to 50%, or even 50 to 60% compared to the untreated control plants. In general, the yield increase may even be higher.

- 5 In yet another particular embodiment the compound of formula (I) and (II) or an agrochemical formulation comprising a compound of formula (I) and (II) are used for increasing the vigor of plants.

Another indicator for the condition of the plant is the plant vigor. The plant vigor becomes manifest in several aspects such as the general visual appearance.

- 10 Improved plant vigor can be characterized, among others, by the following improved properties of the plant: improved vitality of the plant, improved plant growth, improved plant development, improved visual appearance, improved plant stand (less plant verse/lodging), improved emergence, enhanced root growth and/or more developed root system, enhanced nodulation, in particular rhizobial nodulation, bigger leaf blade, bigger size, increased plant height, increased
15 tiller number, increased number of side shoots, increased number of flowers per plant, increased shoot growth, increased root growth (extensive root system), enhanced photosynthetic activity (e.g. based on increased stomatal conductance and/or increased CO₂ assimilation rate), enhanced pigment content, earlier flowering, earlier fruiting, earlier and improved germination, earlier grain maturity, less non-productive tillers, less dead basal leaves, less input needed (such
20 as fertilizers or water), greener leaves, complete maturation under shortened vegetation periods, less fertilizers needed, less seeds needed, easier harvesting, faster and more uniform ripening, longer shelf-life, longer panicles, delay of senescence, stronger and/or more productive tillers, better extractability of ingredients, improved quality of seeds (for being seeded in the following seasons for seed production), reduced production of ethylene and/or the inhibition of its
25 reception by the plant.

According to the present invention, the plant vigor is increased by at least 5 %, preferable by 5 to 10 %, more preferable by 10 to 20 %, or even 20 to 30 % compared to the untreated control plants. In general, the plant vigor increase may even be higher.

- 30 In another embodiment of the invention, the agrochemical formulations comprising the compounds of formula (I) and (II) are used for increasing the total dry matter (TDM) of a plant.

In another embodiment the agrochemical formulations comprising the compounds of formula (I) and (II) increase the vigor of a plant or its products.

In another embodiment the agrochemical formulations comprising the compounds of formula (I) and (II) increase the quality of a plant or its products.

In another embodiment the agrochemical formulations comprising the compounds of formula (I) and (II) increase the digestibility of a plant or its products.

- 5 In another embodiment the agrochemical formulations comprising the compounds of formula (I) and (II) increase the saccharification efficiency of a plant or its products.

The agrochemical formulations comprising the compounds of formula (I) and (II) are employed by treating the plant, plant propagation material, soil, area, material or environment in which a plant is growing or may grow with an effective amount of the active compounds.

- 10 In a particular embodiment the agrochemical formulations comprising the compounds of formula (I) and (II) are used on plants growing in hydroponic culture. The term "hydroponics" refers to the method of growing plants without soil, using mineral nutrient solutions in a water solvent. Typically plants may be grown with only their roots exposed to the mineral solution, or the roots may be supported by an inert medium, such as perlite or gravel.

- 15 When preparing the agrochemical formulations comprising the compounds of formula (I) and (II), it is preferred to employ the pure active compounds, to which further active compounds against pests, such as insecticides, herbicides, fungicides or else herbicidal or growth-regulating active compounds or fertilizers can be added as further active components according to need.

- As stated above, the agrochemical formulations comprising the compounds of formula (I) and
20 (II) are used in "effective amounts". This means that they are used in a quantity which allows to obtain the desired effect which is a synergistic increase of the health of a plant but which does not give rise to any phytotoxic symptom on the treated plant.

- For use according to the present invention, the agrochemical formulations comprising the compounds of formula (I) and (II) can be converted into the customary formulations, for example
25 solutions, emulsions, suspensions, dusts, powders, pastes and granules. The use form depends on the particular intended purpose; in each case, it should ensure a fine and even distribution of the agrochemical formulations comprising the compounds of formula (I) according to the present invention. The formulations are prepared in a known manner to the person skilled in the art.

- The agrochemical formulations may also comprise auxiliaries which are customary in
30 agrochemical formulations. The auxiliaries used depend on the particular application form and active substance, respectively. Examples for suitable auxiliaries are solvents, solid carriers,

dispersants or emulsifiers (such as further solubilizers, protective colloids, surfactants and adhesion agents), organic and inorganic thickeners, bactericides, anti-freezing agents, anti-foaming agents, if appropriate colorants and tackifiers or binders (e.g. for seed treatment formulations).

- 5 Suitable solvents are water, organic solvents such as mineral oil fractions of medium to high boiling point, such as kerosene or diesel oil, furthermore coal tar oils and oils of vegetable or animal origin, aliphatic, cyclic and aromatic hydrocarbons, e.g. toluene, xylene, paraffin, tetrahydronaphthalene, alkylated naphthalenes or their derivatives, alcohols such as methanol, ethanol, propanol, butanol and cyclohexanol, glycols, ketones such as cyclohexanone and
10 gamma-butyrolactone, fatty acid dimethylamides, fatty acids and fatty acid esters and strongly polar solvents, e.g. amines such as N-methylpyrrolidone.

Solid carriers are mineral earths such as silicates, silica gels, talc, kaolins, limestone, lime, chalk, bole, loess, clays, dolomite, diatomaceous earth, calcium sulfate, magnesium sulfate, magnesium oxide, ground synthetic materials, fertilizers, such as, e.g. ammonium sulfate,
15 ammonium phosphate, ammonium nitrate, ureas, and products of vegetable origin, such as cereal meal, tree bark meal, wood meal and nutshell meal, cellulose powders and other solid carriers.

Suitable surfactants (adjuvants, wetters, tackifiers, dispersants or emulsifiers) are alkali metal, alkaline earth metal and ammonium salts of aromatic sulfonic acids, such as ligninsulfonic acid, phenolsulfonic acid, naphthalenesulfonic acid, dibutylnaphthalene-sulfonic acid and fatty acids, alkylsulfonates, alkyl-arylsulfonates, alkyl sulfates, laurylether sulfates, fatty alcohol sulfates, and sulfated hexa-, hepta- and octadecanates, sulfated fatty alcohol glycol ethers, furthermore condensates of naphthalene or of naphthalenesulfonic acid with phenol and formaldehyde, polyoxy-ethylene octylphenyl ether, ethoxylated isooctylphenol, octylphenol, nonylphenol,
20 alkylphenyl polyglycol ethers, tributylphenyl polyglycol ether, tristearyl-phenyl polyglycol ether, alkylaryl polyether alcohols, alcohol and fatty alcohol/ethylene oxide condensates, ethoxylated castor oil, polyoxyethylene alkyl ethers, ethoxylated polyoxypropylene, lauryl alcohol polyglycol ether acetal, sorbitol esters, lignin-sulfite waste liquid and proteins, denatured proteins, polysaccharides (e.g. methylcellulose), hydrophobically modified starches, polyvinyl alcohols,
25 polycarboxylates types, polyalkoxylates, polyvinylamines, polyvinylpyrrolidone and the copolymers thereof. Examples for thickeners (i.e. compounds that impart a modified flowability to formulations, i.e. high viscosity under static conditions and low viscosity during agitation) are polysaccharides and organic and inorganic clays such as Xanthan gum.
30

Bactericides may be added for preservation and stabilization of the formulation. Examples for suitable bactericides are those based on dichlorophene and benzylalcohol hemi formal (Proxel® from ICI or Acticide® RS from Thor Chemie and Kathon® MK from Rohm & Haas) and isothiazolinone derivatives such as alkylisothiazolinones and benzisothiazolinones (Acticide® M BS from Thor Chemie). Examples for suitable anti-freezing agents are ethylene glycol, propylene glycol, urea and glycerin. Examples for anti-foaming agents are silicone emulsions (such as e.g. Silikon® SRE, Wacker, Germany or Rhodorsil®, Rhodia, France), long chain alcohols, fatty acids, salts of fatty acids, fluoroorganic compounds and agrochemical formulations comprising the compounds of formula (I) thereof.

- 10 Suitable colorants are pigments of low water solubility and water-soluble dyes.

Examples for tackifiers or binders are polyvinylpyrrolidons, polyvinylacetates, polyvinyl alcohols and cellulose ethers (Tylose®, Shin-Etsu, Japan).

- 15 Granules, e.g. coated granules, impregnated granules and homogeneous granules, can be prepared by binding the active substances to solid carriers. Examples of solid carriers are mineral earths such as silica gels, silicates, talc, kaolin, attaclay, limestone, lime, chalk, bole, loess, clay, dolomite, diatomaceous earth, calcium sulfate, magnesium sulfate, magnesium oxide, ground synthetic materials, fertilizers, such as, e.g., ammonium sulfate, ammonium phosphate, ammonium nitrate, ureas, and products of vegetable origin, such as cereal meal, tree bark meal, wood meal and nutshell meal, cellulose powders and other solid carriers.

- 20 The agrochemical formulations generally comprise between 0.01 and 95%, preferably between 0.1 and 90%, most preferably between 0.5 and 90%, by weight of active substances. The compounds of the agrochemical formulations comprising the compounds of formula (I) and (II) are employed in a purity of from 90% to 100%, preferably from 95% to 100% (according to their NMR spectrum).

- 25 The compounds of the agrochemical formulations comprising the compounds of formula (I) and (II) can be used as such or in the form of their agricultural compositions, e.g. in the form of directly sprayable solutions, powders, suspensions, dispersions, emulsions, oil dispersions, pastes, dustable products, materials for spreading, or granules, by means of spraying, atomizing, dusting, spreading, brushing, immersing or pouring. The application forms depend
30 entirely on the intended purposes; it is intended to ensure in each case the finest possible distribution of the compounds present in the agrochemical formulations comprising the compounds of formula (I) and (II).

Aqueous application forms can be prepared from emulsion concentrates, pastes or wettable powders (sprayable powders, oil dispersions) by adding water. To prepare emulsions, pastes or oil dispersions, the substances, as such or dissolved in an oil or solvent, can be homogenized in water by means of a wetter, tackifier, dispersant or emulsifier. Alternatively, it is possible to prepare concentrates composed of active substance, wetter, tackifier, dispersant or emulsifier and, if appropriate, solvent or oil, and such concentrates are suitable for dilution with water.

The active substance concentrations in the ready-to-use preparations can be varied within relatively wide ranges. In general, they are from 0.0001 to 10%, preferably from 0.001 to 1 % by weight of compounds of the agrochemical formulations comprising the compounds of formula (I) and (II).

The compounds of the agrochemical formulations comprising the compounds of formula (I) and (II) may also be used successfully in the ultra-low-volume process (ULV), it being possible to apply compositions comprising over 95% by weight of active substance, or even to apply the active substance without additives.

Various types of oils, wetters, adjuvants, herbicides, fungicides, other pesticides, or bactericides may be added to the active compounds, if appropriate not until immediately prior to use (tank mix). These agents can be admixed with the compounds of the agrochemical formulations comprising the compounds of formula (I) in a weight ratio of 1:100 to 100:1, preferably 1:10 to 10:1.

Compositions of this invention may also contain fertilizers such as ammonium nitrate, urea, potash, and superphosphate, phytotoxicants and plant growth regulators and safeners. These may be used sequentially or in combination with the above-described compositions, if appropriate also added only immediately prior to use (tank mix). For example, the plant(s) may be sprayed with a composition of this invention either before or after being treated with the fertilizers.

In the agrochemical formulations comprising the compounds of formula (I) and (II), the weight ratio of the compounds generally depends from the properties of the compounds of the agrochemical formulations comprising the compounds of formula (I) and (II).

The compounds of the agrochemical formulations comprising the compounds of formula (I) and (II) can be used individually or already partially or completely mixed with one another to prepare the composition according to the invention. It is also possible for them to be packaged and used further as combination composition such as a kit of parts.

The user applies the composition according to the invention usually from a pre-dosage device, a knapsack sprayer, a spray tank or a spray plane. Here, the agrochemical composition is made up with water and/or buffer to the desired application concentration, it being possible, if appropriate, to add further auxiliaries, and the ready-to-use spray liquid or the agrochemical composition according to the invention is thus obtained. Usually, 50 to 500 liters of the ready-to-use spray liquid are applied per hectare of agricultural useful area, preferably 50 to 400 liters.

In a particular embodiment the absolute amount of the active compounds, represented by formula (I) and (II), is used in a range between 1mg/liter-100mg/liter, particularly in a range between 1mg/l-20mg/l, particularly in a range between 1mg/l-25mg/l, particularly in a range between 2mg/l-200mg/l, particularly between 2mg/l-100mg/l, particularly between 2mg/l-50mg/l, particularly between 2mg/l-25mg/l, particularly between 4mg/l-40mg/l, particularly between 4mg/l-20mg/l, particularly between 4mg/l-16mg/l, particularly between 4mg/l-12mg/l.

According to one embodiment, individual compounds of the agrochemical formulations comprising the compounds of formula (I) and (II) formulated as composition (or formulation) such as parts of a kit or parts of the inventive mixture may be mixed by the user himself in a spray tank and further auxiliaries may be added, if appropriate (tank mix).

“Agrochemical”, as used herein, means any active substance that may be used in the agrochemical industry (including agriculture, horticulture, floriculture and home and garden uses, but also products intended for non-crop related uses such as public health/pest control operator uses to control undesirable insects and rodents, household uses, such as household fungicides and insecticides and agents, for protecting plants or parts of plants, crops, bulbs, tubers, fruits (e.g. from harmful organisms, diseases or pests); for controlling, preferably promoting or increasing, the growth of plants; and/or for promoting the yield of plants, crops or the parts of plants that are harvested (e.g. its fruits, flowers, seeds etc.).

An “agrochemical composition” as used herein means a composition for agrochemical use, as herein defined, comprising at least one active substance of a compound of formula (I), optionally with one or more additives favoring optimal dispersion, atomization, deposition, leaf wetting, distribution, retention and/or uptake of agrochemicals. As a non-limiting example such additives are diluents, solvents, adjuvants, surfactants, wetting agents, spreading agents, oils, stickers, thickeners, penetrants, buffering agents, acidifiers, anti-settling agents, anti-freeze agents, photo-protectors, defoaming agents, biocides and/or drift control agents.

A “carrier”, as used herein, means any solid, semi-solid or liquid carrier in or on(to) which an active substance can be suitably incorporated, included, immobilized, adsorbed, absorbed,

bound, encapsulated, embedded, attached, or comprised. Non-limiting examples of such carriers include nanocapsules, microcapsules, nanospheres, microspheres, nanoparticles, microparticles, liposomes, vesicles, beads, a gel, weak ionic resin particles, liposomes, cochleate delivery vehicles, small granules, granulates, nano-tubes, bucky-balls, water droplets
5 that are part of an water-in-oil emulsion, oil droplets that are part of an oil-in-water emulsion, organic materials such as cork, wood or other plant-derived materials (e.g. in the form of seed shells, wood chips, pulp, spheres, beads, sheets or any other suitable form), paper or cardboard, inorganic materials such as talc, clay, microcrystalline cellulose, silica, alumina, silicates and zeolites, or even microbial cells (such as yeast cells) or suitable fractions or fragments thereof.

10 The terms "effective amount", "effective dose" and "effective amount", as used herein, mean the amount needed to achieve the desired result or results. More exemplary information about amounts, ways of application and suitable ratios to be used is given below. The skilled artisan is well aware of the fact that such an amount can vary in a broad range and is dependent on various factors such as the treated cultivated plant as well as the climatic and soil conditions.

15 As used herein, the terms "determining", "measuring", "assessing", "monitoring" and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

It is understood that the agrochemical composition is stable, both during storage and during utilization, meaning that the integrity of the agrochemical composition is maintained under storage and/or utilization conditions of the agrochemical composition, which may include
20 elevated temperatures, freeze-thaw cycles, changes in pH or in ionic strength, UV-irradiation, presence of harmful chemicals and the like. More preferably, the compounds of formula (I) as herein described remain stable in the agrochemical composition, meaning that the integrity and the activity of the compounds are maintained under storage and/or utilization conditions of the agrochemical composition, which may include elevated temperatures, freeze-thaw cycles,
25 changes in pH or in ionic strength, UV-irradiation, presence of harmful chemicals and the like. Most preferably, said compounds of formula (I) remain stable in the agrochemical composition when the agrochemical composition is stored at ambient temperature for a period of two years or when the agrochemical composition is stored at 54°C for a period of two weeks. Preferably, the agrochemical composition of the present invention retains at least about 70% activity, more
30 preferably at least about 70% to 80% activity, most preferably about 80% to 90% activity or more. Examples of suitable carriers include, but are not limited to alginates, gums, starch, β -cyclodextrins, celluloses, polyurea, polyurethane, polyester, or clay.

The agrochemical composition may occur in any type of formulation, preferred formulations are powders, wettable powders, wettable granules, water dispersible granules, emulsions,

emulsifiable concentrates, dusts, suspensions, suspension concentrates, suspoemulsions, capsule suspensions, aqueous dispersions, oil dispersions, aerosols, pastes, foams, slurries or flowable concentrates.

According to the method of the present invention, the agrochemical composition according to the invention can be applied once to a crop, or it can be applied two or more times after each other with an interval between every two applications. According to the method of the present invention, the agrochemical composition according to the invention can be applied alone or in mixture with other materials, preferably other agrochemical compositions, to the crop; alternatively, the agrochemical composition according to the invention can be applied separately to the crop with other materials, preferably other agrochemical compositions, applied at different times to the same crop.

In yet another embodiment the invention provides a method for the manufacture ('or the production of' which is equivalent wording) an agrochemical composition according to the invention, comprising formulating a molecule of formula (I) as defined herein before, together with at least one customary agrochemical auxiliary agent. Suitable manufacturing methods are known in the art and include, but are not limited to, high or low shear mixing, wet or dry milling, drip-casting, encapsulating, emulsifying, coating, encrusting, pilling, extrusion granulation, fluid bed granulation, co-extrusion, spray drying, spray chilling, atomization, addition or condensation polymerization, interfacial polymerization, in situ polymerization, coacervation, spray encapsulation, cooling melted dispersions, solvent evaporation, phase separation, solvent extraction, sol-gel polymerization, fluid bed coating, pan coating, melting, passive or active absorption or adsorption.

Customary agrochemical auxiliary agents are well-known in the art and include, but are not limited to aqueous or organic solvents, buffering agents, acidifiers, surfactants, wetting agents, spreading agents, tackifiers, stickers, carriers, fillers, thickeners, emulsifiers, dispersants, sequestering agents, anti-settling agents, coalescing agents, rheology modifiers, defoaming agents, photo-protectors, anti-freeze agents, biocides, penetrants, mineral or vegetable oils, pigments and drift control agents or any suitable combination thereof.

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.

5 Examples

1.Cis-cinammic acid increases the overall Arabidopsis leaf size

To study the underlying molecular mechanism of cis-cinnamic acid (cis-CA) on the growth and development of the vegetative part of the plant, we used *Arabidopsis thaliana* as a model plant. As the photo-isomerization in-between both isoforms of CA (cis-CA and trans-CA) occurs at a relatively fast rate, the low-cost commercially available *trans*-isoform of CA (*t*-CA) was used in all the experiments of the invention, unless otherwise explicitly stated. Since in the growth conditions, *trans*-CA will be largely converted to its *cis*-form, this biologically active mixture is herein further designated as *c/t*-CA. Some further explanation of the isomerization of cinnamic acid is explained further below. The light-mediated isomerization of CA is well described in the art and is induced by UV-B. To determine the isomerization efficiency under the applied plant growth conditions, 2.5 mg commercially available *trans*-CA or *cis*-CA was dissolved in 50 mL Milli-Q-H₂O/DMSO (80/20). Both solutions were subsequently placed in the growth chamber and the isomerization of both isomers was followed over time by ultra-high-pressure liquid chromatography (UHPLC)-mass spectrometry (MS). The chemical equilibrium was in favor of the *cis*-isomer (57%) and was reached after 8 or 15 days, depending on the use of *cis*-CA or *trans*-CA as the initial compound (this is described in Supplemental Fig. S3 from Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943). This indicates that despite the application of *trans*-CA to the growth medium, a substantial amount of the *cis*-isomer is available during the period of plant growth. No spontaneous isomerization was detected in the dark, under deep-red (650-670nm), or far-red illumination (725-750nm). Therefore, experiments to reveal the effect of the pure isomers can be performed under these conditions. *Arabidopsis* seeds were placed on 0.5×MS-medium supplemented with either pure *cis*-CA or *trans*-CA and incubated in darkness to avoid photo-isomerization. Twelve days after germination (DAG) seedlings were screened for phenotypes as before. Whereas no effect on the elongation of the hypocotyl was observed, an inhibitory effect on primary root growth was evident. It was found that *cis*-CA was much more effective than *trans*-CA (IC₅₀ of 3.2 μM and 82.4 μM for *cis*- and *trans*-CA, respectively). To test the metabolism of *trans*- versus *cis*-CA, a yeast heterologous expression system was used to express *Arabidopsis* C4H. In contrast to *trans*-CA, *cis*-CA was not converted to p-coumaric acid by *Arabidopsis* C4H. Therefore, we conclude that only *trans*-CA is an intermediate in the general phenylpropanoid pathway. The *cis*-isomer is the biologically active isomer affecting the leaf

biomass increase in planta. In addition, the functional variants of cis-CA were also purchased as the trans-isoforms and treated with UV-B radiation in order to obtain the cis-variant. In all cases we had a mixture of the cis-variant and the trans-variant wherein the trans-variant was inactive. In addition, compounds with the formulae I and II were purchased as trans-isomers and treated with UV-B to obtain the cis-isomer forms. Mass spectrometric analysis showed that the efficiency for obtaining the cis-isomers was similar for all compounds.

To gain profound insight into growth dynamics, instead of limiting our research to the analysis of end-point measurements, detailed kinematic analysis was done on the rosettes of Arabidopsis plants, using a fully-automated phenotyping platform, named the In Vitro Growth Imaging System (IGIS) (Dhondt et al., 2014). Arabidopsis plants were grown on 0.5xMS-medium supplemented with 1, 2.5 or 5 μ M *c/t*-CA and imaged every hour from germination onwards, until 21 days after stratification (DAS). The final time point was determined by the onset of bolting, resulting in major variability in leaf growth in between different biological repeats, and treatments. The pictures were used to analyze and follow up the rosette area of each individual plant over time. No significant differences in projected area were visualized in-between mock- and *c/t*-CA-treated plants, for any concentration, up to 14 DAS (Fig. 1A). From that moment onwards a dose-dependent increase in rosette area was observed for all the different concentrations tested. 21 DAS, treatment with a doses of 1, 2.5 or 5 μ M *c/t*-CA resulted in a significant increase in rosette area of 15.0%, 16.9% or 24.9% respectively (Fig. 1A/C). By imaging the projected rosette area, overlapping leaves could result in an underestimation of the actual leaf area. To have an idea on the discrepancy between the actual and projected leaf area, leaf series were made of each plant at the final time point (Fig. 1B). The calculation of the actual leaf area clearly indicated that the IGIS strongly underestimated this value. For example, the actual leaf area for mock-treated plants (237.14 mm²) was 1.76-fold higher than the projected rosette area for mock-treated plants (134.79 mm²) (Fig. 1C). For the 1, 2.5 and 5 μ M *c/t*-CA-treated plants the actual leaf area was 1.81-, 1.78- and 1.68-fold higher than the projected rosette area. On the other hand, the actual leaf area of *c/t*-CA-treated plants was still substantially higher than the one of mock-treated plants, which is in line with data obtained with the IGIS. For instance, while a 1.27- and 1.31-fold increase in actual leaf area was obtained at a *c/t*-CA-concentration of 1 and 2.5 μ M, respectively, a *c/t*-CA-concentration of 5 μ M resulted in a somewhat reduced increase in actual leaf area as a 1.21-fold surge was obtained (Fig. 1C). While the IGIS pointed the 5 μ M *c/t*-CA-concentration as giving an optimal effect on leaf growth, leaf series disclosed that a 2.5 μ M concentration of *c/t*-CA was optimal. While analyzing the dose-responsive effect of *c/t*-CA on the fresh (FW) and dry weight (DW) of the entire rosette also here at a *c/t*-CA-concentration of 2.5 μ M a maximum increase in plant biomass was obtained (Fig. 1D-E). The rosettes of Arabidopsis plants treated with 2.5 μ M *c/t*-CA showed an increased biomass, with 49.59 and 27.32% higher FW and DW,

respectively (Fig. 1D-E). Overall, we have evidence that *c/t*-CA induces a dose-dependent increase in leaf growth, and plant yield of *in vitro* grown *Arabidopsis* plants.

2. *c/t*-CA increases the overall leaf size by promoting cell proliferation

To gain insight in the cellular basis of the *c/t*-CA-induced increase in overall leaf area the abaxial epidermis at the basis of the 3rd leaf was analyzed, as leaf series analysis revealed that the 3rd leaf showed the highest increase (45.99%) in area on treatment with 2.5 μ M *c/t*-CA (Fig. 1B). It is also believed that this leaf is fully-grown 21 DAS. The underlying cause for the increase in leaf area could be the result of a significant increase in cell number, and/or, an increase in cell area. In theory, the timing between cell proliferation and cell expansion is another key parameter determining the actual cell number and thus to some extent the final leaf size. This transition occurs when a significant increase in cell size is obtained and when the cell morphology changes from circular for cells proliferating to the typical jigsaw puzzle-shape for expanding and mature cells (Andriankaja et al., 2012). To map these morphological changes and to see which of these processes *c/t*-CA triggers, an automatic image-analysis algorithm was used to quantify the cell number, average cell size and circularity of the cells (Fig. 2A) (Andriankaja et al., 2012). It can be concluded that the increase in leaf area was due to a significant increase in cell number, and not to an increase in cell area (Fig. 2B-C). The shape of the cells was measured with a circularity score, ranging in-between 0 and 1; this with 1 representing a perfect circle and 0 being representative for a progressively lobed cell, indicative of expanding cells. Cells maintained their circularity on *c/t*-CA-treatment indicating cells were mature and fully expanded (Fig. 2D). We conclude that *c/t*-CA promotes cell proliferation, and not expansion and differentiation, thus affecting the final size of the leaf.

3. *c/t*-CA promotes leaf growth in an auxin-dependent manner

The phytohormone auxin regulates numerous aspects of plant growth and development, comprising cell proliferation, elongation, and differentiation (Gonzalez et al., 2012). Nuclear auxin is being perceived by specific receptors, such as TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB) that, upon auxin binding, trigger ubiquitination and subsequent degradation of AUX/IAA auxin signaling repressors allowing the activation of specific target genes (Friml, 2003). The idea that both, *c*-CA and *t*-CA, do not act as an auxin agonist, nor an antagonist at the level of TIR1/AFB, could imply *c/t*-CA acts via an auxin-dependent pathway for leaf growth, this by modifying the spatiotemporal distribution within the plant (Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943). To find further support for the hypothesis that the *c/t*-CA-mediated increase in overall leaf size is due to changes in auxin homeostasis, free IAA itself, as its precursor, catabolite and conjugate levels were measured in *Arabidopsis* seedlings treated

with 2.5, 5 or 10 μM *c/t*-CA 12 DAG. In line, with earlier published data, no big shifts in the IAA biosynthetic metabolome were observed in-between *c/t*-CA- and mock-treated plants (Fig. 8). Interestingly, although no clear shift in free IAA levels (Fig. 3B) was observed on treatment with *c/t*-CA, strongly increased levels of its conjugates, IAA-glutamate and IAA-aspartate were seen (Fig. 3A). This observation is most probably due to the idea that high levels of free IAA are toxic for the plant and will be directly detoxified to its conjugates. The strong increase in IAA-conjugates questions the importance of free IAA for *c/t*-CA-increased leaf growth. Therefore, the role of IAA itself, was re-examined by testing the leaf growth promoting effect of *c/t*-CA in transgenic plants that artificially reduce IAA levels using the IAA lysine synthase overexpressing line *p35S:iaaL*. In contrast, with wild type plants grown under similar conditions, *c/t*-CA-treated *p35S:iaaL* plants did not show any significant increase in leaf area (Fig. 3C-D). Together, these results indicate that *c/t*-CA acts via an auxin-dependent pathway for cell proliferation by altering free auxin levels within the plant.

4. *c/t*-CA affects root architecture in *Nicotiana*

Although, we were able to show that *c/t*-CA has a growth promoting effect on the leaves of *Arabidopsis* rosettes, its effect on the main stem remains unclarified. Here, *Nicotiana* (*Nicotiana benthamiana*) was used as a model system, as in a short period of time in *in vitro* conditions the effect of *c/t*-CA on the shoot can be checked. Before analyzing the effect of *c/t*-CA on the vegetative part of the plant we tested if the effect of *c/t*-CA on the root architecture of *Arabidopsis* was preserved in *Nicotiana* (Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943. Seeds were germinated and grown in vertically positioned petri plates on 0.5xMS-medium supplemented with different concentrations of *c/t*-CA in a similar setup as earlier described in Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943. The level of *c/t*-CA required to reduce the primary root length by 50% ($\text{IC}_{50\text{-root}}$) was determined to be $\pm 3.5 \mu\text{M}$ under the conditions tested (Fig. 4A-B). This confirms the data obtained in *Arabidopsis* ($\text{IC}_{50\text{-root}}$ of $9.2 \mu\text{M}$ CA) (Steenackers *et al.* (2016) *Plant Physiology* Preview, published on November 11, 2016 as DOI:10.1104/pp.16.00943. However, while *c/t*-CA had a robust effect on lateral root formation in *Arabidopsis* (Fig. 4E), *Nicotiana* plants treated with *c/t*-CA only revealed a mild increase in lateral root density (LRD). The increase in LRD is not due to the increase in lateral root number (-) (Fig. 4F), but to the strong inhibition in primary root length (Fig 4B). At a *c/t*-CA-level of 1.0 and $2.5 \mu\text{M}$, respectively an 1.25-fold and 1.24-fold increase in LRD was obtained. However, at these concentrations the LR number (-) staid rather constant or even diminished as a *c/t*-CA-concentration of 1.0 and $2.5 \mu\text{M}$, resulted in an 1.06-fold and 0.73-fold increase and decrease in LR number, respectively. Interestingly, while in *Arabidopsis* the lateral roots shows a drop in length, in *Nicotiana* the length of the lateral roots close to the shoot/root junction strongly

increases, most probably taking over the function of the primary root (Fig. 4A-B). While measuring the lateral root (closest to the junction in-between the root and the shoot) at a *c/t*-CA-level of 1.0 and 2.5 μ M, respectively an 2.05-fold and 2.64-fold increase in length was obtained. Most probably, the most tremendous effect at the root level is the induction of root hairs (Fig. 4C-D). Not only elevates *c/t*-CA the density of the root hairs, but also the length of these root hairs, this way tremendously increasing the actual root surface (Fig. 4E). In line with Arabidopsis, *c/t*-CA also altered the agravitropic response of the main root in *Nicotiana* plants. For this reason, a bending assay was performed and indeed at even minor levels of *c/t*-CA the agravitropic response was perturbed (Fig. 4F). Also, in line with Arabidopsis adventitious roots (Fig. 4H) were observed in *Nicotiana*, although this increase was much lower than in Arabidopsis. These results reveal that *c/t*-CA affects the root architecture of *Nicotiana* seedlings, but in a way dissimilar of Arabidopsis.

5. *c/t*-CA increases plant productivity in *Nicotiana*

To investigate whether *c/t*-CA has a positive effect on leaf growth, and to see which concentration gives the strongest increase in leaf area seeds were germinated and grown in vertically positioned petri plates on 0.5xMS-medium supplemented with different doses of *c/t*-CA (up to 10 μ M). 21 DAS all leaves were harvested and the total leaf area of each plant was quantified (Fig. 5A). A clear positive effect of *c/t*-CA on the total leaf area was found with 1 and 2.5 μ M *c/t*-CA resulting in an increase in overall leaf area of 30.89% and 40.99%, respectively (Fig. 5A). Higher levels of *c/t*-CA resulted in a less substantial increase in leaf area (17.46% and 19.62% for plants treated with 5 and 10 μ M *c/t*-CA, respectively) (Fig. 5A) illustrating the dose-dependent effect of *c/t*-CA on yield improvement. Higher concentrations up to 40 μ M were toxic for the plant, and strongly inhibited rosette growth (data not shown). While analyzing the dose-responsive effect of *c/t*-CA on the fresh (FW) and dry weight (DW) of the entire rosette also here at a *c/t*-CA -concentration of 2.5 μ M a maximum increase in plant yield was obtained (Fig. 5B-C). The rosettes of plants treated with 2.5 μ M *c/t*-CA showed an increased biomass, with 54.13 and 37.52% higher FW and DW, respectively (Fig. 5B-C). Next, we decided to grow *Nicotiana* plants in plastic containers, which provide more space to the growing plants in comparison to the petri plates used for the short-term experiment. Since, we were able to grow *Nicotiana* plants in these boxes for almost three months, it also allowed us to produce a stem, which is also suitable for secondary cell wall analysis. Here, 2.5 μ M *c/t*-CA was selected, as this concentration gave the strongest increase in overall leaf area in the short-term experiment for both Arabidopsis and *Nicotiana* (Fig. 1A and Fig. S2A). All plants were harvested three months after germination (MAG) which corresponded to the moment when some *Nicotiana* plants reached the top lid of their container (Fig. 6A). The *c/t*-CA-mediated change in root architecture was clearly visible (Fig. 6B). No difference in height or branching of the main stem at the base

of the stem was found between *c/t*-CA- and mock-treated plants (Fig. 6C), indicating that the apical dominance of the plant was not altered by *c/t*-CA. Despite these similarities, *c/t*-CA-treated plants had a more robust phenotype. This was reflected in a 54.08% increase in fresh weight (FW) of the aboveground plant biomass (Fig. 6E), as well as a 28.21% increase in stem diameter of *c/t*-CA-treated plants (Fig. 6D). To rule out the possibility that the yield increase was basically the result of an increase in water content, the stems (without leaves) were lyophilized for six days to near complete dryness and samples were reweighted. This revealed an increase in dry weight (DW) of 40.77% for the *c/t*-CA-treated plants, compared to the mock-treated ones, corresponding with an 83.38% increase in FW (Fig. 6F). Altogether, our data provides evidence that exogenously applied *c/t*-CA induces a significant increase in plant biomass of *in vitro* grown *Nicotiana* plants.

6. *c/t*-CA affects stem morphology

In order to evaluate whether the increase in diameter was accompanied by an anatomical change, semi-thin transverse stem sections taken one cm above the root/stem junction of three months old plants were investigated via light-microscopy. Toluidine blue was used to stain the cell walls. Notably, in *c/t*-CA-treated *Nicotiana* plants a spatial loss in alignment of the secondary-thickened xylem cells was observed (Fig. 7A-B), illustrative for a perturbed cell proliferation and/or subsequent cell expansion phase. A semi-automated image-based analysis algorithm was used to calculate the average xylem cell size, cell number and circularity of the cell (Andrianakaja et al., 2012) (Fig. 7C). Interestingly, exogenously applied *c/t*-CA resulted both, in a 32.20% increase in total cell number (Fig. 7E) and a 32.13% increase in cell size (Fig. 7D), whereas the overall shape of the cells remained unaltered (Fig. 7F). When plotting the relative frequency of the cell size, a *c/t*-CA-mediated shift towards bigger cells was observed at the expense of smaller cells (Fig. 7G). Moreover, xylem cells of *c/t*-CA-treated plants displayed a 12.93% decrease in cell wall thickness (Fig. 7H) compared to mock-treated plants. Despite the reduced cell wall thickness, no collapsed xylem cells were observed in the sections.

In conclusion, *c/t*-CA increased cell proliferation and expansion. This change is accompanied by a decrease in cell wall thickness and a loss in spatial organization of the xylem cells.

7. *c/t*-CA alters the secondary cell wall composition

As the lyophilized stem biomass of three months old *Nicotiana* plants is mainly composed of cell walls it is tempting to speculate that the effect of *c/t*-CA on the plant biomass would be reflected at the level of the plant cell wall. To investigate this, cell wall residue (CWR) of *c/t*-CA- and mock-treated stems was prepared by sequential solvent extraction. To our surprise, the dry biomass obtained from *c/t*-CA-treated plants contained only 62.26% CWR, whereas this was 67.08% for mock-treated plants (Table 1). The drop in CWR indicated that the biomass of *c/t*-CA -treated

plants contained higher amounts of (macro)-molecules which are not covalently cross-linked to the cell wall and are removed during extraction. Besides being an intermediate of the phenylpropanoid pathway, *t*-CA (and its isoform *c*-CA), have been described to be a competitive inhibitor of PAL (and C4H). Henceforth, an effect on lignin deposition was expected in *c/t*-CA-treated plants (Boerjan et al., 2003; Chen et al., 2005). Acetyl bromide (AcBr) extraction revealed a no significant decrease in soluble lignin content upon *c/t*-CA-treatment (Table 1). In contrast with to the lignin content, the lignin composition changed as was shown by thioacidolysis and gas-chromatography. Treatment with *c/t*-CA resulted in an increase in the relative proportion of H and G units (31.44% and 23.65%, respectively) (Table 1), whereas an opposite effect was observed for the S units (a decrease of 10.05%). The overall shift in G and S units resulted in a 27.41% decrease in S/G-ratio upon CA-treatment (Table 1). No significant change in the sum of the different subunits (i.e. H+G+S) was observed upon *c/t*-CA-treatment, suggesting no big changes in degree of lignin condensation. To investigate whether the reduction in lignin content of *c/t*-CA -treated plants was compensated for by other cell wall compounds, e.g. polysaccharides, CWR of CA- and mock-treated plants was treated with trifluoroacetic acid (TFA). As TFA hydrolyzes matrix polysaccharides and amorphous cellulose the mass loss during TFA extraction is considered a measure for these polysaccharides. Using this approach, we found that the CWR of *c/t*-CA-treated plants contained on average 43.82% matrix polysaccharides and amorphous cellulose, which corresponds to a 7.80% increase compared to the mock-treated plants (Table 2). The TFA hydrolysate was subsequently used for analysis of the monomeric sugar composition. Treatment with *c/t*-CA significantly altered the amount of all identified monomeric sugars, with the exception of mannose (Table 2). Interestingly, while the relative fraction of xylose was reduced, the relative amount of the other monomeric sugars of the TFA extract was significantly higher upon *c/t*-CA-treatment. The TFA insoluble residues were later used to determine the crystalline cellulose by the phenol-sulfuric acid method, revealing a *c/t*-CA-mediated reduction in crystalline cellulose of 11.74% compared to mock-treated plants (Table 2). In summary, treatment of *Nicotiana* with *c/t*-CA affects lignin and polysaccharide composition of the secondary cell wall.

8.c/t-CA-treated plants show a higher glucose release upon saccharification

As the long-term treatment of *Nicotiana* with *c/t*-CA has a strong effect on the secondary cell wall composition, we anticipated that *c/t*-CA might as well affect the saccharification efficiency of the plant biomass. To test this, dry stem material of *c/t*-CA- and mock-treated plants was pulverized and incubated in an appropriate buffer with a mixture of cellulase and β -glucosidase. The released glucose content was measured after 4h, 8h, 24h and 48h and the cellulose-to-glucose conversion was calculated (Fig. 9A and Table 3). At the final time point, *c/t*-CA-treated *Nicotiana* stems showed a 67.54% increase in cellulose-to-glucose conversion (23.48% and

39.34% for mock- and *c/t*-CA-treated plants, respectively (Fig. 9A and Table 3). When the biomass was subjected to an acid pretreatment prior to the saccharification, the plateau levels corresponding to a maximum glucose release were reached much faster compared to the untreated biomass, indicating a clear positive effect of the treatment on the saccharification efficiency. The biomass of *c/t*-CA-treated *Nicotiana* still outperformed that of mock-treated samples, but the difference was smaller in comparison to the difference between *c/t*-CA- and mock-treated samples which were not subjected to a pretreatment (37.34% increase in efficiency compared to 67.54%). This was mainly due to the fact that the acid treatment improved the saccharification yield of mock-treated plants, whereas the positive effect was less pronounced for the biomass of the *c/t*-CA-treated plants. An alkaline pretreatment had a strong positive effect on the saccharification potential of biomass of mock-treated plants only. As a consequence, biomass of mock-treated plants performed equally well as biomass of *c/t*-CA-treated plants, indicating the alkaline pretreatment abolished the benefits of the *c/t*-CA-treatment (Fig. 9A and Table 3). To calculate the glucose release on a total plant basis, both the cellulose content and the biomass of the plant were taken into account. The higher plant biomass yield combined with an improved saccharification potential of *c/t*-CA-treated plants largely outperformed the drop in cellulose content in these plants, resulting in the release of 790.72 mg glucose per *c/t*-CA-treated plant compared to 487.84 mg glucose for mock-treated plants. This represents a 62.08% increase in glucose release per plant on a dry weight basis (Table 3). When an acid or alkaline pretreatment was performed, the glucose release on a plant basis increased with 34.75% and 4.18%, respectively upon *c/t*-CA-treatment (Fig. 9A and Table 3). In summary, *c/t*-CA significantly improves the saccharification yield by reducing the cell wall recalcitrance and increasing the plant biomass yield.

9. Bio-assay for testing functional variants of cis-cinnamic acid

In Figure 10 several variants of cis-CA are depicted. All compounds have been tested for their effects on the primary root inhibition and on lateral root induction. Compounds which have both a positive effect on the primary root inhibition and on the lateral root induction qualify as functional variants of cis-cinnamic acid which also have a utility to increase the plant biomass. Indeed, compounds 6, 7 and cis-2-phenylcyclopropane-1-carboxylic acid show a clear growth promoting effect on vegetative biomass of *Arabidopsis thaliana*.

10. Cis-cinnamic acid has a positive effect on the root biomass of green lettuce

Figure 11 depicts the measurement of the root growth biomass of green lettuce plants grown in soil (white cupboard boxes were used; each box contains 28 plants). Several concentrations of *c/t*-CA were used. Treatment with *c/t*-CA was done when plantlets were 2 weeks old (*id est* at week 2) and Week 3. Mock (0 mg), 10 mg, 25 mg and 50 mg *c/t*-CA was added to each box

(dissolved in 2.0 liter H₂O). Root biomass (fresh weight (FW)/dry weight (DW) was harvested at Week 4 and Week 5. 10 mg and 25 mg of c/t-CA show a very clear increase in root biomass.

11. Hydroponic cultures of *A. thaliana* and *N. benthamiana*

- 5 Figure 12 depicts *Arabidopsis thaliana* and *Nicotiana benthamiana* plants grown in hydroponic conditions. The picture shows *N. benthamiana* plants which have been treated with 1 μ M c/t-CA. There is a clear shoot increase for the two plant species when treated with c/t-CA.

12. Hydroponic cultures of basil, parsley, green lettuce and red lettuce

- 10 Figure 13 depicts shoot biomass measurements of hydroponic cultures of basil, parsley, green lettuce and red lettuce which have been treated with different concentrations of c/t-CA. A significant increase in shoot biomass can be observed for all four plant species.

Materials and methods

15

1.Plants, chemicals and growth conditions

- Experiments were performed with *Nicotiana benthamiana* (*Nicotiana*) and *Arabidopsis thaliana* Columbia 0 (Col-0). Seeds were vapor-phase sterilized and grown on Hamburg B5 tissue culture medium or 0.5xMS-Medium, respectively. The medium was supplemented with different concentrations of *trans*-cinnamic acid (CA; Sigma Aldrich). CA was dissolved in dimethyl sulfoxide (DMSO) and added to the autoclaved medium prior to pouring the vertical/horizontal plates, and plastic containers. After sowing, seeds were incubated at 4°C for at least 2 days whereupon they were placed in the growth chamber under a 16-hour-light/8-hour-dark photoperiod regime at 21°C for *Arabidopsis* and 24°C for *Nicotiana*.

25

2.Growth analysis and leaf series for *Nicotiana*

- Twelve biological replicates (or *Nicotiana* plants) were randomly positioned in the growth chamber. Plants were grown for 3 months, which allowed the growth and development of a main stem, without any side branches. For all biological replicates, the main stem was harvested 1.0 cm above the junction zone between the main stem and the roots. The fresh weight (mg) of the whole plant as a unit was measured, using a microbalance. After removing leaves and branches, the fresh weight (mg) of the main stem was measured. Both the length and the diameter (at the basis of the stem) were determined for each single stem. Further on, the main stem was chopped in $\pm$ 5 cm pieces and freeze-dried for 8 days. Finally, the dry weight of the main stem was determined.
- 30
- 35 Prior to cell wall preparation main stems were chopped into $\pm$ 2.5 mm pieces, which were used for wet-chemistry cell wall analyses. Leaf series were performed on 12 day old seedlings grown vertically on big plates. Leaves were stripped off the rosette and placed sequentially on plastic

plates containing agar, which were photographed, and further analyzed using ImageJ. The total rosette area represents the sum of all leaves.

3.IGIS and leaf series for *Arabidopsis*

5 For automated phenotypic analysis plants were grown on the In Vitro Growth Imaging System (IGIS) platform in the same growth conditions as mentioned before. The platform allows for a detailed rosette growth analysis of *in vitro* grown *Arabidopsis* plants and can hold up to 10 petri dishes. Images were taken on an interval-basis of 6 minutes, using near-infrared technology to visualize plants in the dark. Individual rosettes were extracted automatically by image analysis
10 processing. A data analysis pipeline compiles the measurements and constructs rosette growth curves. 21 days after stratification (DAS) leaves were stripped off the rosette and placed serially on agar plates, which were photographed, and further analyzed using ImageJ. The rosette area represents the sum of all cotyledon and leaf areas (Dhondt et al., 2014).

4.Microscopy for epidermal cell size measurements and image analysis

The third leaf was harvested for cellular analysis, and after clearing with 70% ethanol and mounted in lactic acid on microscopy slides. The total leaf blade area was measured for 15 representative leaves from each treatment under a dark-field binocular microscope. Abaxial epidermal cells at the basis of the leaves were drawn with a microscope equipped with
20 differential interference contrast optics (DM LB with 40× and 63× objectives; Leica) and a drawing tube. The microscopic drawings of the abaxial epidermis were scanned for digitalization. Processing of the microscopic images was done according to (Andriankaja et al., 2012).

5.Light and electron microscopy

25 For light and scanning electron microscopy, transverse stem sections (250 - 500 mm) at the root/shoot junction were made from the embedded stem segments, using a tabletop scanning electron microscope (Hitachi). Samples were excised and immersed in a fixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde and post fixed in 1% OsO₄ with 1.5% K₃Fe(CN)₆ in 0.1 M sodium cacodylate buffer, pH 7.2, for 1h under vacuum infiltration at room temperature
30 and 4h at room temperature rotating followed by fixation overnight at 4°C. After washing three times for 20min in buffer, samples were dehydrated through a graded ethanol series, including a bulk staining with 2% uranyl acetate at the 50% ethanol step, followed by embedding in Spurr's resin. Semithin sections were made and stained with toluidine blue. Light microscopy pictures were captured with a BX51P polarizing microscope (Olympus, Tokyo, Japan) equipped with a
35 20x air objective or 40x oil-immersion objective (Olympus, Tokyo, Japan), depending on the assay. Electron microscopy pictures were made with a transmission electron microscope (JEOL 1010; JEOL). Light microscopy pictures were made with a 20x air objective or 40x oil-immersion

objective. The microscopic drawings (20x) were used for digitalization. Processing of the microscopic images was done according to (Andriankaja et al., 2012). Outcome is the measures cell size, cell number and circularity. Cell thickness was measured by using ImageJ on transmission electron microscopy pictures and light microscopy pictures made with a 40x oil immersion objective.

6. Cell wall preparation, lignin content and composition

Aliquots of 50 mg scissor-chopped main stem material were subjected to a sequential extraction to obtain purified cell wall residue (CWR). Extractions were performed in 2 ml vials (Eppendorf®), for a time-interval of each 30min, at near boiling temperatures for water (98°C), ethanol (76°C), chloroform (59°C) and acetone (54°C). The remaining CWR was dried under vacuum. Lignin content was quantified according to a modified version of the acetyl bromide method, optimized for small amounts of plant tissue (Van Acker et al., 2013). The lignin composition was investigated with thioacidolysis as previously described (Van Acker et al., 2013).

7. Polysaccharide composition and saccharification

Aliquots of 5 mg chopped main stem material were subjected to a sequential extraction to obtain purified cell wall residue (CWR). This biomass was pretreated with 1ml of 1M HCl (acid) or 62.5 mM NaOH (alkaline) at 80°C for 2 h, while shaking (850 rpm). Acid or alkaline extract was removed and pretreated material was washed three times with 1 ml water to obtain a neutral pH. The polysaccharide composition and saccharification was investigated as previously described (Van Acker et al., 2013).

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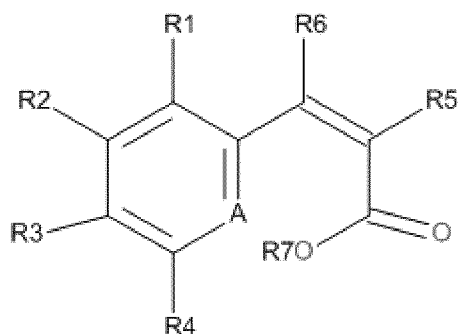
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Claims

1. An agrochemical formulation comprising a compound of formula (I), or a stereoisomer, a tautomer, a hydrate, a solvate or a salt thereof, or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof,

5



(I)

wherein in (I):

R1 is hydrogen, halogen, C1-C4 alkane, or a C1-C4-alkoxy group, and

10 R2 is hydrogen, halogen, CF₃, C1-C4-alkane or a C1-C4-alkoxy group, and

R3 is hydrogen, halogen, C1-C4 alkane or a C1-C4-alkoxy group, and

R4 is hydrogen, CF₃ or a C1-C4-alkoxy group, and

R5 is hydrogen or methyl, and

R6 is hydrogen or methyl, and

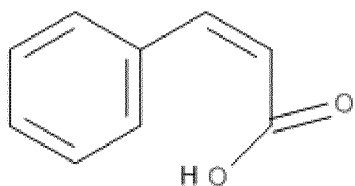
15 R7 is hydrogen, and

R5 and R6 can form a closed ring structure to form a cyclopropyl group or R1 and R6 can form a closed ring structure selected from the list consisting of cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, and

A can be C or N.

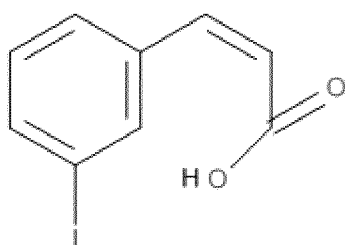
20 for inducing yield increase in plants.

2. An agrochemical formulation according to claim 1 wherein the compound is selected from the list consisting of:



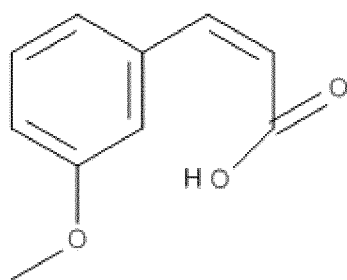
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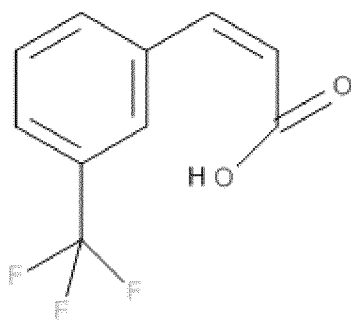


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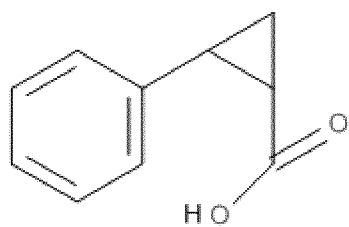
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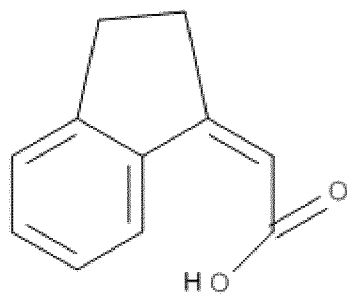
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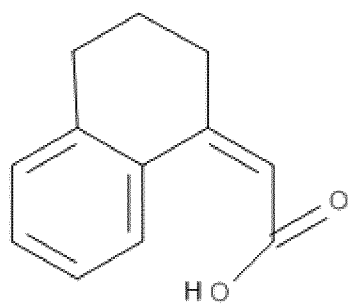
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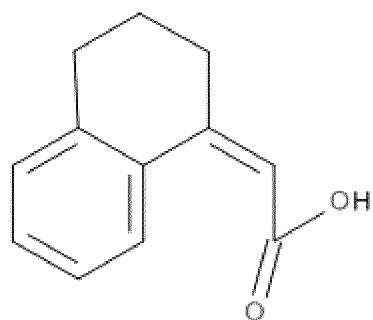
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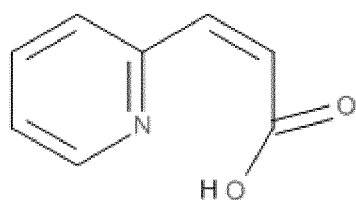
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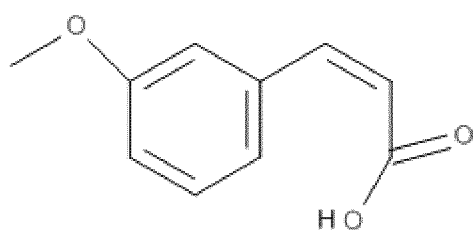
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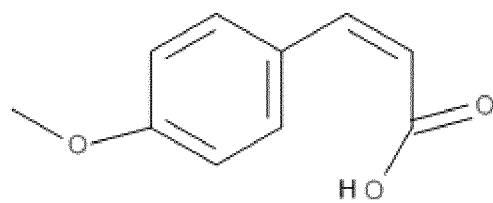
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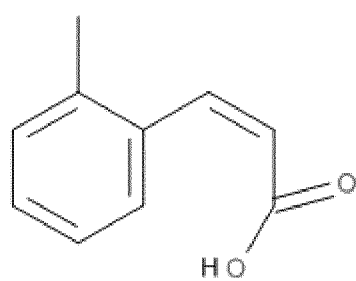
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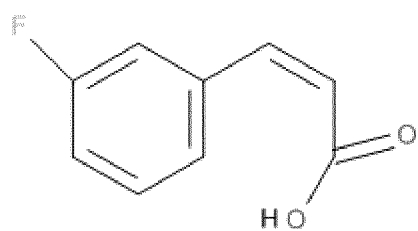


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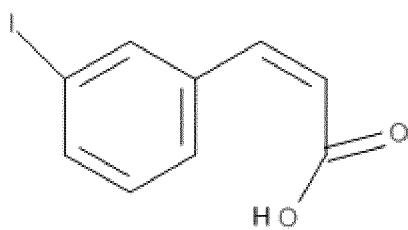
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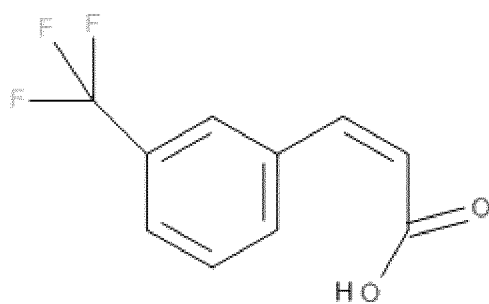
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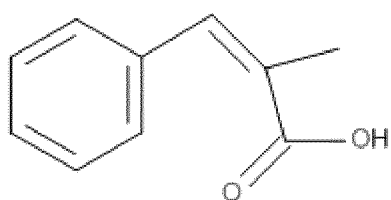
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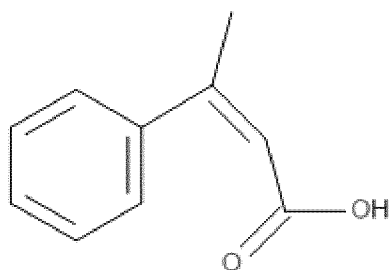


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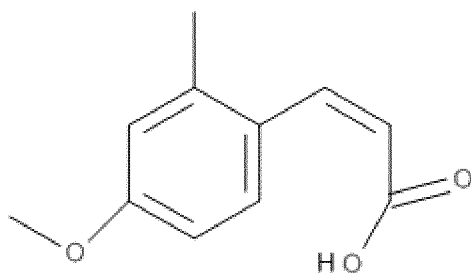


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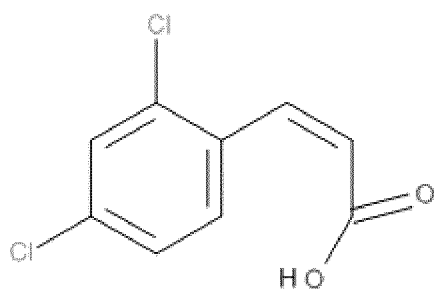


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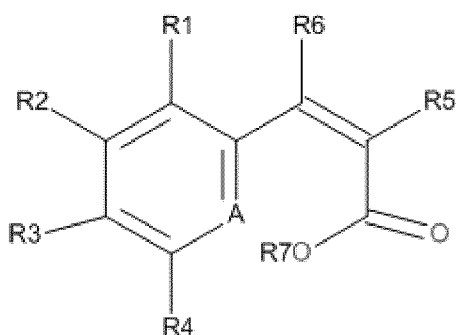
and



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3. Use of a compound of formula (I) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof,



(I)

wherein in (I):

R1 is hydrogen, halogen, C1-C4 alkane, or a C1-C4-alkoxy group, and

5 R2 is hydrogen, halogen, CF₃, C1-C4-alkane or a C1-C4-alkoxy group, and

R3 is hydrogen, halogen, C1-C4 alkane or a C1-C4-alkoxy group, and

R4 is hydrogen, CF₃ or a C1-C4-alkoxy group, and

R5 is hydrogen or methyl, and

R6 is hydrogen or methyl, and

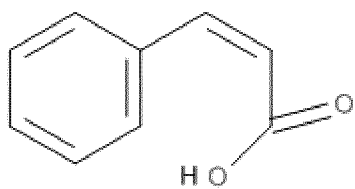
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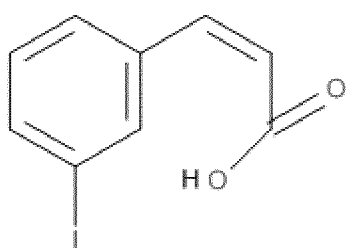
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4. Use of a compound according to claim 3 wherein said compound is selected from the list consisting of:

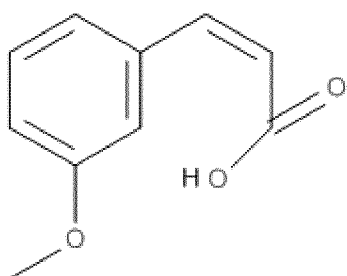


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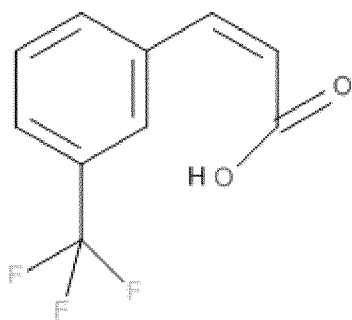
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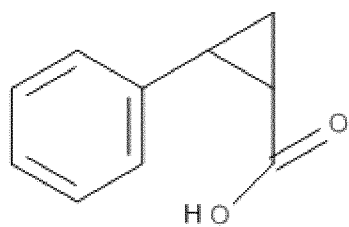


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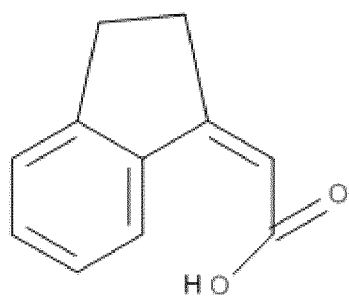


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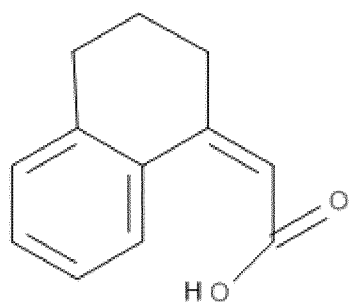
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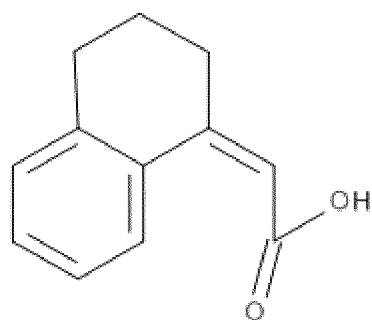
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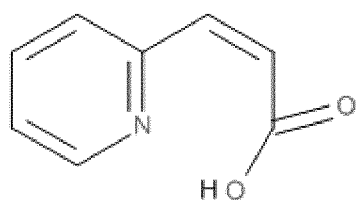
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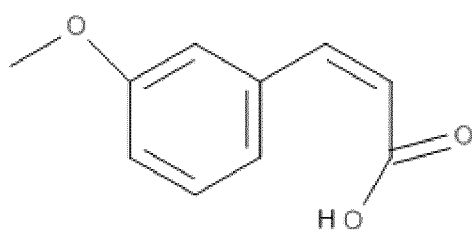
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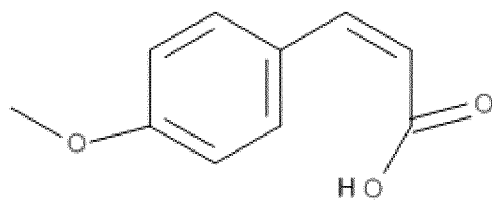
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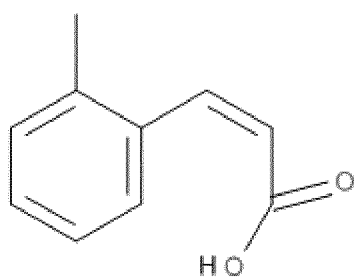


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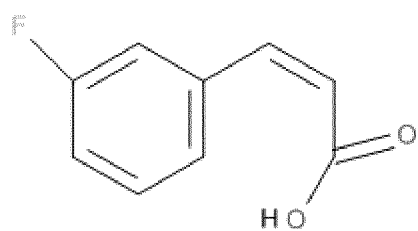


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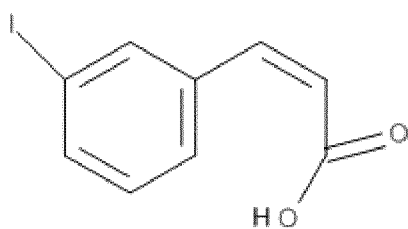
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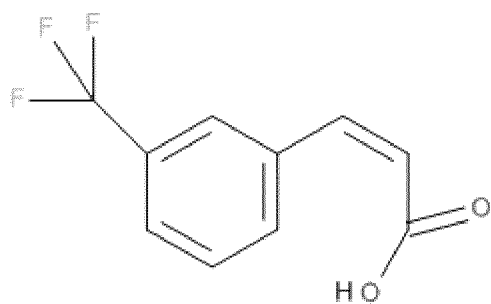
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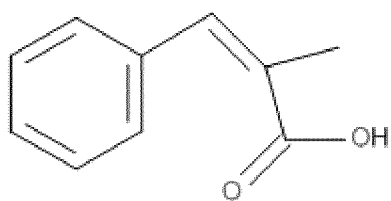
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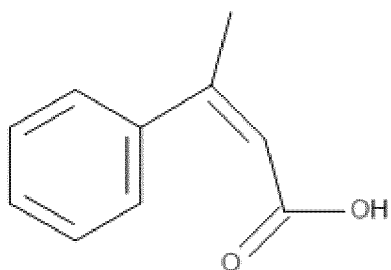


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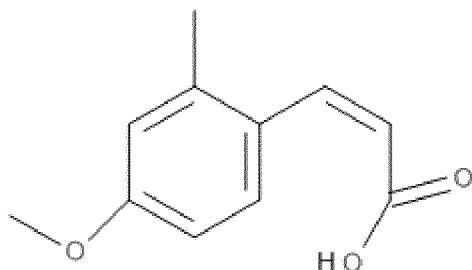


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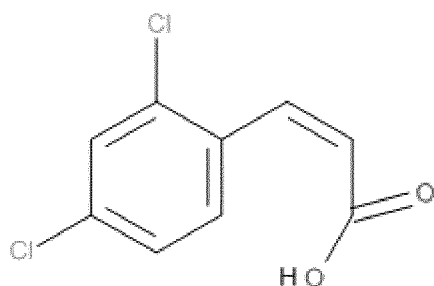
;



;



and



5

5. An agrochemical formulation according to claim 1 or 2 for use in stimulating yield increase in plants wherein said yield increase is vegetative biomass increase.
6. An agrochemical formulation according to claim 1 or 2 for use in stimulating yield increase in plants wherein said yield increase is vegetative biomass increase and wherein the saccharification efficiency of said biomass is increased.
7. An agrochemical formulation according to claim 1 or 2 for use in stimulating yield increase in plants wherein said yield increase is leaf biomass increase.
8. An agrochemical formulation according to claim 1 or 2 for use in in a hydroponic plant culture.

Figure 1

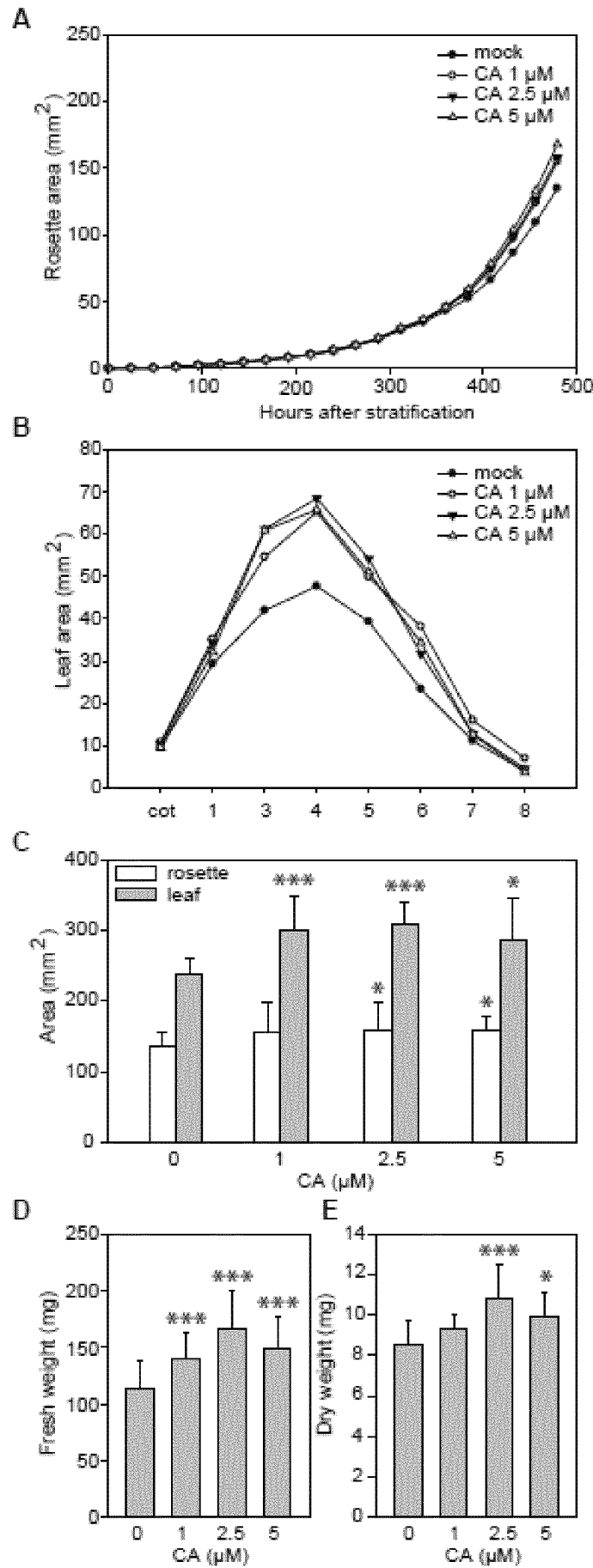


Figure 2

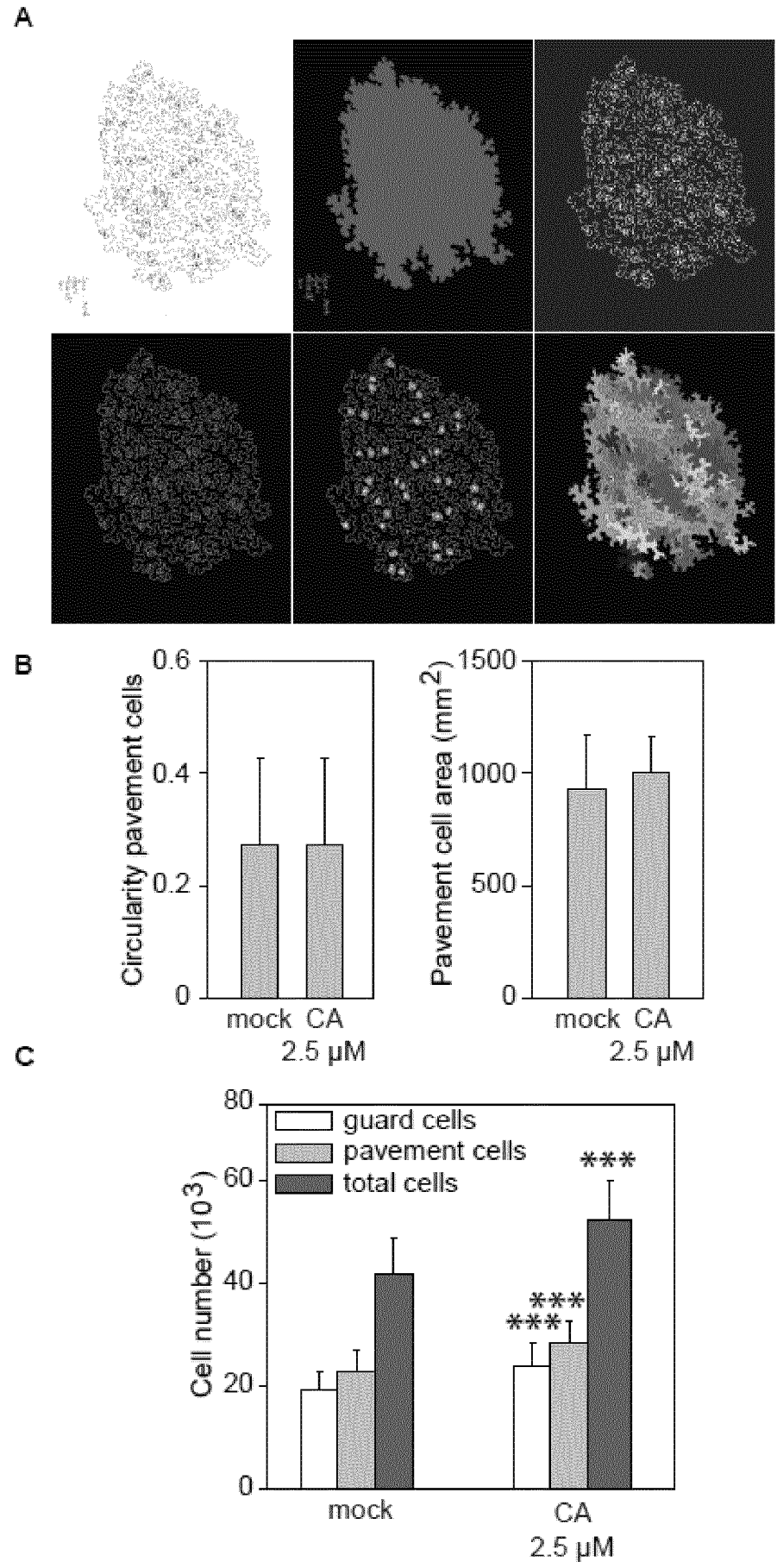


Figure 3

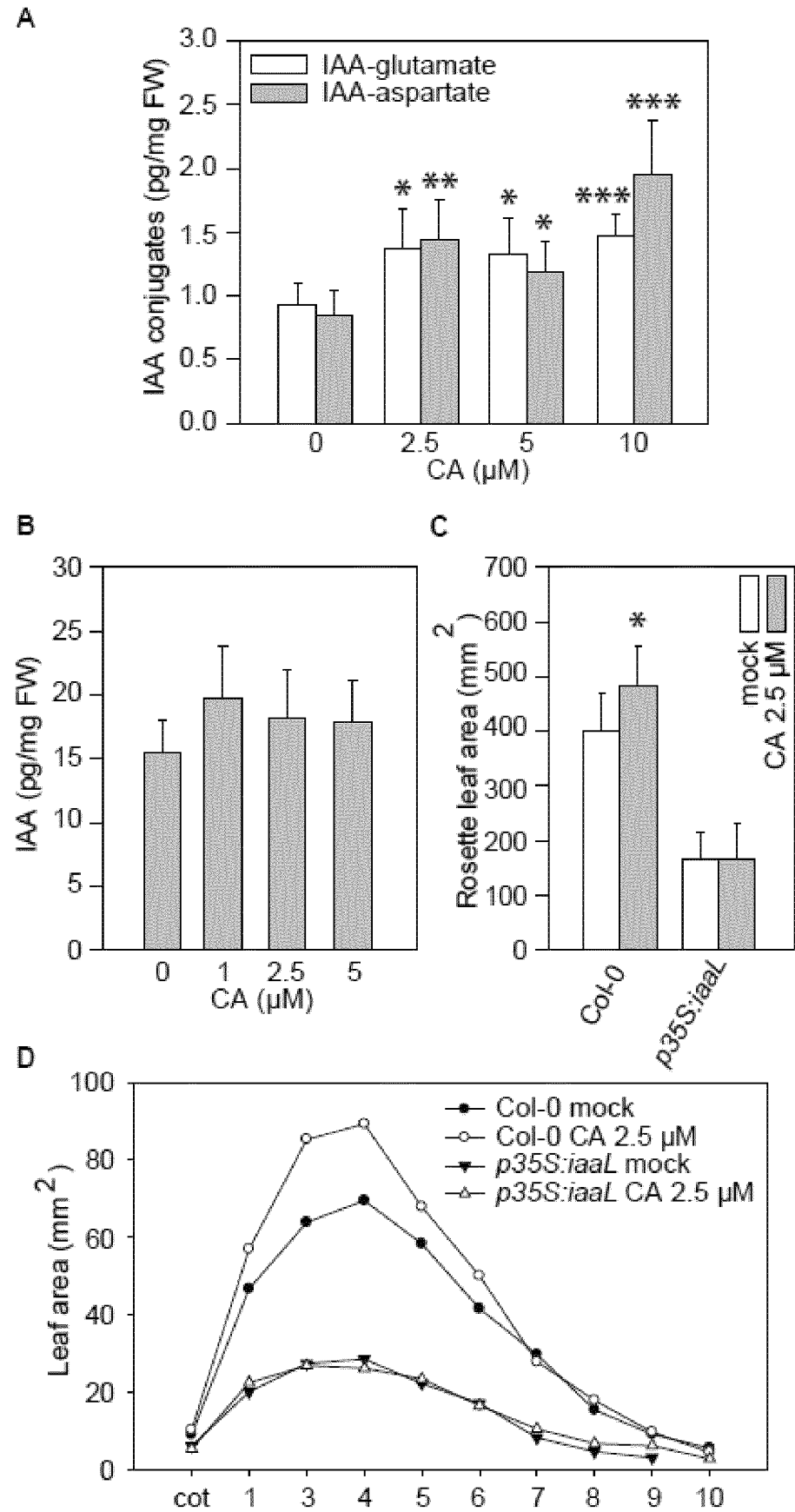


Figure 4

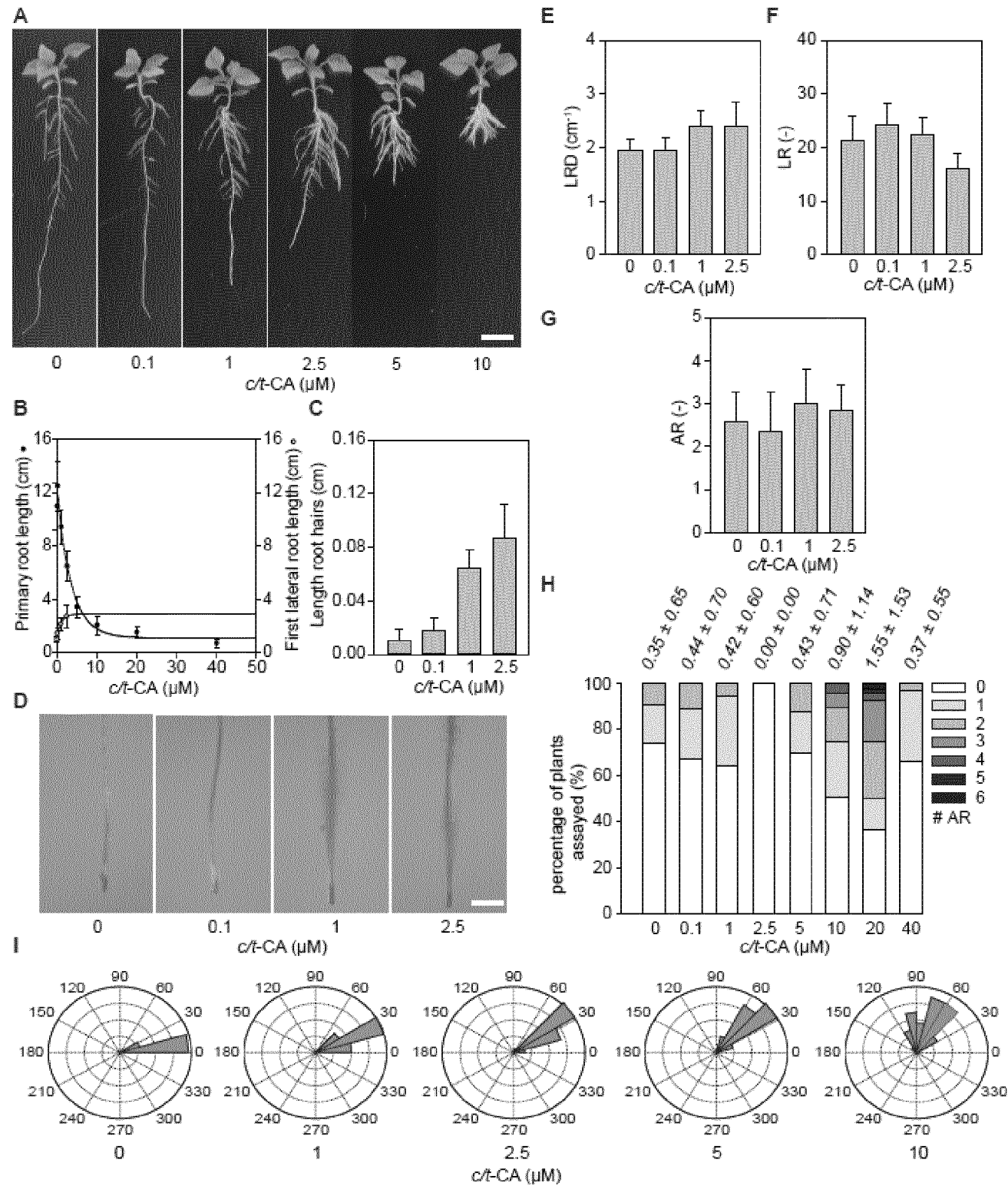


Figure 5

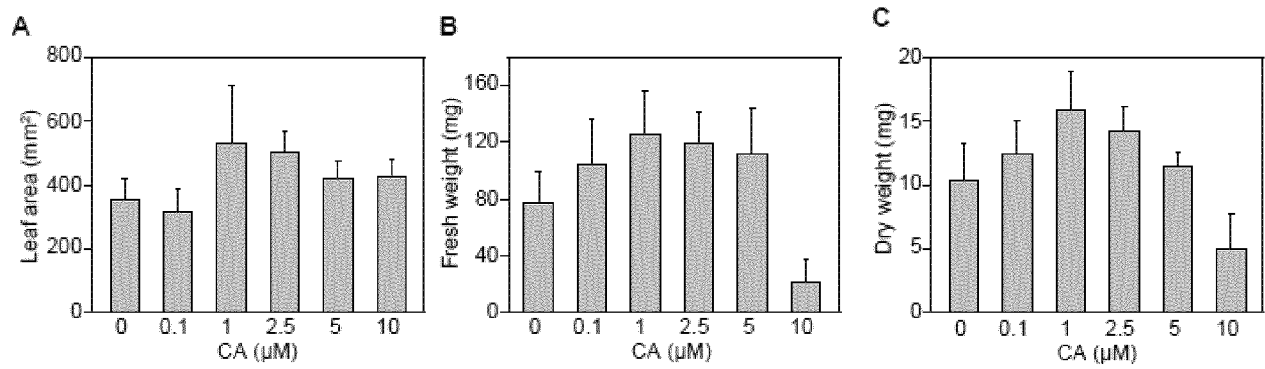


Figure 6

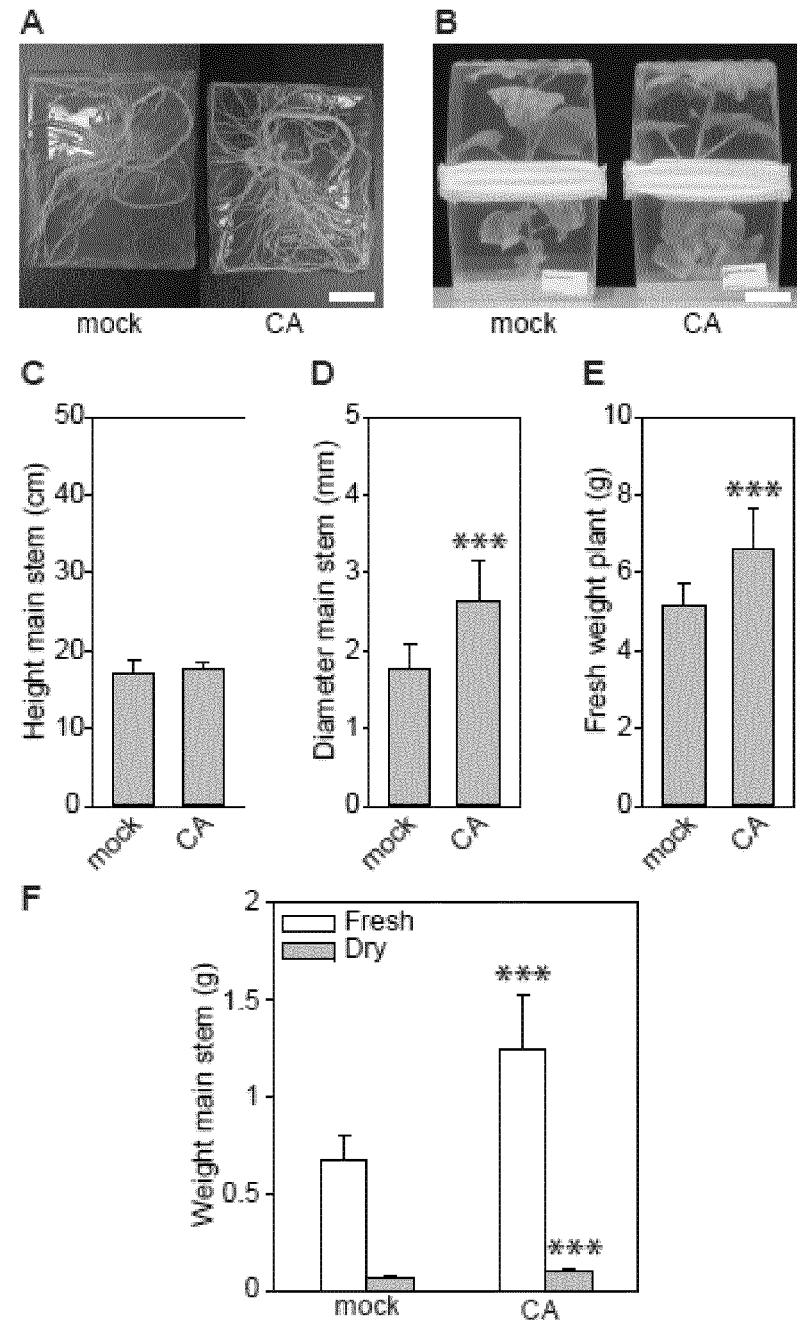


Figure 7

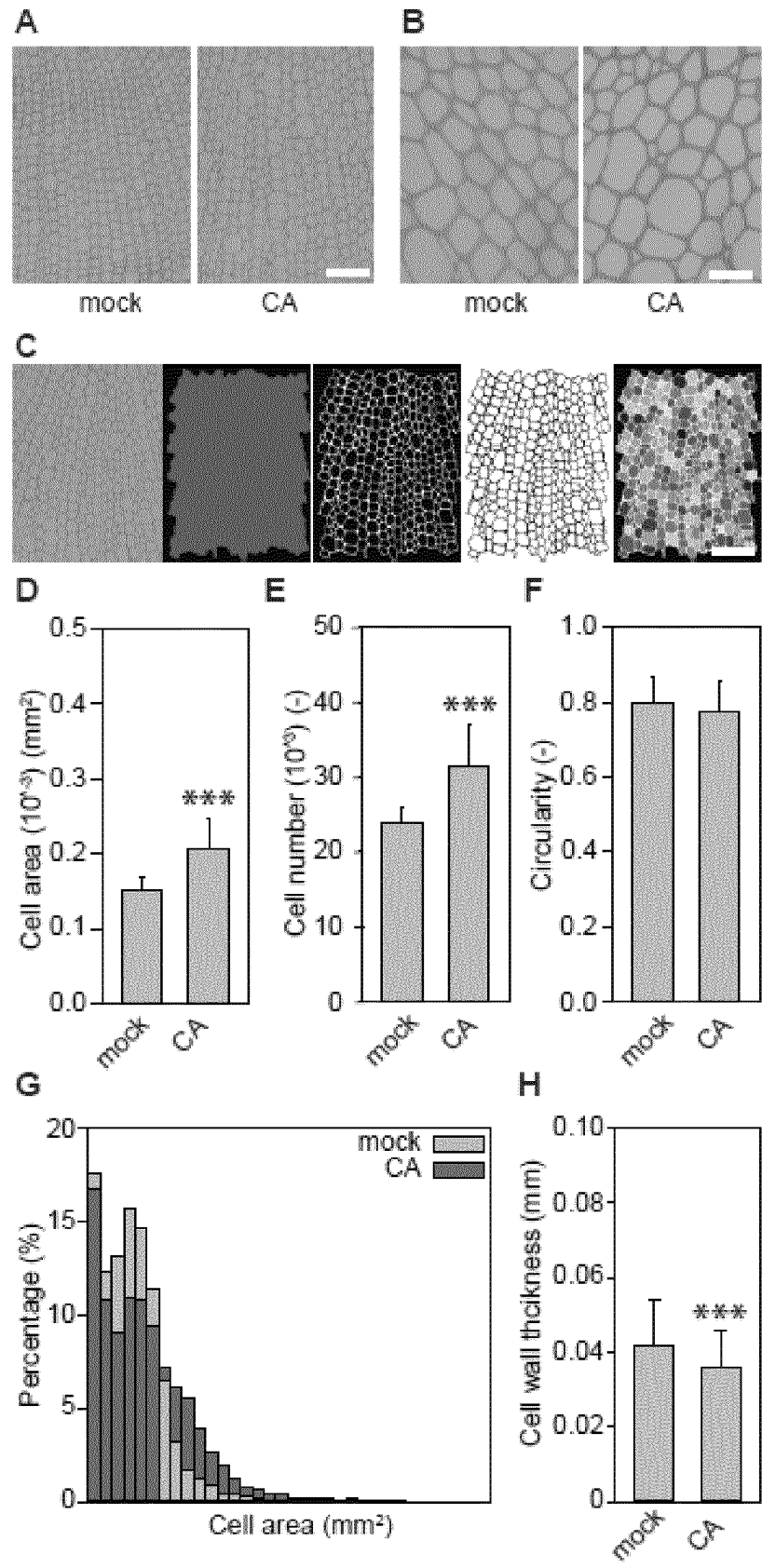


Figure 8

indole-3-acetic acid, precursors, conjugates and catabolites levels (pg/mg fresh weight)

	mock	CA 2.5 μ M	CA 5 μ M	CA 10 μ M
indole-3-acetic acid	15.5 $\pm$ 2.5	19.7 $\pm$ 4.1	18.2 $\pm$ 3.8	17.8 $\pm$ 3.3
precursors				
anthranilate	26.5 $\pm$ 6.4	32.3 $\pm$ 5.8	23.9 $\pm$ 4.8	28.7 $\pm$ 7.3
L-tryptophan	2170.9 $\pm$ 508.9	3112.8 $\pm$ 785.7 *	2591.9 $\pm$ 614.3	2749.8 $\pm$ 712.8
tryptamine	0.18 $\pm$ 0.05	0.23 $\pm$ 0.05	0.19 $\pm$ 0.04	0.19 $\pm$ 0.04
indole-3-acetamide	1.13 $\pm$ 0.26	1.11 $\pm$ 0.16	0.97 $\pm$ 0.25	0.99 $\pm$ 0.26
indole-3-acetonitrile	388.3 $\pm$ 79.5	320.7 $\pm$ 65.3	316.5 $\pm$ 46.8	359.6 $\pm$ 80.5
indole-3-acetaldoxime	12.5 $\pm$ 2.4	12.4 $\pm$ 2.2	12.2 $\pm$ 2.7	14.0 $\pm$ 2.5
indole-3-acetaldehyde	95.3 $\pm$ 24.6	77.7 $\pm$ 14.0	77.5 $\pm$ 19.0	74.9 $\pm$ 15.0
indole-3-pyruvic acid	129.5 $\pm$ 15.3	115.2 $\pm$ 12.7	108.2 $\pm$ 11.1 *	111.7 $\pm$ 13.3
catabolites, conjugates:				
2-oxindole-3-acetic acid	109.17 $\pm$ 26.10	105.77 $\pm$ 19.72	87.95 $\pm$ 12.45	105.02 $\pm$ 6.41
IAA-glutamate	0.94 $\pm$ 0.16	1.38 $\pm$ 0.31 *	1.33 $\pm$ 0.29 *	1.47 $\pm$ 0.17 ***
IAA-aspartate	0.85 $\pm$ 0.20	1.45 $\pm$ 0.30 **	1.19 $\pm$ 0.24 *	1.96 $\pm$ 0.42 ***

Figure 9

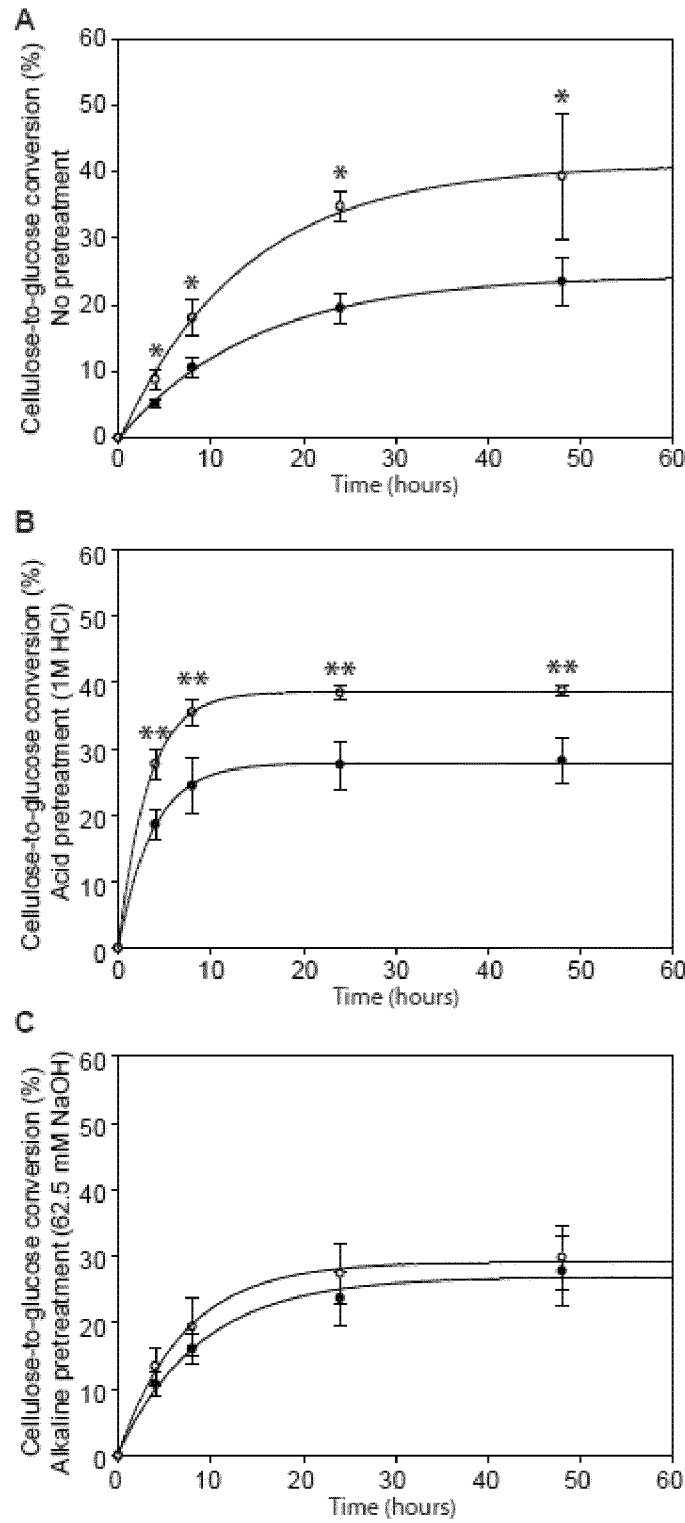


Figure 10

compound	Phenotype - Root			Phenotype - Rosette (IGIS)	
	Concentration (µM)	Primary root inhibition	Lateral root induction	Concentration (µM)	Growth Promoting
<i>trans</i> -cinnamic acid	0-2.5-5-10-20-40	No	No		
<i>cis</i> -cinnamic acid	0.1-1-2.5-5-10	Yes	Yes		
<i>trans/cis</i> -cinnamic acid	0-2.5-5-10-20-40	Yes	Yes	0-1-2.5-5-10	Yes
<i>trans</i> -2-phenylcyclopropane-1-carboxylic acid	0-0.25-0.5-1-1.5-2-5	No	No		
<i>cis</i> -2-phenylcyclopropane-1-carboxylic acid	0-0.25-0.5-1-1.5-2-5	Yes	Yes	0-0.25-0.5-1	Yes
compound 1	0-1-2.5-5-10	Yes	Yes		
compound 2	0-1-2.5-5-10	No	No		
compound 3	0-1-2.5-5-10	No	No		
compound 4	0-1-2.5-5-10	Yes	Yes		
compound 5	0-1-2.5-5-10	Yes	Yes		
compound 6	0-1-2.5-5-10	Yes	Yes	0-1-2.5-5-10	Yes
compound 7	0-1-2.5-5-10	Yes	Yes	0-0.5-1-1.5-2.5	Yes
compound 8	0-1-2.5-5-10	Yes	Yes		
compound 9	0-1-2.5-5-10	Yes	Yes		
Ferulic acid	0-2.5-10-20-40	No	No		
<i>p</i> -Coumaric acid	0-2.5-10-20-40	No	No		
Caffeic acid	0-2.5-10-20-40	No	No		

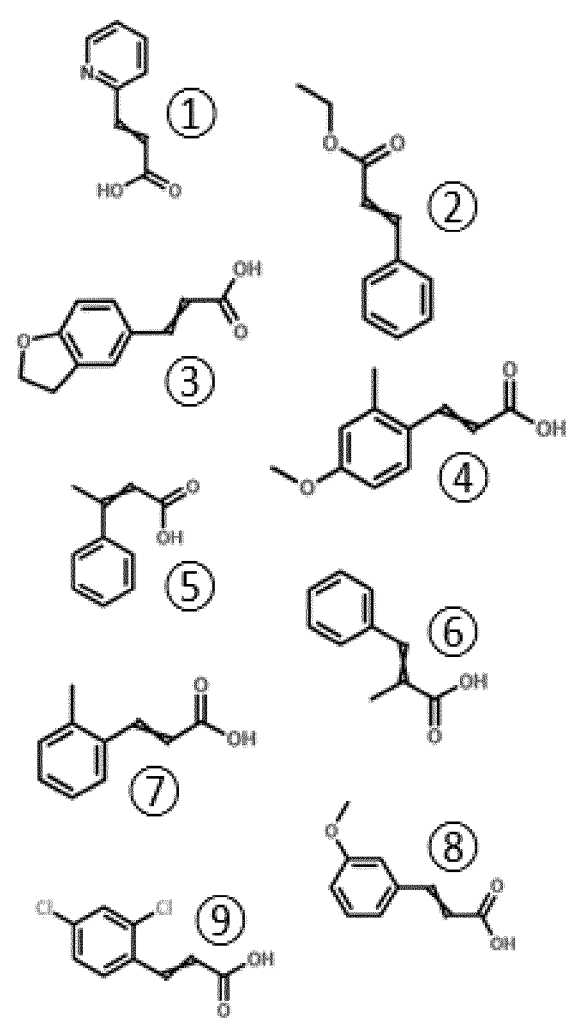
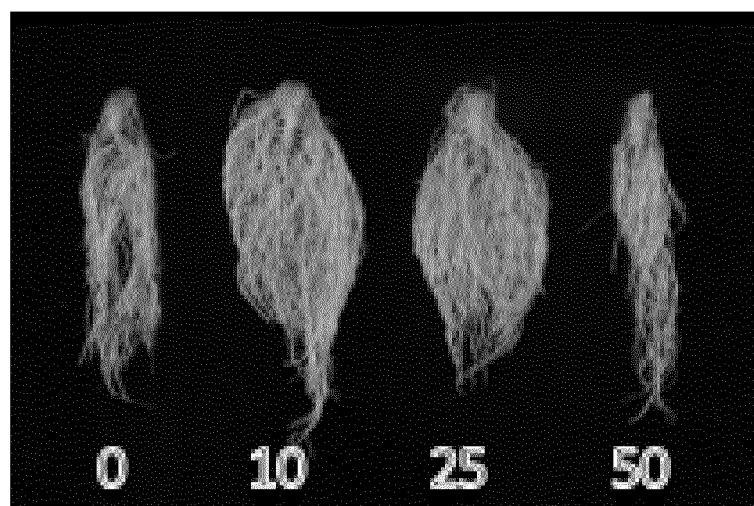


Figure 10 continued

compound 1	3-(pyridin-2-yl)prop-2-enoic acid
compound 2	ethyl 3-phenylprop-2-enoate
compound 3	3-(2,3-dihydro-1-benzofuran-5-yl)prop-2-enoic acid
compound 4	3-(4-methoxy-2-methylphenyl)prop-2-enoic acid
compound 5	3-phenylbut-2-enoic acid
compound 6	2-methyl-3-phenylprop-2-enoic acid
compound 7	3-(2-methylphenyl)prop-2-enoic acid
compound 8	3-(3-methoxyphenyl)prop-2-enoic acid
compound 9	3-(2,4-dichlorophenyl)prop-2-enoic acid

Figure 11



CA mg/2L

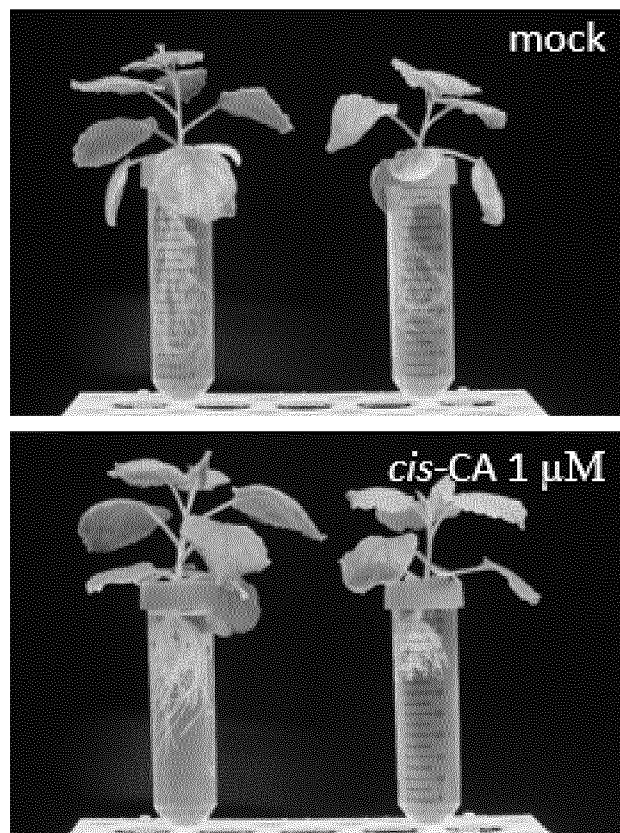
Root FW

CA (mg)	Average	Std.Dev.	Increase (%)	Significance
0	1063,7	445,2	0	
10	2996,3	1049,5	182	***
25	2454,4	872,4	131	***
50	1323,7	624,5	24	

Root DW

CA (mg)	Average	Std.Dev.	Increase (%)	Significance
0	50,9	23,8	0	
10	231,1	101,7	354	***
25	203,6	101,2	300	***
50	76,1	33,6	49	

Figure 12

***Arabidopsis thaliana***

c/t-CA (μ M)	Average	Std.Dev.	Increase (%)	
0	6766	558,762	0	
0.1	6669	1210,244	-1,4	
0.25	7758	1108,186	14,6	*

Nicotiana benthamiana

c/t-CA (μ M)	Average	Std.Dev.	Increase (%)	
0	1508	514	0	
0.25	1730	807	14.7	
1	1869	516	23.9	

Figure 13

Basil	CA (mg)	Average	Std.Dev	Increase (%)	Significance
	0	5,19	0,59	0	
	1	6,30	0,65	21	***
	10	6,43	0,88	24	***
	25	6,72	0,65	30	***
	50	6,33	0,53	22	***
	100	5,83	1,05	12	
	250	5,03	0,66	-3	
Parsley	CA (mg)	Average	Std.Dev	Increase (%)	Significance
	0	0,70	0,22	0	
	1	0,82	0,18	17	
	10	1,06	0,27	51	***
	25	1,14	0,25	63	***
	50	0,86	0,21	23	
	100	0,95	0,26	35	*
	250	0,79	0,21	13	
Green Lettuce	CA (mg)	Average	Std.Dev	Increase (%)	Significance
	0	5,88	1,29	0	
	1	5,98	0,90	2	
	10	8,06	0,76	37	***
	25	8,04	0,97	37	***
	50	7,11	1,16	21	*
	100	7,21	1,31	23	*
	250	6,73	0,89	15	
Red Lettuce	CA (mg)	Average	Std.Dev	Increase (%)	Significance
	0	5,11	0,86	0	
	1	5,49	0,75	7	
	10	6,10	0,46	19	**
	25	6,55	0,61	28	***
	50	5,96	0,56	16	*
	100	5,46	1,00	7	
	250	4,98	0,89	-3	

hydroponics

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/081258A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N37/10
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)
A01N

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, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WARD STEENACKERS ET AL: "cis-Cinnamic Acid Is a Novel, Natural Auxin Efflux Inhibitor That Promotes Lateral Root Formation", PLANT PHYSIOLOGY, vol. 173, no. 1, 11 November 2016 (2016-11-11), pages 552-565, XP055439425, Rockville, Md, USA ISSN: 0032-0889, DOI: 10.1104/pp.16.00943 cited in the application page 6 page 8, lines 178-189 ----- -/--	1-8



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

11 January 2018

Date of mailing of the international search report

19/01/2018

Name and mailing address of the ISA/

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Authorized officer

Götz, Gerhard

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/081258

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IMAM TALAAT: "Physiological Response of Sweet Basil Plants (<i>Ocimum basilicum</i> L.) to Putrescine and Trans-Cinnamic Acid", AMERICAN-EURASIAN J. AGRIC. & ENVIRON. SCI., vol. 8, no. 4, 1 January 2010 (2010-01-01), pages 438-445, XP055439427, abstract tables 1, 2 -----	1-8
X	I M TALAAT: "Changes in growth, hormones levels and essential oil content of <i>Ammi visnaga</i> L. plants treated with some bioregulators", SAUDI JOURNAL OF BIOLOGICAL SCIENCES, vol. 21, 2014, pages 355-365, XP002777190, abstract tables 3-7, 10, 12 page 364: conclusion -----	1-8
X	EP 1 459 627 A2 (DAINICHISEIKA COLOR CHEM [JP]) 22 September 2004 (2004-09-22) the whole document -----	1
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X	DATABASE WPI Week 199910 Thomson Scientific, London, GB; AN 1999-114775 XP002777192, & JP H10 338603 A (DAINICHISEIKA COLOR & CHEM MFG) 22 December 1998 (1998-12-22) abstract -----	1-8
X	E N UGOCHUKWU: "Studies on plant growth-regulating substances. XXV. The plant growth-regulating activity of cinnamic acids", ANN APPL BIOL, vol. 61, 1968, pages 121-130, XP002777193, table 2 ----- -/--	1-8

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International application No
PCT/EP2017/081258

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/081258

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