

19



Octrooi Centrum
Nederland

11

2014879

12 B1 OCTROOI

21

Aanvraagnummer: **2014879**

51

Int. Cl.:
A01G 1/06 (2016.01) **A01N 3/04** (2016.01)

22

Aanvraag ingediend: **28/05/2015**

43

Aanvraag gepubliceerd:
08/06/2016

73

Octrooihouder(s):
Fides B.V. te De Lier.

47

Octrooi verleend:
14/06/2016

72

Uitvinder(s):
Pankaj Bacharam Dhonukshe te Renkum.
Tobias Günter Dümmer te Rheinberg (DE).
Johannes Franciscus Johanna Maria van den
Heuvel te Rotterdam.

45

Octrooischrift uitgegeven:
21/10/2016

74

Gemachtigde:
ir. P.J. Hylarides c.s. te Den Haag.

54

Partially encapsulated plant cuttings.

57

The present invention relates to methods for providing improved vigour, and especially priming or improving root formation, of plant cuttings and to plant cuttings, amongst others, obtainable by the present methods. Specifically, the present invention relates to methods for improving the vigour of plant cuttings, wherein the method comprises the steps of a) contacting a plant cutting with a polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds, thereby providing a coated plant cutting; b) allowing the polymeric solution, or suspension, of the coated plant cutting to solidify into a complexed coating thereby providing an encapsulated plant cutting; wherein said encapsulated plant cutting is coated with the complexed coating from the basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting to partially encapsulate the plant cutting with the complexed coating.

PARTIALLY ENCAPSULATED PLANT CUTTINGS

Description

5 The present invention relates to methods for providing improved vigour, and especially faster root development and/or improving adventitious root formation, of plant cuttings and to plant cuttings, amongst others, obtainable by the present methods.

 Plant cutting, also known as striking or cloning, is a technique for vegetatively or asexually propagating plants in which a part of the stem or root of the parent plant generally
10 ranging from 1 to 3 cm to 25 cm, is placed in a suitable growth substrate such as moist soil, potting mix, coir or rock wool. The cutting produces new roots, stems, or both, and thus becomes a new plant independent of the parent. Typically, striking is a simple process in which a small amount of the parent plant is removed. The removed part, called the cutting, is then induced to grow as an independent plant.

15 Since most plant cuttings will initially have no roots these roots need to be developed, which is a critical component for survival of the cutting. Some roots, called adventitious roots, arise mainly from an organ other than the root usually a stem and in some cases a leaf. They are especially numerous on underground stems. The formation of adventitious roots makes it possible to vegetatively propagate many plants from stem or leaf cuttings. These cuttings
20 do not have a primary root (emerging from an embryo's radicle), but the adventitious roots give rise to branch or lateral roots.

 Plant cuttings also generally require a moist growth substrate, which, however, cannot be too wet to prevent rotting of the plant cutting. A number of growth substrates are generally used in this process, including but not limited to, soil, perlite, vermiculite, coir, rock
25 wool and expanded clay pellets. The environment should be humid and partial shade is preferably provided to prevent the plant cutting from drying out. After plant cuttings are placed in a suitable growth substrate, they can be watered thoroughly such as with a fine mist. After initial watering, the growth medium can be allowed to almost dry out before subsequent watering with the aim to keep the soil moist but not wet and waterlogged.

30 If initiation of root development for the plant cutting is difficult to achieve, a rooting hormone to promote plant growth can be administered. Though not essential, several compounds may be used to promote the formation of roots through the signalling activity of plant hormone auxins. Among the commonly used chemicals is an auxin precursor indole-3-butyric acid (IBA) used as a powder, liquid solution or gel. This compound is applied either to the basal cut
35 surface or tip of the plant cutting or as a foliar spray.

Although plant cutting is a relatively simple technique for asexually propagating plants at a small scale, at a commercial scale however, a major problem is to ensure the general quality of a plant cutting and more specifically to ensure that a plant cutting is actually capable of developing into a mature plant in a timely manner a condition generally designated in the art as vigour. One cause of this problem is that plants cuttings are generally produced at a nursery or farm in one location and subsequently transported to another location where they are allowed to develop into mature plants.

It is an object of the present invention, amongst other objects, to obviate at least partially the above problems associated with the production of plant cuttings at a commercial scale.

This object of the present invention, amongst other objects, is met by providing methods and plant cuttings as outlined in the appended claims.

Specifically, this object of the present invention, amongst other objects, is, according a first aspect, met by a method for improving the vigour of plant cuttings, wherein the method comprises the steps of:

- a) contacting a plant cutting with a polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds, thereby providing a coated plant cutting;
- b) allowing the polymeric solution, or suspension, of the coated plant cutting to solidify into a complexed coating thereby providing an encapsulated plant cutting;

wherein said encapsulated plant cutting is coated with the complexed coating from basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting to partially encapsulate the plant cutting with the complexed coating.

The present inventors have surprisingly discovered that partially coating a plant cutting with a polymeric coat optionally comprising one or more plant growth promoting and/or plant protecting compounds significantly improves the vigour of the plant cutting especially when the plant cutting is produced at, for example, a nursery and subsequently transported over relatively large distances before being cultivated into a mature plant elsewhere. Further, the present inventors have surprisingly discovered that the present partially encapsulated plant cutting requires no, or a relatively small growth substrate volume in order to remain viable thereby significantly reducing transport costs. Further, the present inventors have surprisingly discovered that the present partially encapsulated plant cutting produces roots quicker thereby significantly reducing greenhouse growing costs.

According to the present invention, it is essential that the plant cutting is only partially encapsulated to avoid the development of necrotic patches negatively influencing the vigour of the plant cutting.

According to a preferred embodiment of this first aspect of the present invention,
 5 the present polymeric solution, or suspension, is selected from the group consisting of sodium alginate, agar, polyacrylamide, agarose, gelatin, and biodegradable plastics.

Sodium alginate, also designated as alginic acid or align, is an anionic polysaccharide found in the cell walls of brown algae, where, through binding with water, it forms a viscous gum. In extracted form, sodium alginate absorbs water quickly and is capable of
 10 absorbing 200 to 300 times water of its own weight. The general structure of sodium alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked β -D-mannuronate (M) and its C-5 epimer α -L-guluronate (G) residues covalently linked in different sequences or blocks. The constituting monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks).

15 Agar is a jelly-like substance obtained from algae. Agar is derived from the polysaccharide agarose, which forms the supporting structure in the cell walls of certain species of algae. Agar is the resulting mixture of the linear polysaccharide agarose and a heterogeneous mixture of smaller molecules called agarpectin. Chemically agar can be designated as a polymer composed of subunits of galactose.

20 Acrylamide is a chemical compound with the chemical formula C_3H_5NO . It is a white odorless crystalline solid, soluble in water, ethanol, ether, and chloroform. Acrylamide can be prepared by the hydrolysis of acrylonitrile by nitrile hydratase. In industry, most acrylamide is used to synthesize polyacrylamides, which find many uses as water-soluble thickeners. These include use in wastewater treatment, gel electrophoresis (SDS-PAGE), papermaking, ore
 25 processing, tertiaryoil recovery.

Agarose is a linear polymer with a molecular weight of about 120,000, consisting of alternating D-galactose and 3,6-anhydro-L-galactopyranose linked by α -(1 $\rightarrow$ 3) and β -(1 $\rightarrow$ 4) glycosidic bonds. The 3,6-anhydro-L-galactopyranose is an L-galactose with an anhydro bridge between the 3 and 6 positions, although some L-galactose unit in the polymer may not contain the
 30 bridge. Some D-galactose and L-galactose units can be methylated. Each agarose chain contains approximately 800 molecules of galactose, and the agarose polymer chains form helical fibers that aggregate into a supercoiled structure. When solidified, the fibers form a three-dimensional mesh of channels with a diameter depending on the concentration of agarose used.

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of
 35 collagen extracted from the skin, bones, and connective tissues of animals. During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that

rearranges more easily. Its chemical composition is, in many respects, closely similar to that of collagen.

According to an especially preferred embodiment of this first aspect of the present invention, the present polymeric solution or suspension is sodium alginate. The present plant cuttings can be readily provided with a sodium alginate coat by contacting, for example by dipping, the basal cut surface of plant cutting with a sodium alginate solution of, for example, 1 to 10% w/v. Sodium alginate is especially preferred considering its non-toxic nature and biocompatibility characteristics. Further, a sodium alginate coat can be readily complexed by contacting the coated plant cutting with a Ca^{2+} comprising solution, such as a 0.5 to 5% w/v solution of CaCl_2 for a sufficient amount of time, for example 10 minutes to 1 hour, to allow an exchange of the sodium ions by calcium ions.

According to another preferred embodiment of this first aspect of the present invention, the present method further comprises, after complexing, a wash or rinsing step, for example using water, to remove leftover potential toxic residual compounds such as ions.

According to the present invention, the present one or more plant growth promoting and/or plant protecting compounds are selected from the group consisting of auxin and derivatives thereof, plant hormones, antibiotics, sugars, minerals and trace elements.

A particularly suitable plant growth promoting and/or plant protecting compound is auxin or a derivative thereof such as 1-naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid.

1-Naphthaleneacetic acid or NAA is an organic compound with the formula $\text{C}_{10}\text{H}_7\text{CH}_2\text{CO}_2\text{H}$. The colorless solid is soluble in organic solvents. NAA is a synthetic plant hormone of the auxin family and is an ingredient in many commercial plant rooting horticultural products; it is a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting. It is also used for plant tissue culture. The hormone NAA does not occur naturally, and, like all auxins, is toxic to plants at high concentrations.

2,4-Dichlorophenoxyacetic acid, also designated as 2,4-D, hedonal or trinoxol is a common systemic herbicide used in the control of broadleaf weeds. It is one of the most widely used herbicides in the world. 2,4-D is a synthetic auxin and as such it is often used in laboratories for plant research and as a supplement in plant cell culture media such as MS medium.

According to a most preferred embodiment of this first aspect of the present invention, the present plant cuttings are encapsulated with a complexed coating from basal cut surface or tip of the plant cutting to less than 50% of the total length of said plant cutting, preferably less than 40%, more preferably less than 20%, most preferably less than 10% to minimize, amongst others, the development of necrotic patches negatively influencing the vigour of the present plant cuttings.

For example, the general length a plant cutting is between 1 cm to 25 cm thus within the context of the present invention, a plant cutting encapsulated with a complexed coating from basal cut surface or tip of the plant cutting to less than 50% indicates that less than 0.5 cm to 12.5 cm of the plant cutting is encapsulated with the present complexed coating measured from the tip of the plant cutting where the plant cutting is separated from the parent plant. As another example, a plant cutting encapsulated with a complexed coating from basal cut surface or tip of the plant cutting to less than 10% indicates that less than 0.1 cm to 2.5 cm of the plant cutting is encapsulated with the present complexed coating measured from the tip where the plant cutting is separated from the parent plant.

According to the present invention, the vigour of the present plant cutting is especially improved with respect to faster root development and/or improving adventitious root formation of said plant cuttings. Although some of the one or more plant growth promoting and/or plant protecting compounds according to the invention have been implicated in root development, present within the present complexed polymeric coat, these compound still provide a surprising beneficial development of “true roots”, i.e. adventitious root formation as opposed to the development of callus and immature, non-functional or partially functional roots. Inherently, the formation, or the induction, of a functional root system, i.e. a root system allowing to support the development of a plant cutting into a mature plant, strongly contributes to the vigour of the present plant cuttings.

According to a second aspect, the present invention relates to encapsulated plant cutting comprising from the basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting a complexed coating comprising a solidified polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds. These encapsulated plant cuttings can suitably be produced using the methods as outlined above.

According to a preferred embodiment of this second aspect of the present invention, the present polymeric solution, or suspension, is selected from the group consisting of sodium alginate, agar, polyacrylamide, agarose, biodegradable polymers and gelatin.

According to an especially preferred embodiment of this second aspect of the present invention, the present polymeric solution, or suspension, is sodium alginate. The present plant cuttings can be readily provided with a sodium alginate coat by contacting, for example by dipping the basal cut surface of plant cutting into a sodium alginate solution of, for example, 1 to 10% w/v. Sodium alginate is especially preferred considering its non-toxic nature and biocompatibility characteristics. Further, the sodium alginate coat can be readily complexed by contacting the coated plant cutting with a Ca^{2+} comprising solution, such as a 0.5 to 5% w/v

solution of CaCl_2 for a sufficient amount of time, for example 10 minutes to 1 hour, to allow an exchange of the sodium ions by calcium ions.

According to the present invention, the present one or more plant growth promoting and/or plant protecting compounds are selected from the group consisting of auxin and derivatives thereof, plant hormones, antibiotics, sugars, minerals and trace elements.

A particularly suitable and influential plant growth promoting and/or plant protecting compound is auxin or a derivative thereof such as 1-naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid.

According to a most preferred embodiment of this second aspect of the present invention, the present plant cuttings are encapsulated with a complexed coating from the basal cut surface or tip of the plant cutting to less than 50% of the total length of said plant cutting, preferably less than 40%, more preferably less than 20%, most preferably less than 10% to, for example, minimize the development necrotic patches negatively influencing the vigour of the present plant cuttings.

According to a third aspect, the present invention relates to encapsulated plant cuttings as outlined above in a growth substrate, preferably a growth substrate selected from the group consisting of vermiculite, glass wool, peat, rock wool, soil, coir and clay.

The present invention will be further detailed in the following example of especially preferred embodiments of the present invention. In the example, reference is made to figures wherein:

Figure 1: provides exemplary photographs of different type of root development used to assess the vigour of the present plant cuttings;

Figure 2: shows a graphical representation of the data shown in Table 1;

Figure 3: shows a graphical representation of the data shown in Table 2;

Figure 4: shows a graphical representation of the data shown in Table 3;

Figure 5: shows a graphical representation of the data shown in Table 4;

Figure 6: shows a graphical representation of the data shown in Table 5;

Figure 7: shows a graphical representation of the data shown in Table 6;

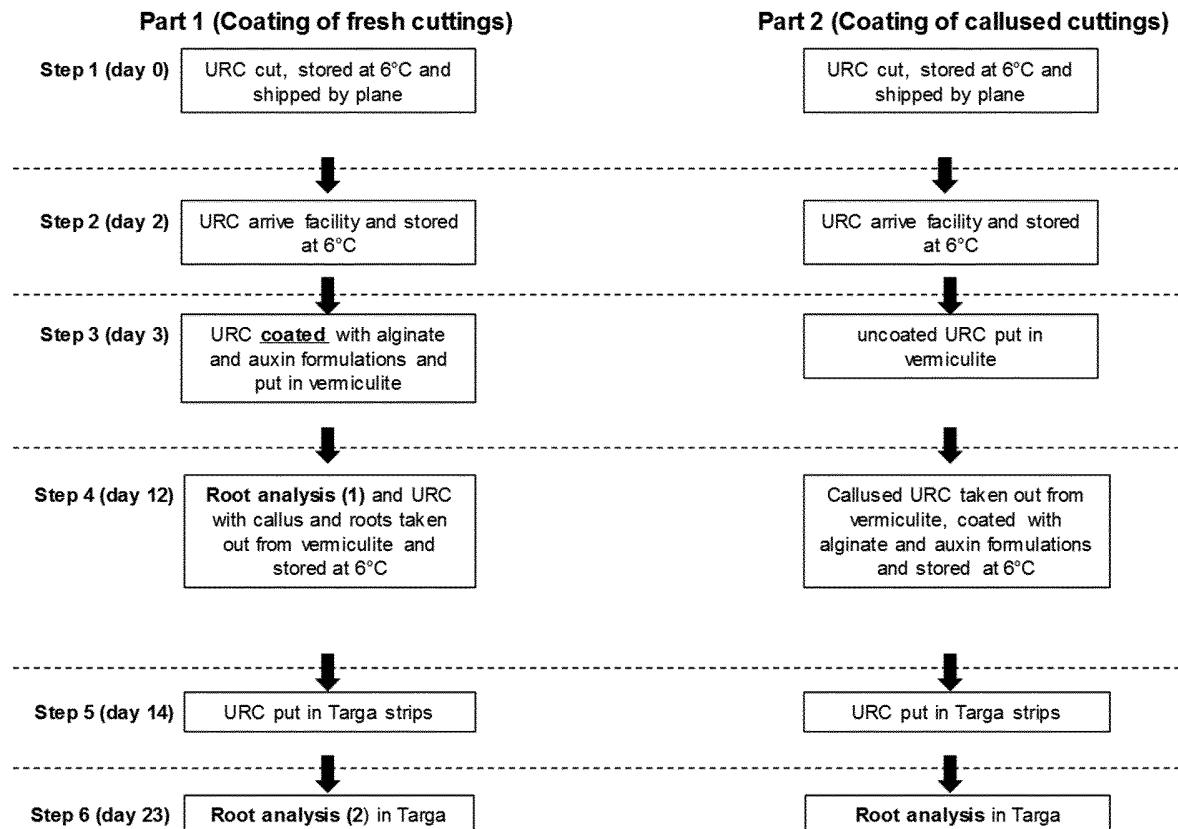
Figure 8: shows a graphical representation of the data shown in Table 7;

Figure 9: shows a graphical representation of the data shown in Table 8;

Figure 10: shows a graphical representation of the data shown in Table 9.

Example

Three two parts [1) coating of fresh cuttings and 2) coating of callused cuttings] trials were performed for assessing plant cutting vigour and especially root formation (**Figure 1**) by following the experimental protocol as depicted in a below scheme (URC denotes plant cutting):



- 10 Plant cuttings from two species were used, i.e. *Pelargonium peltatum* (20629) and *P. zonale* (20261 and 20309).

For the trials, fresh plant cuttings were divided into 4 groups:

- 15 Group 1: non-encapsulated control group;
 Group 2: encapsulated plant cuttings with no plant growth promoting and/or plant protecting compounds in the complexed coat;
 Group 3: encapsulated plant cuttings with NAA (10 mg/l) in the complexed coat;
 Group 4: encapsulated plant cuttings with 2,4-D (10 mg/l) in the complexed coat.

Encapsulation was performed by dipping, cutting face first, the plant cutting (part 1, as mentioned in scheme above) and the callused cuttings (part 2, as mentioned in scheme above) in a 2.5% w/v sodium alginate solution thereby coating over approximately 10% of the length of the plant cutting with sodium alginate. Subsequently, the coated plant cuttings were incubated in a

5 CaCl_2 bath (1.1% w/v) for 25 to 30 minutes allowing the exchange of the sodium ions in the alginate by calcium ions for providing a complexed alginate coat. Then, the encapsulated plant cuttings were rinsed in water to remove residual calcium and sodium ions.

All four groups of plant cuttings and callused cuttings were stored at 6 °C. For part 1 of the trials, the plant cuttings were placed in vermiculite and callus and root initiation were

10 assayed by visually analyzing individual cuttings and categorizing them based on four successive stages of callus and root initiation: callus, mature callus, roots emerging, roots grown. These cuttings from part 1 of the trials and the callused cuttings from part 2 of the trials were subsequently shifted to soil present in the targa strips and vigour was assayed by visually analyzing individual cuttings and categorizing them based on stages of root formation.

15

Root formation was divided in five categories:

- - No roots
- - Root tips

20

- - Roots emerging
- - Roots developing
- - Extended root development

A representative example of the above categories is presented in **Figure 1**.

25

Below, the results of the three trials are presented in **Tables 1 to 9**. The numbers depict number of cuttings belonging to a specific category. Graphical presentation of the results is shown for **Table 1** in **Figure 2**, for **Table 2** in **Figure 3**, for **Table 3** in **Figure 4**, etc.

30

Table 1: *Part 1: callus and root initiation analysis in vermiculite – variety 20309*

	callus	mature callus	roots emerging	roots grown
Control	24	21	13	1
A (Coating)	24	35	4	7
B (Coating + NAA)	1	21	23	25
C (Coating + 2,4-D)	5	40	18	7

5 **Table 2:** *Part 1: root analysis in Targa – variety 20309*

20309	no roots	root tips	roots emerging	roots developing	extended root development
Control	0	3	11	48	3
A (Coating)	0	0	7	43	20
B (Coating + NAA)	0	0	0	23	48
C (Coating + 2,4-D)	0	1	6	25	42

Table 3: *Part 2: 1 root analysis in Targa – variety 20309*

10

20309	no roots	root tips	roots emerging	roots developing	extended root development
Control	0	3	11	48	3
D (Callus Coating)	0	0	5	42	26
E (Callus Coating + NAA)	0	0	8	36	18
F (Callus Coating + 2,4-D)	0	1	0	45	23

Table 4: *Part 1: callus and root initiation analysis in vermiculite – variety 20261*

	callus	mature callus	roots emerging	roots grown
Control	30	30	6	3
A (Coating)	22	39	8	3
B (Coating + NAA)	5	30	27	8
C (Coating + 2,4-D)	0	65	3	0

5 **Table 5:** *Part 1: root analysis in Targa – variety 20261*

20261	no roots	root tips	roots emerging	roots developing	extended root development
Control	1	1	8	42	18
A (Coating)	0	0	8	46	17
B (Coating + NAA)	0	1	0	35	34
C (Coating + 2,4-D)	0	0	2	36	32

Table 6: *Part 2: 1 root analysis in Targa – variety 20261*

10

20261	no roots	root tips	roots emerging	roots developing	extended root development
Control	1	1	8	42	18
D (Callus Coating)	0	0	9	41	20
E (Callus Coating + NAA)	0	0	3	50	17
F (Callus Coating + 2,4-D)	2	1	11	52	4

Table 7: *Part 1: callus and root initiation analysis in vermiculite – variety 20629*

	callus	mature callus	roots emerging	roots grown
Control	8	17	20	23
A (Coating)	5	15	29	22
B (Coating + NAA)	0	10	18	42
C (Coating + 2,4-D)	0	8	38	23

5 **Table 8:** *Part 1: root analysis in Targa – variety 20629*

20629	no roots	root tips	roots emerging	roots developing	extended root development
Control	0	2	2	32	32
A (Coating)	1	2	3	26	40
B (Coating + NAA)	0	1	6	25	37
C (Coating + 2,4-D)	6	1	10	30	24

Table 9: *Part 2: 1 root analysis in Targa – variety 20629*

20629	no roots	root tips	roots emerging	roots developing	extended root development
Control	0	2	2	32	32
D (Callus Coating)	1	3	6	32	28
E (Callus Coating + NAA)	0	1	7	35	27
F (Callus Coating + 2,4-D)	0	0	9	41	18

CLAUSES

1. Method for improving the vigour of plant cuttings, said method comprises the steps of:

- 5 a) contacting a plant cutting with a polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds, thereby providing a coated plant cutting;
- b) allowing the polymeric solution, or suspension, of the coated plant cutting to solidify into a complexed coating thereby providing an encapsulated
- 10 plant cutting;

wherein said encapsulated plant cutting is coated with the complexed coating from the basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting to partially encapsulate the plant cutting with the complexed coating.

15 2. Method according to clause 1, wherein said polymeric solution, or suspension, is selected from the group consisting of sodium alginate, agar, polyacrylamide, agarose, biodegradable plastics, and gelatin.

20 3. Method according to clause 1 or clause 2, wherein said polymeric solution, or suspension, is sodium alginate.

25 4. Method according to clause 3, wherein said sodium alginate solution, or suspension, is solidified by contacting the sodium alginate solution, or suspension, coated plant cutting with a solution comprising calcium ions, preferably CaCl_2 .

 5. Method according to any of the clauses 1 to 4, wherein said method further comprises a washing or rinsing step after step (b).

30 6. Method according to any of the clauses 1 to 5, wherein said one or more plant growth promoting and/or plant protecting compounds are selected from the group consisting of auxin and derivatives thereof, plant hormones, antibiotics, sugars, minerals and trace elements.

35 7. Method according to any of the clauses 1 to 6, wherein said one or more plant growth promoting and/or plant protecting compounds growth promoting and/or plant protecting compounds comprise 1-naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid.

8. Method according to any of the clauses 1 to 7, wherein said complexed coating is from basal cut surface or tip of the plant cutting to less than 50% of the total length of said plant cutting, preferably less than 40%, more preferably less than 20%, most preferably less than 10%.

5 9. Method according to any of the clauses 1 to 8, wherein said improved vigour comprises faster root development and/or improving adventitious root formation of said plant cuttings.

10 10. Encapsulated plant cutting comprising from basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting a complexed coating comprising a solidified polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds.

15 11. Encapsulated plant cutting according to clause 10, wherein said polymeric solution, or suspension, is selected from the group consisting of sodium alginate, agar, acrylamide, agarose, biodegradable polymers and gelatin.

20 12. Encapsulated plant cutting according to clause 10 or clause 11, wherein said polymeric solution, or suspension, is sodium alginate.

 13. Encapsulated plant cutting according to clause 12, wherein said sodium alginate solution, or suspension, is solidified by contacting the sodium alginate solution, or suspension, coated plant cutting with a solution comprising calcium ions, preferably CaCl_2 .

25 14. Encapsulated plant cutting according to any of the clauses 10 to 13, wherein said one or more plant growth promoting and/or plant protecting compounds are selected from the group consisting of auxin, plant hormones, antibiotics and sugars.

30 15. Encapsulated plant cutting according to any of the clauses 10 to 14, wherein said one or more plant growth promoting and/or plant protecting compounds growth promoting and/or plant protecting compounds comprise 1-naphthaleneacetetic acid or 2,4-dichlorophenoxyacetetic acid.

35 16. Encapsulated plant cutting according to any of the clauses 10 to 15, wherein said complexed coating is from basal cut surface or tip of the plant cutting to less than 50% of the

total length of said plant cutting, preferably less than 40%, more preferably less than 20%, most preferably less than 10%.

17. A plant cutting obtainable by a method according to any of the clauses 1 to 9
5 or an encapsulated plant cutting according to any of the clauses 10 to 16 in a growth substrate.

18. A plant cutting according to clause 17 wherein said growth substrate is
selected from the group consisting of vermiculite, glass wool, peat, rock wool, soil, coir and clay.

CONCLUSIES

1. Werkwijze voor het verbeteren van de ontwikkelcapaciteit van plantstekken, de genoemde werkwijze omvat de stappen van:

- 5 a) het in contact brengen van een plantstek met een polymere oplossing, of suspensie, optioneel omvattende een of meer plantgroei bevorderende en/of plant beschermende verbindingen, waardoor een beklede plantstek wordt verschaft;
- 10 b) het verschaffen dat de polymere oplossing, of suspensie, van de beklede plantstek stolt of vernet tot een gecomplexeerde bekleding waardoor een omhulde plantstek wordt verschaft;

waarbij de genoemde omhulde plantstek is bekleed met de gestolde of vernette bekleding vanaf het snijvlak tot en met minder dan 60% van de totale lengte van de genoemde plantstek voor het gedeeltelijk omhullen van de plantstek met de gecomplexeerde bekleding.

15

2. Werkwijze volgens conclusie 1 waarbij de genoemde polymere oplossing, of suspensie, wordt geselecteerd uit de groep die bestaat uit natrium alginaat, agar, polyacrylamide, agarose, biologisch afbreekbare kunststoffen en gelatine.

20 3. Werkwijze volgens conclusie 1 of conclusie 2, waarbij de genoemde polymere oplossing, of suspensie, natrium alginaat is.

25 4. Werkwijze volgens conclusie 3, waarbij de genoemde natrium alginaat oplossing, of suspensie, stolt of wordt vernet door middel van het in contact brengen van de natrium alginaat oplossing, of suspensie, beklede plantstek met een oplossing omvattende calcium ionen, bij voorkeur CaCl_2 .

30 5. Werkwijze volgens een van de conclusies 1 tot en met 4, waarbij de genoemde werkwijze verder omvat een was- of spoelstap na stap (b).

35 6. Werkwijze volgens een van de conclusies 1 tot en met 5, waarbij de genoemde een of meer plantgroei bevorderende en/of plant beschermende verbindingen worden geselecteerd uit de groep die bestaat uit auxine of derivaten daarvan, planthormonen, antibiotica, suikers, mineralen en sporenelementen.

7. Werkwijze volgens een van de conclusies 1 tot en met 6, waarbij de genoemde een of meer plantgroei bevorderende en/of plant beschermende verbindingen 1-naphthaleen-azijnzuur of 2,4-dichloorfenox-azijnzuur omvatten.

5 8. Werkwijze volgens een van de conclusies 1 tot en met 7, waarbij de genoemde gecomplexeerde bekleding is vanaf het snijvlak van de plantstek tot en met minder dan 50% van de totale lengte van de genoemde plantstek, bij voorkeur minder dan 40%, meer bij voorkeur minder dan 20%, meest bij voorkeur minder dan 10%.

10 9. Werkwijze volgens een van de conclusies 1 tot en met 8, waarbij de genoemde verbeterde groeicapaciteit een snellere wortelvorming omvat en/of een “adventitious” wortelvorming van de genoemde plantstek.

15 10. Omhulde plantstek omvattende vanaf het snijvlak tot en met minder dan 60% van de totale lengte van de genoemde plantstek een gestolde of vernette polymere oplossing, of suspensie, optioneel omvattende een of meer plantgroei bevorderende en/of plant beschermende verbindingen.

20 11. Omhulde plantstek volgens conclusie 10, waarbij de genoemde polymere oplossing, of suspensie, wordt geselecteerd uit de groep die bestaat uit natrium alginaat, agar, polyacrylamide, agarose, biologisch afbreekbare kunststoffen en gelatine.

25 12. Omhulde plantstek volgens conclusie 10 of conclusie 11, waarbij de genoemde polymere oplossing, of suspensie, natrium alginaat is.

30 13. Omhulde plantstek volgens conclusie 12, waarbij de genoemde natrium alginaat oplossing, of suspensie, stolt of wordt vernet door middel van het in contact brengen van de natrium alginaat oplossing, of suspensie, beklede plantstek met een oplossing omvattende calcium ionen, bij voorkeur CaCl_2 .

35 14. Omhulde plantstek volgens een van de conclusies 1 tot en met 4, waarbij de genoemde een of meer plantgroei bevorderende en/of plant beschermende verbindingen worden geselecteerd uit de groep die bestaat uit auxine of derivaten daarvan, planthormonen, antibiotica, suikers, mineralen en sporenelementen.

15. Omhulde plantstek volgens een van de conclusies 10 tot en met 14, waarbij de genoemde een of meer plantgroei bevorderende en/of plant beschermende verbindingen 1-naphthaleen- azijnzuur of 2,4-dichloorfenox-azijnzuur omvatten.

5 16. Omhulde plantstek volgens een van de conclusies 10 tot en met 15, waarbij de genoemde gecomplexeerde bekleding is vanaf het snijvlak van de plantstek tot en met minder dan 50% van de totale lengte van de genoemde plantstek, bij voorkeur minder dan 40%, meer bij voorkeur minder dan 20%, meest bij voorkeur minder dan 10%.

10 17. Plantstek verkrijgbaar door middel van een van de conclusies 1 tot en met 9 of een omhulde plantstek volgens een van de conclusies 1 tot en met 16 in een groeistruaat.

18. Plantstek volgens conclusie 17, waarbij het genoemde groeistruaat wordt geselecteerd uit de groep die bestaat uit vermiculiet, glaswol, turf, steenwol, kokosvezel en klei.

15

FIGURE 1

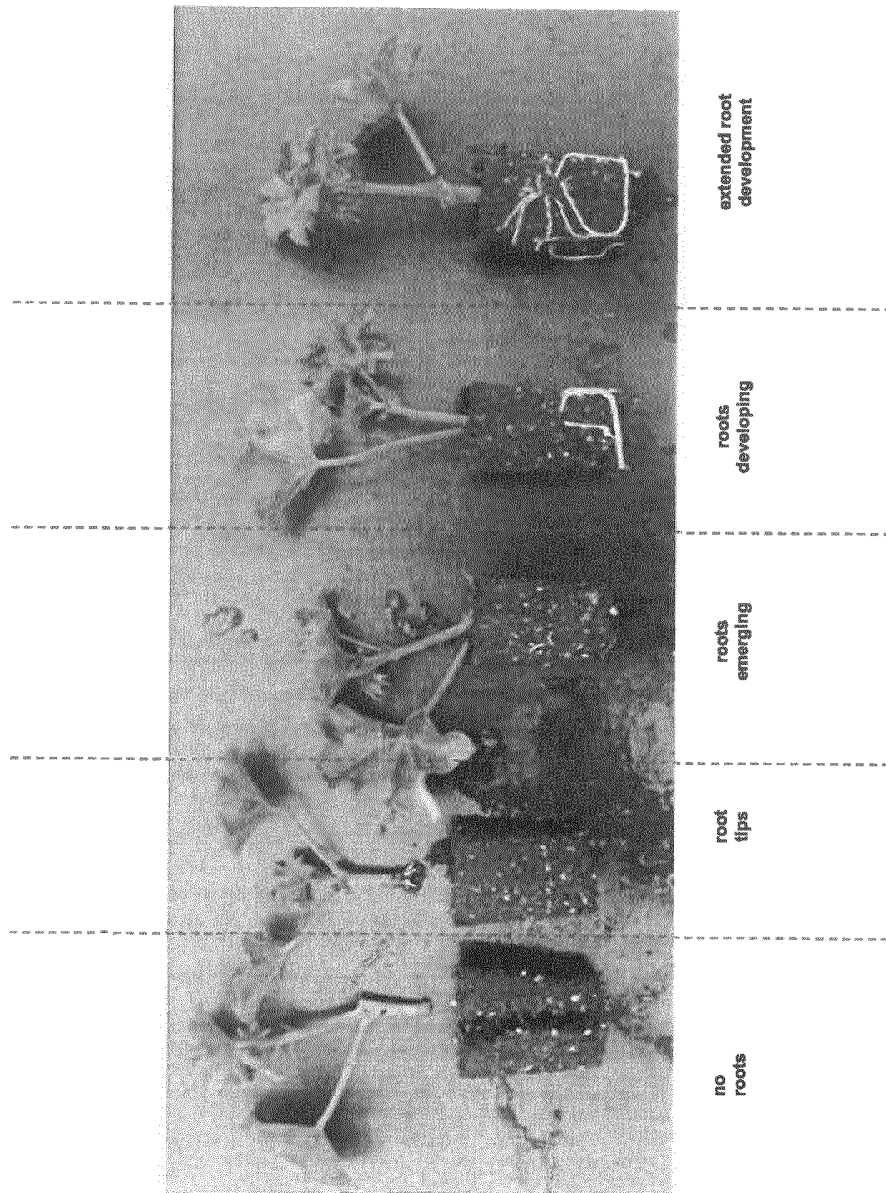


FIGURE 2

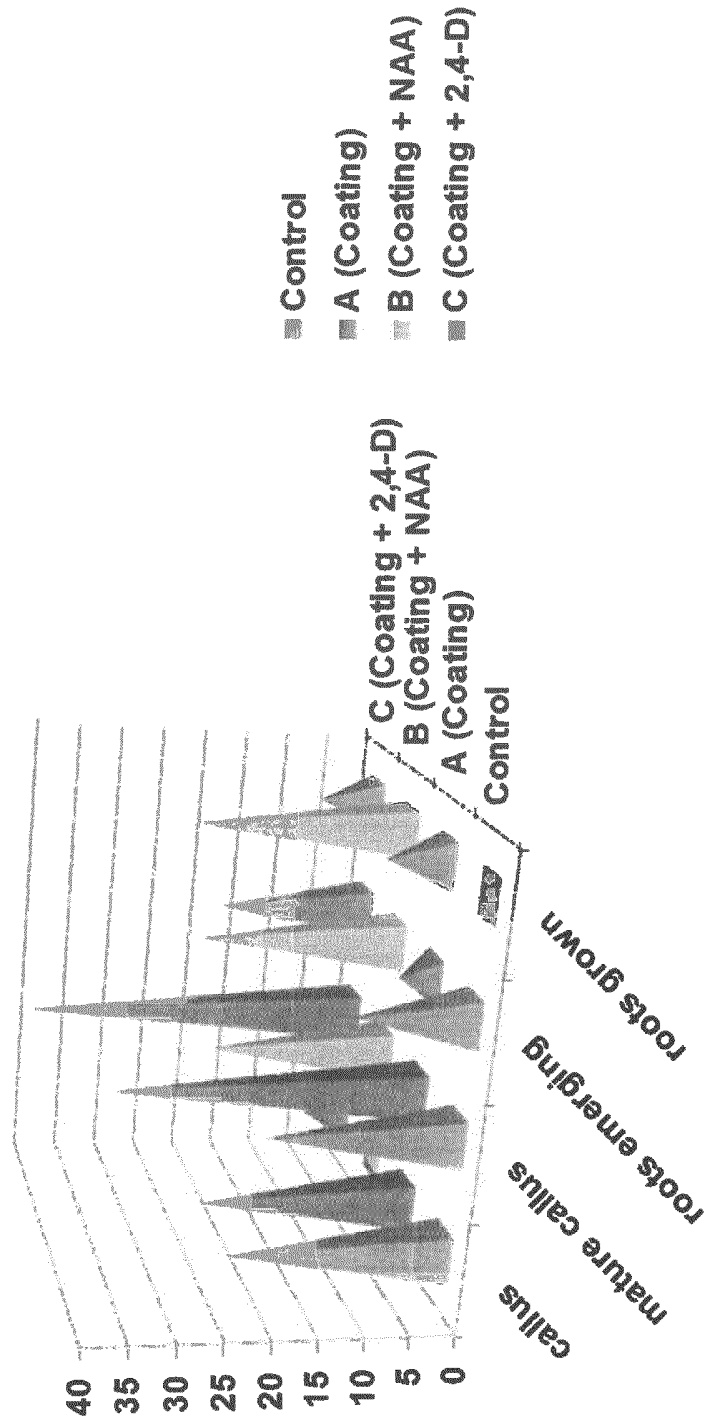


FIGURE 3

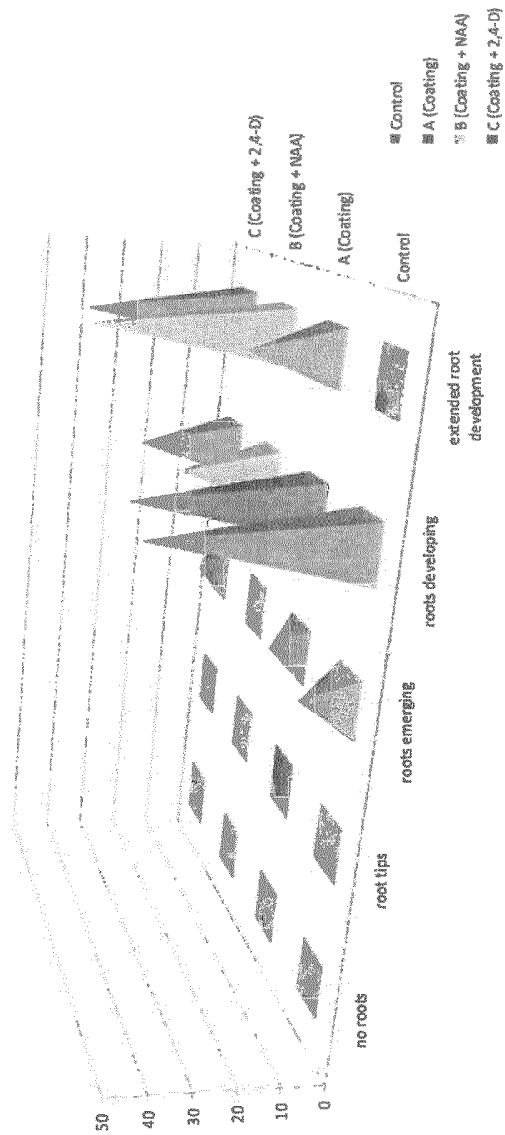


FIGURE 4

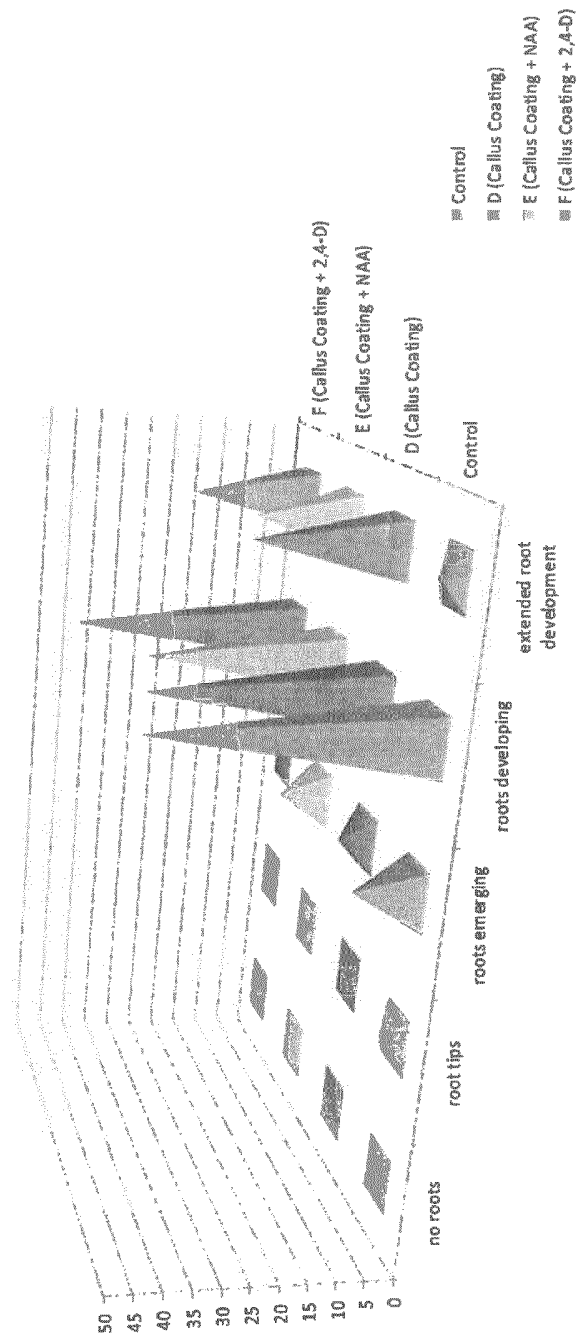


FIGURE 5

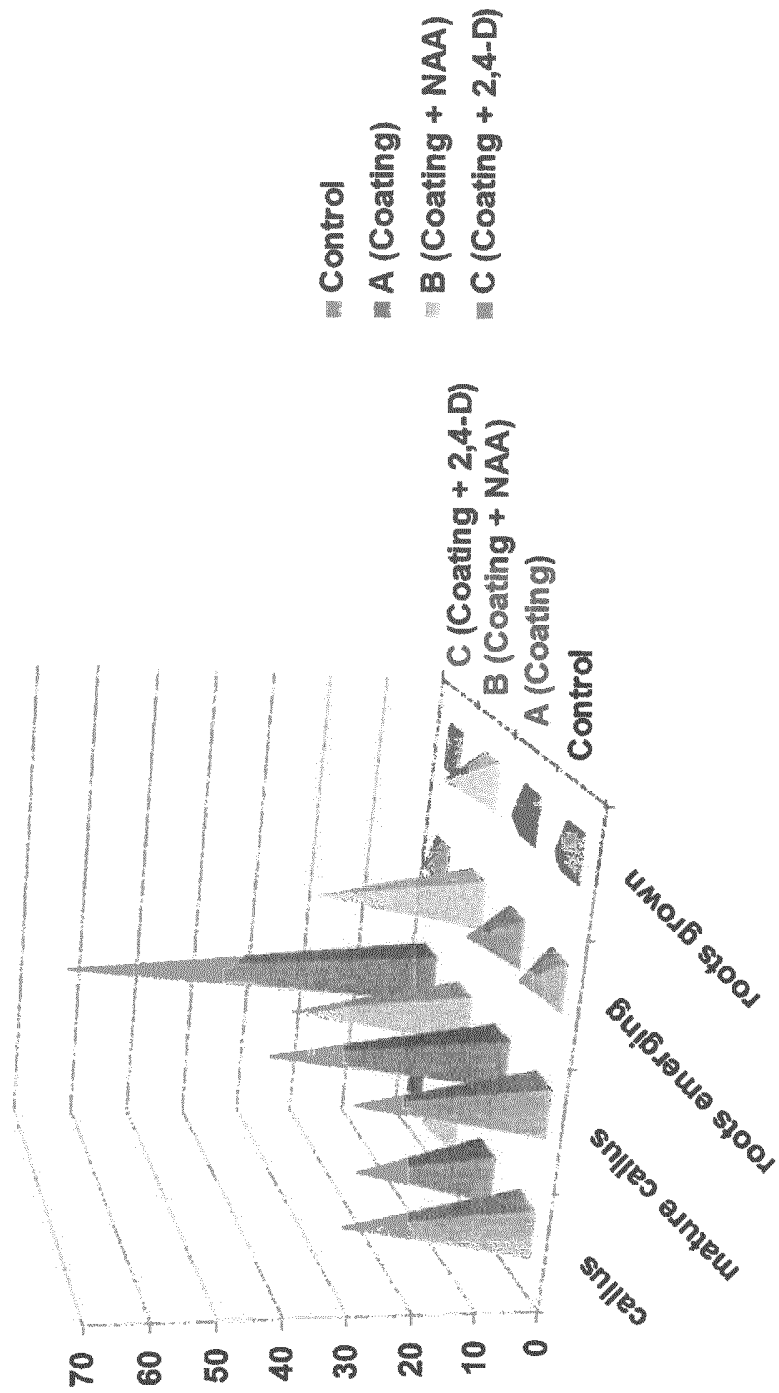


FIGURE 6

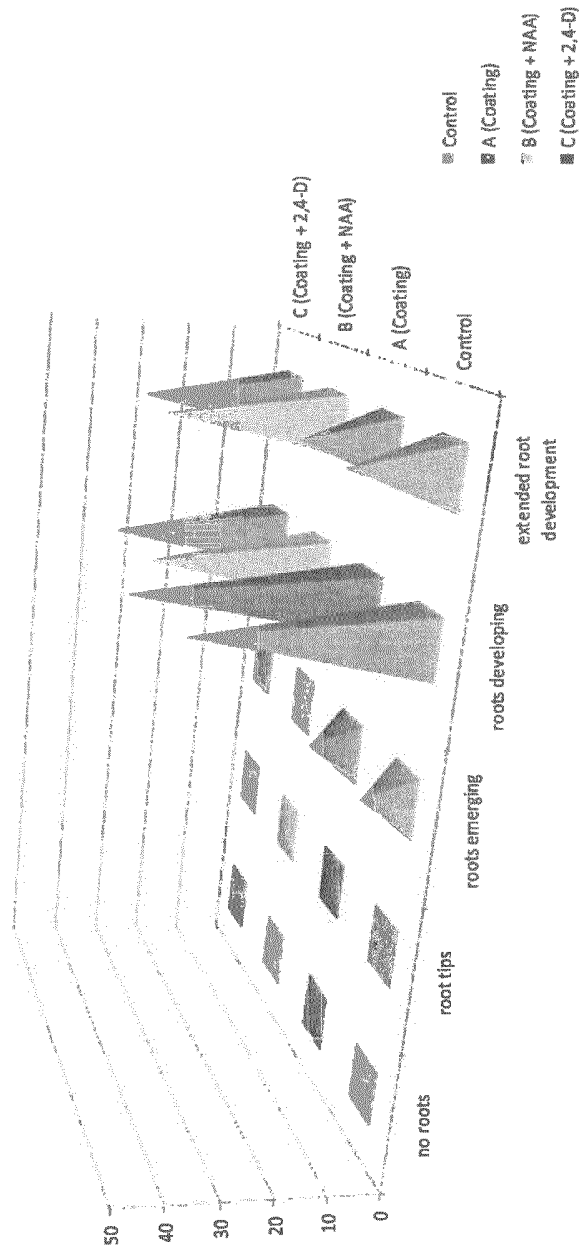


FIGURE 7



FIGURE 8

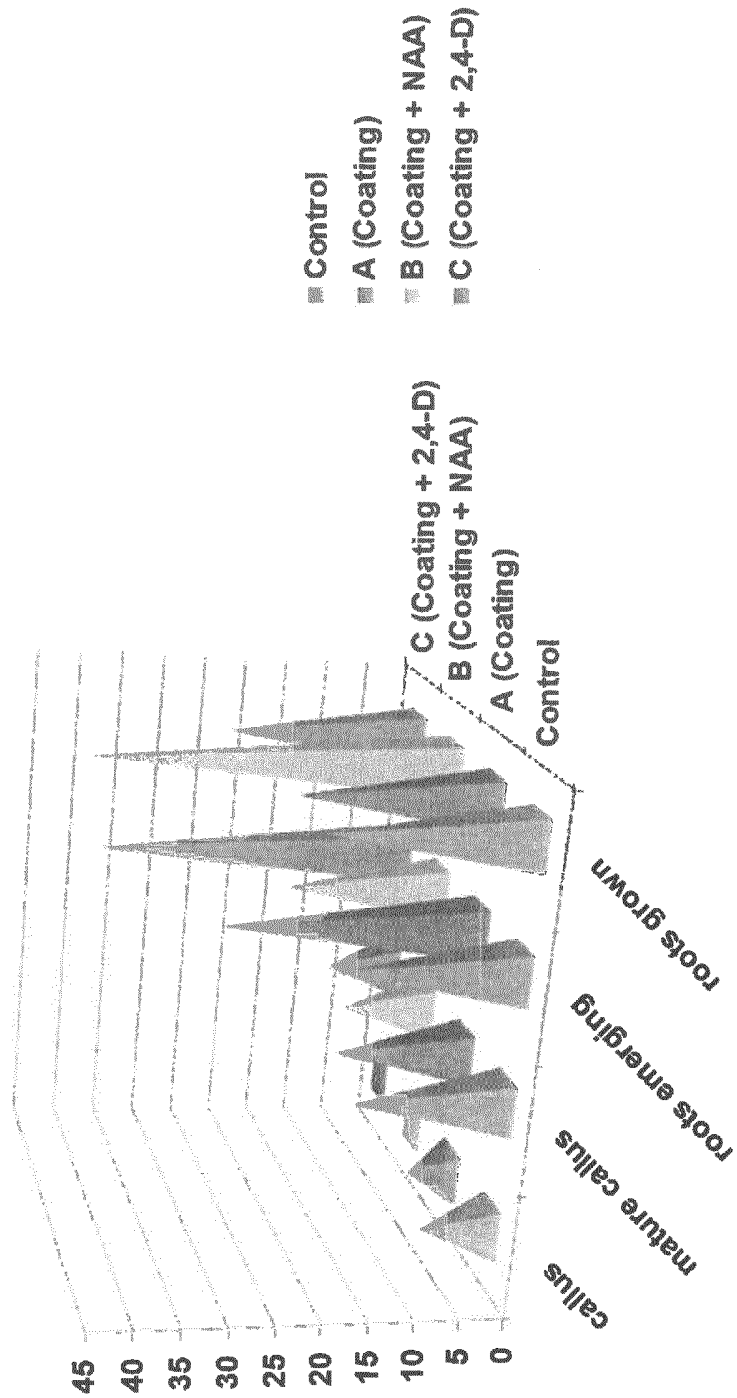


FIGURE 9

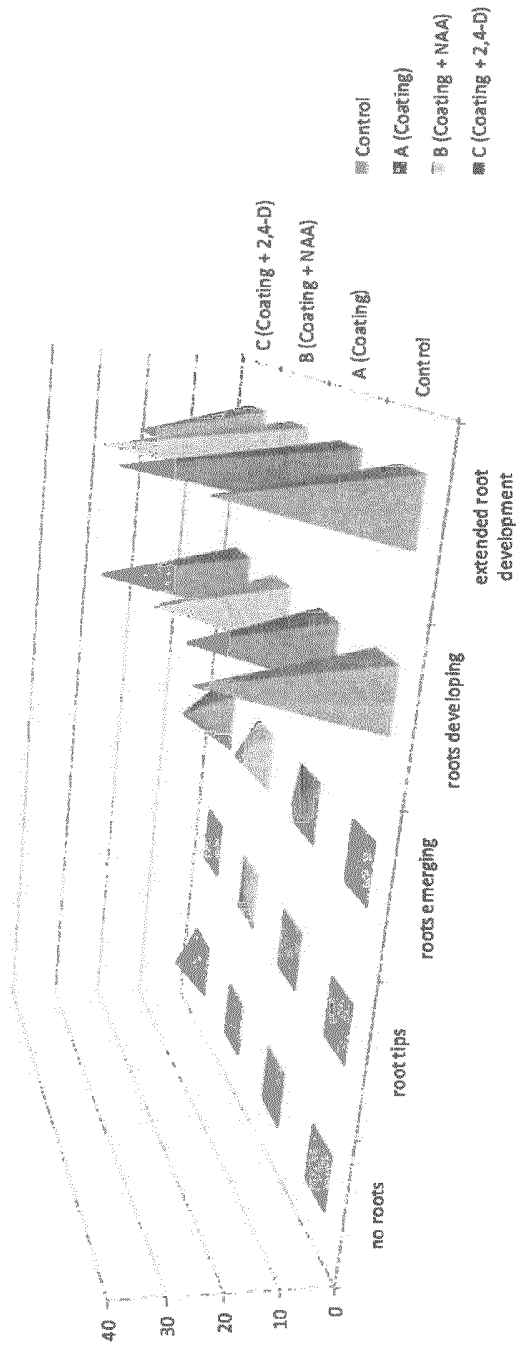
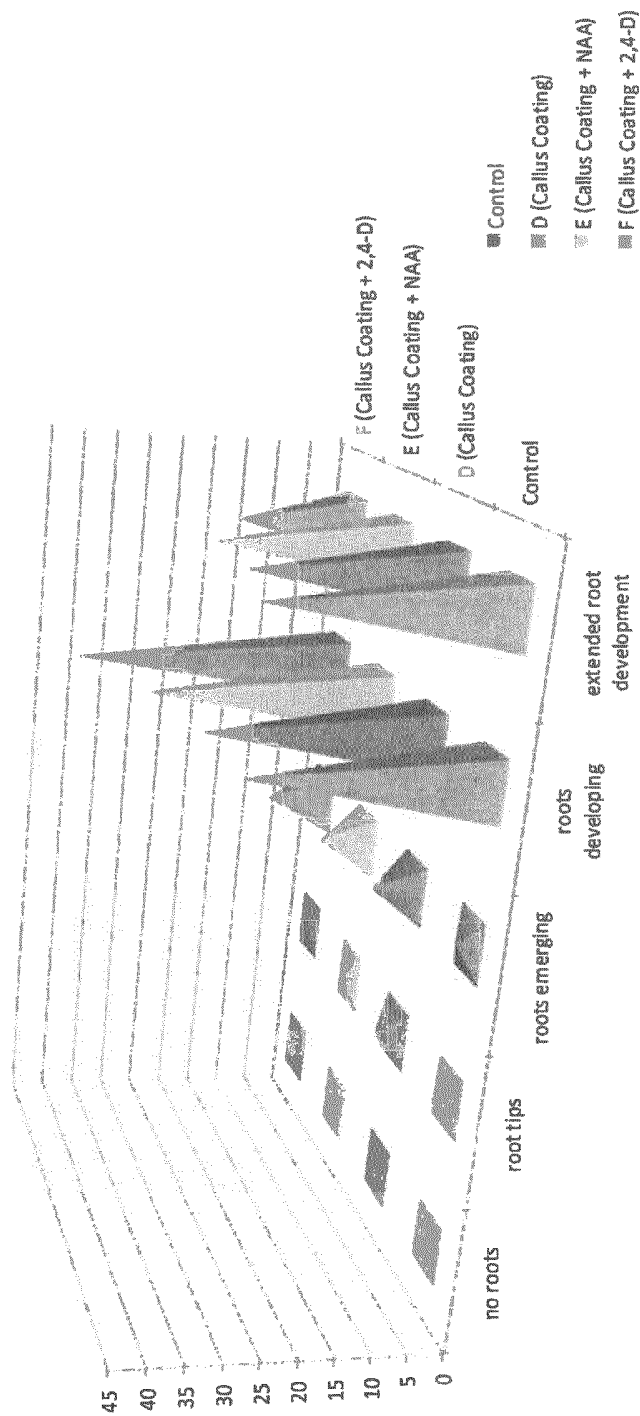


FIGURE 10



ABSTRACT

The present invention relates to methods for providing improved vigour, and especially priming or improving root formation, of plant cuttings and to plant cuttings, amongst
5 others, obtainable by the present methods. Specifically, the present invention relates to methods for improving the vigour of plant cuttings, wherein the method comprises the steps of a) contacting a plant cutting with a polymeric solution, or suspension, optionally comprising one or more plant growth promoting and/or plant protecting compounds, thereby providing a coated plant cutting; b)
10 allowing the polymeric solution, or suspension, of the coated plant cutting to solidify into a complexed coating thereby providing an encapsulated plant cutting; wherein said encapsulated plant cutting is coated with the complexed coating from the basal cut surface or tip of the plant cutting to less than 60% of the total length of said plant cutting to partially encapsulate the plant cutting with the complexed coating.

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE 	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE 4/2RR83/11
Nederlands aanvraag nr. 2014879	Indieningsdatum 28-05-2015
	Ingevoerd voorrangsdatum
Aanvrager (Naam) Fides B.V.	
Datum van het verzoek voor een onderzoek van internationaal type 11-07-2015	Door de instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. SN64519
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)	
Volgens de internationale classificatie (IPC) A01G1/06;A01N3/04	
II. ONDERZOCHETE GEBIEDEN VAN DE TECHNIEK	
Onderzochte minimumdocumentatie	
Classificatiesysteem	Classificatiesymbolen
IPC	A01G;A01N
Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen:	
III.	☐ GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES (opmerkingen op aanvullingsblad)
IV.	☐ GEBREK AAN EENHEID VAN UITVINDING (opmerkingen op aanvullingsblad)

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2014879

A. CLASSIFICATIE VAN HET ONDERWERP

INV. A01G1/06 A01N3/04
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimum documentatie (plaatscode gevolgd door classificatiesymbolen)

A01G A01N

Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)

EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie *	Geaciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	GB 2 425 530 A (SUPAPLANTS LTD [GB]) 1 november 2006 (2006-11-01) * samenvatting; figuur 2 * * bladzijde 3 - bladzijde 13 *	1-18
A	US 2007/163173 A1 (SAVICH MILAN H [US]) 19 juli 2007 (2007-07-19) * het gehele document *	1-18
	----- -/-	



Verdere documenten worden vermeld in het vervolg van vak C.



Leden van dezelfde octroofamilie zijn vermeld in een bijlage

*** Speciale categorieën van aangehaalde documenten**

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"C" in de octrooiaanvraag vermeld

"E" eerdere octrooi(aanvraag), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermeldde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorafgedatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorafgedatum gepubliceerde literatuur die niet bezwaarlijk is voor de octrooiaanvraag, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geaciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"Z" lid van dezelfde octroofamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid

3 februari 2016

Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type

Naam en adres van de instantie

European Patent Office, P.O. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040
Fax: (+31-70) 340-3016

De bevoegde ambtenaar

Balzar, Maarten

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2014879

C (Verzoek) VAN BELANG GEACHTTE DOCUMENTEN

Categorie *	Geachte documenten, eventueel met aanduiding van specifiek van belang zijnde passages	Van belang voor conclusie nr.
A	NAIK S K ET AL: "Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (Punica granatum L.) for germplasm distribution and exchange". SCIENTIA HORTICULTURAE, ELSEVIER SCIENCE PUBLISHERS, XX, deel 108, nr. 3, 8 mei 2006 (2006-05-08), bladzijden 247-252, XP024983761, ISSN: 0304-4238, DOI: 10.1016/J.SCIENTA.2006.01.030 [gevonden op 2006-05-08] * het gehele document * -----	1-18
A	WO 2011/125045 A2 (VOLTERRANI MARCO [IT]; PACINI MAURIZIO [IT]) 13 oktober 2011 (2011-10-13) * het gehele document * -----	1-18

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**
informatie over leden van dezelfde oestroefamilie

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2014879

In het rapport genoemd oestroefgeeschrift	Datum van publicatie	Overeenkomstig(e) geschrift(en)	Datum van publicatie	
GB 2425530	A	01-11-2006	GB 2425530 A	01-11-2006
			US 2008216404 A1	11-09-2008
			WO 2006114615 A1	02-11-2006

US 2007163173	A1	19-07-2007	GEEN	

WO 2011125045	A2	13-10-2011	EP 2555608 A2	13-02-2013
			IT 1402789 B1	18-09-2013
			US 2014075604 A1	13-03-2014
			WO 2011125045 A2	13-10-2011

WRITTEN OPINION

File No. SN64519	Filing date (day/month/year) 28.05.2015	Priority date (day/month/year)	Application No. NL2014879
International Patent Classification (IPC) INV. A01G1.06 A01N3/04			
Applicant Fides B.V.			

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

	Examiner Balzar, Maarten
--	-----------------------------

WRITTEN OPINION

Application number
NL2014879

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the application as filed.
 - ☐ filed together with the application in electronic form.
 - ☐ furnished subsequently for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes: Claims	1-18
	No: Claims	
Inventive step	Yes: Claims	
	No: Claims	1-18
Industrial applicability	Yes: Claims	1-18
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1 GB 2 425 530 A (SUPAPLANTS LTD [GB]) 1 november 2006 (2006-11-01)
- D3 NAIK S K ET AL: "Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange",
SCIENTIA HORTICULTURAE, ELSEVIER SCIENCE PUBLISHERS, XX, deel 108, nr. 3, 8 mei 2006 (2006-05-08), bladzijden 247-252, XP024983761,
ISSN: 0304-4238, DOI: 10.1016/J.SCIENTA.2006.01.030
[gevonden op 2006-05-08]

- 1 The present application does not meet the criteria of patentability, because the subject-matter of claim 1-18 does not involve an inventive step.
- 1.1 Document D1 is regarded as being the prior art closest to the subject-matter of claim 1, and discloses a

werkwijze voor het verbeteren van de ontwikkelcapaciteit van plantstekken (22) (abstract, note "... for the improved propagation of plant cuttings, ..."; page 5, lines 4-10; figure 2), de genoemde werkwijze omvat de stappen van:

het in contact brengen van een plantstek (22) met een polymere oplossing of suspensie (page 5, lines 8-9, note "placing an appropriate portion of a plant cutting suitable for propagation into the viscous aqueous planting fluid ..."; page 5, lines 31-33; page 10, lines 5-7, note "The viscous aqueous planting fluid may be provided in the form of hydrateable precursor ..., such as polymer granules ... to form a homogeneous fluid"), optioneel omvattende een of meer plantgroei bevorderende en/of plant beschermende verbindingen (page 6, lines 2-11), waardoor een beklede plantstek (22) wordt verschaft (page 5, lines 14-33; page 10, lines 31-34; figure 2);

het verschaffen dat de oplossing of suspensie, van de beklede plantstek (22) stolt of vernet tot een gecomplexeerde bekleding waardoor een omhulde plantstek (22) wordt verschaft (page 5, lines 25-27, note "... as such gels retain their integrity in-situ and do not break down ...");

waarbij de genoemde omhulde plantstek (22) is bekleed met de gestolde of vernette bekleding vanaf het snijvlak tot en met minder dan 60% van de totale lengte van de genoemde plantstek (22) voor het gedeeltelijk omhullen van de plantstek (22) met de gecomplexeerde bekleding (figure 2, note that the upper leafed portion 24 of cutting 22 must represent over 60% of the total length of cutting 22; page 10, line 21 - page 11, line 2).

1.1.1 The subject-matter of claim 1 therefore differs from this known "werkwijze voor het verbeteren van de ontwikkelcapaciteit van plantstekken" in that the "werkwijze" comprises "het verschaffen dat de polymere oplossing of suspensie, van de beklede plantstek stolt of vernet tot een gecomplexeerde bekleding", and is therefore new.

1.1.2 The problem to be solved by the present invention may therefore be regarded as how to modify the "werkwijze voor het verbeteren van de ontwikkelcapaciteit van plantstekken" to provide an alternative way of providing a "complexed coating" around a part of the cutting.

1.1.3 The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step for the following reasons:

Document D1 discloses that "the plant cutting (22) comprises a first, upper, leafed portion (24) and a lower, rooting portion (26) which is inserted into the viscous aqueous planting fluid (12)" (page 10, lines 32-34). Additionally, document D1 discloses that "the viscous aqueous planting fluid may be a gel derived from the use of one or more organic gellation aids" and that "suitable organic gellation aids are known to persons skilled in the art" (page 5, lines 31-33).

The feature "het verschaffen dat de polymere oplossing of suspensie, van de beklede plantstek stolt of vernet tot een gecomplexeerde bekleding" is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of

inventive skill, in order to solve the problem posed. For example, the use of agar gel (polymer), wherein the gel is at first liquid and solidifies after cooling, is very well-known in plant biology (e.g. root development). Additionally, document D3 discloses the use of sodium alginate in combination with a complexing agent for the encapsulation of nodal segments in relation to root/shoot development (page 2, left column, paragraph 2.2).

Therefore, the subject-matter of independent claim 1 does not involve an inventive step.

- 1.2 The additional features of claims 2-5 are merely some of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to obtain the to-be-expected advantages (see e.g. document D3). Consequently, the subject-matter of dependent claims 2-5 does not involve an inventive step.
- 1.3 Concerning claim 6, document D1 further discloses that said one or more plant growth promoting and/or plant protecting compounds are selected from the group consisting of auxin and derivatives thereof (page 6, lines 2-11). Consequently, the subject-matter of dependent claim 6 does not involve an inventive step.
- 1.4 The additional features of claim 7 are merely some of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to obtain the to-be-expected advantages (see e.g. document D3, note that, for example, naphthalene acetic acid is disclosed in the abstract). Consequently, the subject-matter of dependent claim 7 does not involve an inventive step.
- 1.5 The additional features of claim 8 are merely some of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to obtain the to-be-expected advantages. Consequently, the subject-matter of dependent claim 8 does not involve an inventive step.

- 1.6 Concerning claim 9, document D1 discloses that said improved vigour comprises faster root development of said plant cuttings (abstract, note "improved rooting"; pages 12 and 13). Therefore, the subject-matter of dependent claim 9 does not involve an inventive step.
- 1.7 The same reasoning (see above points 1.1-1.1.3) applies, mutatis mutandis, to the subject-matter of the corresponding independent claim 10, which therefore is also considered not inventive.
- 1.8 The same reasoning (see above points 1.2-1.6) applies, mutatis mutandis, to the subject-matter of the (resp.) corresponding dependent claims 11-16, which therefore is also considered not inventive.
- 1.9 Concerning claim 17, document D1 furthermore discloses an encapsulated plant cutting (22,12) in a growth substrate (4) (figure 2; page 10, lines 21-35, note "The container acts as a matrix suitable for supporting root growth"; page 9, lines 15-20). Therefore, the subject-matter of dependent claim 17 does not involve an inventive step.
- 1.10 Concerning claim 18, document D1 also discloses that said growth substrate is selected from the group consisting of vermiculite, glass wool, peat, rock wool, soil, coir and clay (page 9, lines 15-20). Therefore, the subject-matter of dependent claim 18 does not involve an inventive step.