

Methods for Producing Proteins In Plants

The present ~~invention~~-disclosure relates to methods for expressing proteins of interest, particularly pharmaceutically valuable proteins, transiently in plants.

- 5 The large scale production of recombinant proteins is an important application of transgenic plants. Although many plants can be successfully used for production of recombinant proteins, few systems have the potential to produce substantial amounts of a recombinant protein within a short time period.

Transient gene expression in plant cells has been developed as a rapid means to
10 produce small amounts of a given protein and for testing genetic constructs. Methods to transiently produce a protein in a plant cell include for example particle gun delivery of a nucleic acid molecule comprising the gene coding for a desired protein in an expressible manner, Agrobacterium-mediated delivery of a binary vector comprising the expressible gene, electroporation of protoplasts, and
15 polyethylene glycol-mediated delivery of naked DNA into plant protoplasts.

Particle bombardment usually reaches only a few cells and the DNA must reach the cell nucleus for transcription to be accomplished, and is thus not very efficient for transient expression.

The use of Agrobacterium delivered by infiltration (agro-infiltration) can deliver
20 foreign genes to significantly higher number of cells. The original system of Agrobacterium infiltration for transient expression was described by Kapila et al., Plant Sci. 122: 101-108 (1997) and was developed for rapid testing of the functionality of a protein thought to be useful for disease resistance of the plant tissue. For this application the protein would not need to be purified or
25 characterized since the entire plant tissue could be used in a bioassay. This system was later used to express pharmaceutically important proteins (Vaquero

The present ~~invention~~-disclosure provides the means and methods to meet this need. In particular, the present ~~invention~~-disclosure provides in one embodiment a method for producing a heterologous peptide or protein of interest comprising:

5 (i) contacting a whole plant, particularly a whole and intact plant or a part of a whole and intact plant, or a plurality of whole and intact plants or a plurality of parts of whole and intact plants, with Agrobacterium cells suspended in a fluid, wherein the Agrobacterium cells comprise an expressible, particularly a plant expressible construct encoding the heterologous peptide or protein of interest;

10 (ii) treating said plant or plant part, or the plurality of plants or plant parts and the Agrobacterium cells with one or more pressure cycle(s) whereby the Agrobacterium cells infiltrate the whole plant or the plant part, and

wherein the expressible construct is selected to provide transient expression of the heterologous peptide or protein of interest and at least one of the pressure cycles comprises an increase in pressure relative to atmospheric pressure.

15 In certain embodiments of the ~~invention~~disclosure, the plants are treated with one or more pressure cycles, which all comprise an increase in pressure relative to atmospheric pressure.

In a specific embodiment, the pressure per cycle is maintained for a period of
20 between 0.5 seconds and 60 seconds.

In one aspect of the present disclosure, there is provided a method for producing a heterologous peptide or protein of interest comprising:

25 a) contacting a whole and intact plant, or a part of a whole and intact plant, with Agrobacterium cells suspended in a fluid, wherein the Agrobacterium cells comprise an expressible construct encoding the heterologous peptide or protein of interest;

b) treating the whole and intact plant or a part of the whole and intact plant and the Agrobacterium cells with two or more cycle(s) of positive pressure within a system comprising a chamber configured for receiving the whole
30 plant or the plant part, and a means configured for adjustably increasing air

and/or fluid pressure in the chamber, whereby the Agrobacterium cells infiltrate the whole plant, or the plant part,

wherein a pressure of at least 2.0 bar is maintained for a period of between 0.5 seconds and 60 seconds per cycle.

5 In one embodiment, the present ~~invention~~disclosure provides a method for infiltrating Agrobacteria into a plant or plant part, or a plurality of plants or plant parts, comprising:

- 10 (i) contacting a whole plant, particularly a whole and intact plant or a part of a whole and intact plant, or a plurality of whole and intact plants or a plurality of parts of whole and intact plants, with Agrobacterium cells suspended in a fluid;
- (ii) treating said plant or plant part, or the plurality of plants or plant parts and the Agrobacterium cells with one or more pressure cycle(s),

wherein the expressible construct is selected to provide transient expression of the heterologous peptide or protein of interest and at least one of the pressure cycles comprises an increase in pressure relative to atmospheric pressure.

In certain embodiments of the ~~invention~~disclosure, the plants are treated with one or more pressure cycles, which all comprise an increase in pressure relative to atmospheric pressure.

In a specific embodiment, the pressure per cycle is maintained for a period of between 0.5 seconds and 60 seconds.

In another aspect of the present disclosure, there is provided a method for infiltrating Agrobacterium cells into a whole and intact plant, or a part of a whole intact plant comprising:

a) contacting a whole and intact plant, or a part of a whole and intact plant, with Agrobacterium cells suspended in a fluid;

b) treating the whole plant or a part of the whole and intact plant and the Agrobacterium cells with two or more cycle(s) of positive pressure within a system comprising a chamber configured for receiving the whole plant or the plant part, and a means configured for adjustably increasing air and/or fluid pressure in the chamber,

wherein a pressure of at least 2.0 bar is maintained for a period of between 0.5 seconds and 60 seconds per cycle.

In one embodiment of the ~~invention~~disclosure, the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, and the Agrobacterium cells are treated with one or more pressure cycle(s) within a closed system, particularly a system comprising a chamber configured for receiving a whole plant, particularly a whole and intact plant or a part of the whole and intact plant, and a means for adjustably increasing air and/or fluid pressure in the chamber.

In one embodiment of the ~~invention~~disclosure, the chamber is configured such that the entire plant body including its aerial and underground parts is submerged

in the infiltration medium containing the Argobacterium cells and subjected to the pressure applied during the one or more pressure cycle(s).

5 | In another embodiment of the ~~invention~~disclosure, the chamber is configured such that only all or part of the aerial parts of the plant are submerged in the infiltration medium containing the Argobacterium cells, but the entire plant including the aerial parts as well as the underground parts of the plant is subjected to the pressure applied during the one or more pressure cycle(s).

10 | In still another embodiment of the ~~invention~~disclosure, the chamber is configured such that all or part of the aerial parts of the plant are submerged in the infiltration medium containing the Argobacterium cells and exposed to pressure in the one or more pressure cycles, whereas the underground parts of the plant, particularly the plant root, are positioned outside of the chamber such that they are not

submerged in the infiltration medium containing the Agrobacterium cells and not subjected to the pressure applied during the one or more pressure cycle(s).

5 In one embodiment of the ~~invention~~disclosure, the pressure treatment comprising one or more pressure cycle(s) is applied to the whole and intact plant or a part of the whole and intact plant, while said plant or plant part is in contact with the Agrobacterium cells in the bacterial cell suspension.

10 In one embodiment of the ~~invention~~disclosure, the suspension of Agrobacterium cells comprises an OD600 of at least 1.0, particularly of at least 1.5, particularly of at least 2.0, particularly of at least 2.5, particularly of at least 3.0, particularly of at least 3.5, particularly of at least 4.0, particularly of at least 4.5.

15 In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises subjecting the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, to a pressure which is increased relative to atmospheric pressure, particularly the whole and intact plant or a part of the whole and intact plant is subjected to a pressure of at least 0.5 bar, particularly of at least 1.0 bar, particularly of at least 1.5 bar, particularly of at least 2.0 bar, particularly of at least 2.5 bar, particularly of at least 3.0 bar, particularly of at least 3.5 bar, particularly of at least 4.0 bar, particularly of at least 4.5 bar, 20 particularly of at least 5.0 bar, particularly of at least 5.5 bar, particularly of at least 6.0 bar, particularly of at least 7.0 bar, particularly of at least 8.0 bar, particularly of at least 9.0 bar, particularly of at least 10.0 bar, particularly of at least 11.0 bar, and particularly of at least 12.0 bar.

25 The optimal pressure, which allows a maximal infiltration of the bacterial suspension into the plant or plant part without damaging the plant, may vary depending on the plant species and, within a given plant species, also depending on the variety, used in the infiltration method. The optimal pressure conditions

can be easily determined by the person skilled in the art by using a test system as disclosed herein in the Examples.

In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, comprises at least 2 cycles, particularly at least 3 cycles, particularly at least 4 cycles, particularly at least 5 cycles, particularly at least 6 cycles, particularly at least 7 cycles, particularly at least 8 cycles, particularly at least 9 cycles, particularly at least 10 cycles, particularly at least 11 cycles, wherein at least one of said cycles, or, in a specific embodiment, all of said cycles, comprise a pressure of at least 0.5 bar, particularly of at least 1.0 bar, particularly of at least 1.5 bar, particularly of at least 2.0 bar, particularly of at least 2.5 bar, particularly of at least 3.0 bar, particularly of at least 3.5 bar, particularly of at least 4.0 bar, particularly of at least 4.5 bar, particularly of at least 5.0 bar, particularly of at least 5.5 bar, particularly of at least 6.0 bar, for at least 0.1 seconds/cycle, particularly for at least 0.2 second/cycle, particularly for at least 0.3 second/cycle, particularly for at least 0.4 second/cycle, particularly for at least 0.5 second/cycle, particularly for at least 1 second/cycle, particularly for at least 1.5 seconds/cycle, particularly for at least 2.0 seconds/cycle, particularly for at least 2.5 seconds/cycle, particularly for at least 3.0 seconds/cycle, particularly for at least 3.5 seconds/cycle, particularly for at least 5.0 seconds/cycle.

In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants, or the plant part or the plurality of plant parts comprises at least 2 cycles.

In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts comprises at least 4 cycles.

In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts comprises at least 5 cycles.

5 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts comprises at least 6 cycles.

In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts comprises at least 7 cycles.

10 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts comprises at least 8 cycles.

15 In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 2.5 bar.

In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 3.5 bar.

20 In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 4.5 bar.

In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 6 bar.

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In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 8 bar.

5 In one embodiment of the ~~invention~~disclosure, at least one of the pressure cycles comprises treating the whole plant or the plurality of whole plants or the plant part or the plurality of plant parts, with a pressure of at least 12 bar.

In one embodiment of the ~~invention~~disclosure, the pressure is applied for between 0.5 seconds/cycle and 10 seconds/cycle.

10 In one embodiment of the ~~invention~~disclosure, the pressure is applied for between 1 second/cycle and 5 seconds/cycle.

In one embodiment of the ~~invention~~disclosure, the pressure is applied for between 0.5 seconds/cycle and 1 second/cycle.

15 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, with a pressure that is increased relative to atmospheric pressure comprises at least 5 cycles, at a pressure of at least 3.0 bar, for at least 0.5 seconds/cycle.

20 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, with a pressure that is increased relative to atmospheric pressure comprises at least 8 cycles, at a pressure of at least 4.5 bar, for at least 1 second/cycle.

25 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, with

a pressure that is increased relative to atmospheric pressure comprises at least 1 cycle, at a pressure of at least 8.0 bar, for at least 0.5 seconds/cycle.

5 In one embodiment of the ~~invention~~disclosure, the step of treating the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, with a pressure that is increased relative to atmospheric pressure comprises at least 2 cycles, at a pressure of at least 6.0 bar, for at least 0.5 seconds/cycle.

10 In one embodiment of the ~~invention~~disclosure, the step of contacting the whole plant, particularly the whole and intact plant or a part of the whole and intact plant, or the plurality of whole and intact plants or of parts of whole and intact plants, with Agrobacterium cells comprises (i) dipping the plant or a part thereof in a suspension of Agrobacterium cells, wherein the suspension comprises an OD600 of at least 1.0, particularly of at least 1.5, particularly of at least 2.0,
15 particularly of at least 2.5, particularly of at least 3.0, particularly of at least 3.5, particularly of at least 4.0, particularly of at least 4.5, (ii) exposing the plant or a part thereof to an aerosol generated by using a suspension of Agrobacterium cells comprising an OD600 of at least 1.0, particularly of at least 1.5, particularly of at least 2.0, particularly of at least 2.5, particularly of at least 3.0, particularly of
20 at least 3.5, particularly of at least 4.0, particularly of at least 4.5.

In one embodiment of the ~~invention~~disclosure, the plant is a *Nicotiana* species, particularly a *Nicotiana tabacum* species, at a development stage of 8, 9, or 10.

25 In one embodiment, the present ~~invention~~disclosure provides a system for infiltrating Agrobacteria into a whole plant, particularly a whole and intact plant or a part of a whole and intact plant, or a plurality of whole and intact plants or of parts of whole and intact plants, and/or for producing a heterologous peptide or protein comprising a chamber configured for receiving a whole plant, particularly a whole and intact plant or a part of a plant, and a means configured for adjustably increasing air and/or fluid pressure in the chamber.

In one embodiment of the ~~invention~~disclosure, the system comprises a compressor and a pressure reducer.

5 In one embodiment of the ~~invention~~disclosure, the system comprises a plurality of inlets, outlets and conduits for providing a fluid path between the interior and exterior of the chamber, wherein the flow of fluid is regulated.

In one embodiment of the ~~invention~~disclosure, the chamber comprises, in the interior, a plurality of nozzles that are dimensioned for atomizing or aerosolizing a liquid.

10 In one embodiment of the ~~invention~~disclosure, the chamber is configured such that the entire plant body including its aerial and underground parts is submerged in the infiltration medium containing the Argobacterium cells and subjected to the pressure applied during the one or more pressure cycle(s).

15 In one embodiment of the ~~invention~~disclosure, the chamber is configured such that only all or part of the aerial parts of the plant are submerged in the infiltration medium containing the Argobacterium cells, but the entire plant including the aerial parts as well as the underground parts of the plant is subjected to the pressure applied during the one or more pressure cycle(s).

20 In one embodiment of the ~~invention~~disclosure, the chamber is configured such that all or part of the aerial parts of the plant are submerged in the infiltration medium containing the Argobacterium cells and exposed to pressure in the one or more pressure cycles, whereas the underground parts of the plant, particularly the plant root, are positioned outside of the chamber such that they are not submerged in the infiltration medium containing the Agrobacterium cells and not subjected to the pressure applied during the one or more pressure cycle(s).

25 In one embodiment of the ~~invention~~disclosure, the chamber comprises one or more openings to allow passage of aerial parts of the plants during insertion or removal, which openings are lined with an elastic seal, particularly an elastic pneumatic or hydraulic seal.

In one embodiment, the ~~invention disclosure~~ relates to the use of a system according to the ~~invention disclosure~~ and as described herein for infiltrating Agrobacterium bacteria into a whole plant, particularly a whole and intact plant or a plant part and/or for producing a heterologous peptide or protein.

5 Definitions

The technical terms and expressions used within the scope of this application are generally to be given the meaning commonly applied to them in the pertinent art of plant biology.

10 All of the following term definitions apply to the complete content of this application. The word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. A single step may fulfil the functions of several features recited in the claims. The terms "essentially", "about", "approximately" and the like in connection with an attribute or a value particularly also define exactly the attribute or exactly the value,
15 respectively. The term "about" in the context of a given numerate value or range refers to a value or range that is within 20 %, within 10 %, within 5 %, or within 2 % of the given value or range.

A "plant" as used within the present invention refers to an intact plant, a substantially intact plant, or to a plurality of intact or substantially intact plants; a
20 whole plant, a substantially whole plant, or a plurality of whole or substantially whole plants, and its progenies, at any stage of its development. For the purpose of the present application, an intact plant is understood to refer to a plant comprising an essentially intact and closed vascular system, which does not show drainage of the vascular fluids due to injury or lesions.

25 A "plant part" as used within the present invention refers to a any part of a plant including cuttings, a plant organ, a plant tissue, or a plant cell, which plant part may be an isolated part of the plant or a part of the whole and intact plant.

CLAIMS

1. A method for producing a heterologous peptide or protein of interest comprising:

5 a) contacting a whole and intact plant, or a part of a whole and intact plant, with *Agrobacterium* cells suspended in a fluid, wherein the *Agrobacterium* cells comprise an expressible construct encoding the heterologous peptide or protein of interest;

10 b) treating the whole and intact plant or a part of the whole and intact plant and the *Agrobacterium* cells with one or more pressure cycle(s) whereby the *Agrobacterium* cells infiltrate the whole plant, or the plant part,

wherein at least one of the pressure cycle(s) comprises an increase in pressure relative to atmospheric pressure and is maintained for a period of between 0.5 seconds and 60 seconds.

15 2. A method for infiltrating *Agrobacterium* cells into a whole and intact plant, or a part of a whole intact plant comprising:

a) contacting a whole and intact plant, or a part of a whole and intact plant, with *Agrobacterium* cells suspended in a fluid;

20 b) treating the whole plant or a part of the whole and intact plant and the *Agrobacterium* cells with one or more pressure cycle(s),

wherein at least one of the pressure cycle(s) comprises an increase in pressure relative to atmospheric pressure and is maintained for a period of between 0.5 seconds and 60 seconds.

25 3. The method of any one of the preceding claims, wherein the expressible construct is a plant expressible construct and is selected to provide transient expression of the heterologous peptide or protein of interest.

4. The method of any one of the preceding claims, wherein the pressure is maintained for a period of between 0.5 seconds and 60 seconds.
5. The method of any one of the preceding claims, wherein the pressure is applied to the whole and intact plant or a part of the whole and intact plant, while said plant or plant part is in contact with the *Agrobacterium* cells in the bacterial cell suspension.
6. The method of any one of the preceding claims, wherein the suspension of *Agrobacterium* cells comprises an OD600 of at least 1.0.
7. The method of any one of the preceding claims, wherein the whole plant or the plant part and the *Agrobacterium* cells are treated with one or more pressure cycle(s) within a closed system, particularly a system comprising a chamber configured for receiving a whole plant, or a plant part, and a means for adjustably increasing air and/or fluid pressure in the chamber.
8. The method of claim 7, wherein the chamber is configured such that the entire plant body including its aerial and underground parts is submerged in the infiltration medium containing the *Agrobacterium* cells and subjected to the pressure applied during the one or more pressure cycle(s).
9. The method of claim 7, wherein the chamber is configured such that only all or part of the aerial parts of the plant is submerged in the infiltration medium containing the *Agrobacterium* cells, but the entire plant including the aerial parts as well as the underground parts of the plant is subjected to the pressure applied during the one or more pressure cycle(s).
10. The method of claim 7, wherein the chamber is configured such that all or part of the aerial parts of the plant are submerged in the infiltration medium containing the *Agrobacterium* cells and exposed to pressure in the one or more pressure cycles, whereas the underground parts of the plant, particularly the plant root, are positioned outside of the chamber such that they are not submerged in the

~~infiltration medium containing the Agrobacterium cells and not subjected to the pressure applied during the one or more pressure cycle(s).~~

11. The method of any one of the preceding claims, wherein the step of treating the whole plant or the plant part comprises

- 5 a) at least 2 cycles; or
 b) at least 3 cycles; or
 c) at least 4 cycles; or
 d) at least 8 cycles.

12. The method of any one of the preceding claims, wherein at least one of the pressure cycles comprises treating the whole plant or the plant part with a pressure of

- 10 a) at least 3.5 bar; or
 b) at least 4.5 bar; or
 c) at least 6.5 bar; or
15 d) at least 8 bar; or
 e) at least 12 bar.

13. The method of any one of the preceding claims, wherein the pressure is applied for between

- 20 a) 0.5 seconds/cycle and 10 seconds/cycle; or
 b) 1 second/cycle and 5 seconds/cycle; or
 c) 0.5 seconds/cycle and 1 second/cycle.

14. The method of any one of the preceding claims, wherein the step of treating the whole plant or the plant part with a pressure that is increased relative to atmospheric pressure comprises

- 25 a) at least 1 cycle, at a pressure of at least 8 bar, for at least 0.5 seconds/cycle; or
 b) at least 2 cycles, at a pressure of at least 6 bar, for at least 0.5 seconds/cycle; or

~~c) at least 8 cycles, at a pressure of at least 4.5 bar, for at least 1 second/cycle.~~

15. The method any one of the preceding claims, wherein the step of treating the whole plant or the plant part with a pressure that is increased relative to atmospheric pressure comprises at least 8 cycles, at a pressure of at least 4.5 bar, for at least 1 second/cycle.

16. The method of any one of the preceding claims, wherein the step of contacting the whole plant, or the plant part with *Agrobacterium* cells comprises

- a) dipping the plant or a part thereof in a suspension of *Agrobacterium* cells, wherein the suspension comprises an OD600 of at least 2.5,
- b) exposing the plant or a part thereof to an aerosol generated by using a suspension of *Agrobacterium* cells comprising an OD600 of at least 2.5.

17. The method of any one of the preceding claims, wherein the steps comprise

- a) submerging the plant or a part thereof in a suspension of *Agrobacterium* cells in an enclosed chamber, and
- b) applying liquid fluid pressure to the suspension of *Agrobacterium* cells comprising the submerged plant or part thereof.

18. The method of any one of the preceding claims, wherein the plant is a *Nicotiana* species, particularly a *Nicotiana tabacum* species, at a development stage of 8, 9, or 10, wherein the plants have an average height of between about 6.5 cm and about 16.5 cm.

19. The method of any one of the preceding claims, wherein all the pressure cycles applied comprise an increase in pressure relative to atmospheric pressure.

20. The method of any of the preceding claims, wherein the pressure treatment comprising one or more pressure cycle(s) is applied while the whole and intact

~~plant or a part of the whole and intact plant are submerged in the bacterial cell suspension.~~

21. The method of any one of the preceding claims, wherein a plurality of plants or plant parts is contacted with *Agrobacterium* cells suspended in a fluid and treated with one or more pressure cycles.

22. A system for infiltrating *Agrobacterium* bacteria into a whole and intact plant or a part of a whole and intact plant and/or for producing a heterologous peptide or protein in a whole and intact plant or a part of a whole and intact plant comprising a chamber configured for receiving the whole plant or the plant part, and a means configured for adjustably increasing air and/or fluid pressure in the chamber.

23. The system of claim 22, comprising a compressor and a pressure reducer.

24. The system of claim 22, comprising a plurality of inlets, outlets and conduits for providing a fluid path between the interior and exterior of the chamber, wherein the flow of fluid is regulated.

25. The system of any one of claims 22 or 24, wherein the chamber comprises, in the interior, a plurality of nozzles that are dimensioned for atomizing or aerosolizing a liquid.

26. The system of any one of claims 22 to 25, wherein the chamber is configured such that the entire plant body including its aerial and underground parts is submerged in the infiltration medium containing the *Agrobacterium* cells and subjected to the pressure applied during the one or more pressure cycle(s).

27. The system of any one of claims 22 to 25, wherein the chamber is configured such that only all or part of the aerial parts of the plant are submerged in the infiltration medium containing the *Agrobacterium* cells, but the entire plant

including the aerial parts as well as the underground parts of the plant is subjected to the pressure applied during the one or more pressure cycle(s).

28. The system of any one of claims 22 to 25, wherein the chamber is configured such that all or part of the aerial parts of the plant are submerged in the infiltration medium containing the *Agrobacterium* cells and exposed to pressure in the one or more pressure cycles, whereas the underground parts of the plant, particularly the plant root, are positioned outside of the chamber such that they are not submerged in the infiltration medium containing the *Agrobacterium* cells and not subjected to the pressure applied during the one or more pressure cycle(s).

29. The system of claim 28, wherein the chamber comprises one or more openings to allow passage of the aerial parts of the plant during insertion or removal, which openings are lined with elastic seals.

30. The system of claim 29, wherein said elastic seal is an elastic pneumatic or hydraulic seal.

31. Use of a system according any of claims 22 to 30 for infiltrating *Agrobacterium* bacteria into a whole plant or a plant part and/or for producing a heterologous peptide or protein in a whole plant or a plant part.

CLAIMS

1. A method for producing a heterologous peptide or protein of interest
5 comprising:

a) contacting a whole and intact plant, or a part of a whole and intact plant,
with *Agrobacterium* cells suspended in a fluid, wherein the *Agrobacterium*
cells comprise an expressible construct encoding the heterologous peptide
or protein of interest;

10 b) treating the whole and intact plant or a part of the whole and intact plant
and the *Agrobacterium* cells with two or more cycle(s) of positive pressure
within a system comprising a chamber configured for receiving the whole
plant or the plant part, and a means configured for adjustably increasing air
and/or fluid pressure in the chamber, whereby the *Agrobacterium* cells
15 infiltrate the whole plant, or the plant part,

wherein a pressure of at least 2.0 bar is maintained for a period of between
0.5 seconds and 60 seconds per cycle.

2. A method for infiltrating *Agrobacterium* cells into a whole and intact plant, or a
part of a whole intact plant comprising:

20 a) contacting a whole and intact plant, or a part of a whole and intact plant,
with *Agrobacterium* cells suspended in a fluid;

b) treating the whole plant or a part of the whole and intact plant and the
Agrobacterium cells with two or more cycle(s) of positive pressure within a
system comprising a chamber configured for receiving the whole plant or the
25 plant part, and a means configured for adjustably increasing air and/or fluid
pressure in the chamber,

wherein a pressure of at least 2.0 bar is maintained for a period of between
0.5 seconds and 60 seconds per cycle.

3. The method of claim 1, wherein the expressible construct is a plant expressible
30 construct and is selected to provide transient expression of the heterologous
peptide or protein of interest.

4. The method of any one of claims 1 to 3, wherein the infiltration of the whole plant or a part of the whole and intact plant and the Agrobacterium cells is carried out in the presence of a surfactant.

5. The method of any one of the preceding claims, wherein the pressure is applied to the whole and intact plant or a part of the whole and intact plant, while said plant or plant part is in contact with the Agrobacterium cells in the bacterial cell suspension.

6. The method of any one of the preceding claims, wherein the suspension of Agrobacterium cells comprises an OD600 of at least 1.0.

7. The method of any one of the preceding claims, wherein the chamber is configured such that the entire plant body including its aerial and underground parts is submerged in the infiltration medium containing the Agrobacterium cells and subjected to the pressure applied during the one or more pressure cycle(s).

8. The method of any one of the preceding claims, wherein the chamber is configured such that only all or part of the aerial parts of the plant is submerged in the infiltration medium containing the Agrobacterium cells, but the entire plant including the aerial parts as well as the underground parts of the plant is subjected to the pressure applied during the one or more pressure cycle(s).

9. The method of any one of the preceding claims, wherein the chamber is configured such that all or part of the aerial parts of the plant are submerged in the infiltration medium containing the Agrobacterium cells, while the underground parts of the plant are not submerged in the infiltration medium.

10. The method of any one of the preceding claims, wherein the step of treating the whole plant or the plant part comprises

- a) at least 2 cycles; or
- b) at least 3 cycles; or
- c) at least 4 cycles; or
- d) at least 8 cycles.

11. The method of any one of the preceding claims, wherein at least one of the pressure cycles comprises treating the whole plant or the plant part with a pressure of

- a) at least 3.5 bar; or
- b) at least 4.5 bar; or
- c) at least 6.5 bar; or
- d) at least 8 bar; or
- e) at least 12 bar.

12. The method of any one of the preceding claims, wherein the pressure is applied for between

- a) 0.5 seconds/cycle and 10 seconds/cycle; or
- b) 1 second/cycle and 5 seconds/cycle; or
- c) 0.5 seconds/cycle and 1 second/cycle.

13. The method of any one of the preceding claims, wherein the step of treating the whole plant or the plant part with a pressure that is increased relative to atmospheric pressure comprises

- a) at least 1 cycle, at a pressure of at least 8 bar, for at least 0.5 seconds/cycle; or
- b) at least 2 cycles, at a pressure of at least 6 bar, for at least 0.5 seconds/cycle; or
- c) at least 8 cycles, at a pressure of at least 4.5 bar, for at least 1 second/cycle.

14. The method any one of the preceding claims, wherein the step of treating the whole plant or the plant part with a pressure that is increased relative to atmospheric pressure comprises at least 8 cycles, at a pressure of at least 4.5 bar, for at least 1 second/cycle.

15. The method of any one of the preceding claims, wherein the step of contacting the whole plant, or the plant part with *Agrobacterium* cells comprises

- a) dipping the plant or a part thereof in a suspension of *Agrobacterium* cells, wherein the suspension comprises an OD600 of at least 2.5,

b) exposing the plant or a part thereof to an aerosol generated by using a suspension of *Agrobacterium* cells comprising an OD600 of at least 2.5.

16. The method of any one of the preceding claims, wherein the steps comprise

a) submerging the plant or a part thereof in a suspension of *Agrobacterium* cells in an enclosed chamber, and

b) applying liquid fluid pressure to the suspension of *Agrobacterium* cells comprising the submerged plant or part thereof.

17. The method of any one of the preceding claims, wherein the plant is a *Nicotiana* species, particularly a *Nicotiana tabacum* species, at a development stage of 8, 9, or 10, wherein the plants have an average height of between about 6.5 cm and about 16.5 cm.

18. The method of any one of the preceding claims, wherein all the pressure cycles applied comprise an increase in pressure relative to atmospheric pressure.

19. The method of any of the preceding claims, wherein the pressure treatment comprising one or more pressure cycle(s) is applied while the whole and intact plant or a part of the whole and intact plant are submerged in the bacterial cell suspension.

20. The method of any one of the preceding claims, wherein a plurality of plants or plant parts is contacted with *Agrobacterium* cells suspended in a fluid and treated with one or more pressure cycles.