

The present disclosure provides the means and methods to meet this need. In particular, the present 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 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.

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

25 b) treating the whole and intact plant or a part of the whole and intact plant and the *Agrobacterium* cells with two or more cycles 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

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.

5 In certain embodiments of the 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.

10 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;

15 b) treating the whole plant or a part of the whole and intact plant and the Agrobacterium cells with two or more cycles 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,

20 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 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,
25 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 disclosure, the chamber is configured such that the entire plant body including its aerial and underground parts is submerged

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 cycles 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 cycles 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 two or more pressure cycles.
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 two or more pressure cycles.
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 2 cycles, at a pressure of at least 6 bar, for at least 0.5 seconds/cycle; or
- b) 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 6.5 cm and 16.5 cm.

18. The method of any of the preceding claims, wherein the pressure treatment comprising two or more pressure cycles is applied while the whole and intact plant or a part of the whole and intact plant are submerged in the bacterial cell suspension.

19. 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 two or more pressure cycles.