yields by at least a factor of 10, which minimizes the costs for extraction and purification of the protein of interest.

A technique of this kind for transient expression in plants is now used on a large scale for producing vaccines by certain companies, which are currently developing extensive production units in the United States.

However, despite these efforts, there is still a need for an efficient and reproducible method for producing recombinant allergens that allows recombinant allergens to be obtained having a composition and conformation similar to those of their natural homologs. Moreover, there is a need for a method that has a good production yield.

The present invention makes it possible to obtain complex recombinant proteins, in particular complex recombinant allergens, which have never been obtained previously in recombinant form. These allergens are, moreover, copies identical to their natural homologs.

The present invention therefore relates to a method for producing a recombinant protein in a plant, preferably a tobacco plant, preferably *Nicotiana benthamiana*, comprising the following steps:

a) culturing the plants aeroponically or hydroponically, preferably on mobile floats, and under LED lighting,

b) agroinfiltration of the plants obtained in step a), under vacuum, by agrobacteria comprising a DNA fragment coding for the recombinant protein, then

c) returning the plants to culturing after step b), in the same conditions as for step a), then

d) extraction and purification of the recombinant protein from the aerial parts of the plants produced in step c).

The present invention also relates to a recombinant protein obtainable by the method according to the invention.

The present invention also relates to an expression vector comprising the SEQ ID NO: 21 and an insert, said insert comprising at least one nucleic acid sequence coding for a peptide selected from SEQ ID NO: 1 to 20, said nucleic acid sequence being directly fused with a second nucleic acid sequence encoding the protein of interest.

The present invention also relates to a method for producing a recombinant allergen in a plant, notably a tobacco plant, preferably *Nicotiana benthamiana*, comprising the following steps:

a) culture of the plant aeroponically or hydroponically, preferably on mobile floats, and under LED lighting, then

b) agroinfiltration of the plant obtained in a), under vacuum, by agrobacteria comprising a DNA fragment coding for the recombinant allergen, then

c) returning the plants to culturing after step b), in the same conditions as for step a), then

d) extraction and purification of the recombinant allergen from the aerial parts of the plants produced in step c).

The plant usable in the method according to the invention is notably a tobacco plant selected from *Nicotiana benthamiana* and *Nicotiana tabacum* or any other plant usable for transient expression, such as a lettuce (genus Lactuca) or a spinach plant (Spinacia oleracea). Among the lettuces, we may mention the lettuce Appia, Grosse Blonde Paresseuse, Lollo Rosso, Merveille

CLAIMS

1. A method for producing a recombinant protein in a plant, notably a tobacco plant, preferably *Nicotiana benthamiana*, comprising the following steps:
a) culture of the plant aeroponically or hydroponically, preferably on mobile floats, and under LED lighting, then
b) agroinfiltration of the plant obtained in a), under vacuum, by agrobacteria comprising a DNA fragment coding for the recombinant protein, then
c) returning the plants to culturing after step b), in the same conditions as for step a), then
d) extraction and purification of the recombinant protein from the aerial parts of the plants produced in step c).

2. The method as claimed in claim 1, characterized in that the recombinant protein is a recombinant allergen, preferably a recombinant mite allergen.

3. The method as claimed in claim 1 or 2, characterized in that the recombinant protein is selected from the allergens of *Dermatophagoides farinae*, the allergens of *Dermatophagoides pteronyssinus*, the allergens of *Euroglyphus manei*, the allergens of *Acarus siro*, the allergens of *Blomia tropicalis*, cockroach allergens, tree or grass pollen allergens, allergens of animals, allergens of molds, allergens of hevea latex and allergens responsible for food allergies.

4. The method as claimed in one of claims 1 to 3, characterized in that the recombinant protein is selected from Der f 10, Der f 11, Der f 13, Der f 14, Der f 15, Der f 16, Der f 17, Der f 18, Der f 2, Der f 2.0101, Der f 2.0102, Der f 2.0103, Der f 2.0104, Der f 2.0105, Der f 2.0106, Der f 2.0107, Der f 2.0108, Der f 2.0109, Der f 2.0110, Der f 2.0111, Der f 2.0112, Der f 2.0113, Der f 2.0114, Der f 2.0115, Der f 2.0116, Der f 2.0117, Der f 20, Der f 3, Der f 4, Der f 5, Der f 6, Der f 7, Der f 8, Der f 9 and Der f HSP70.

5. The method as claimed in one of claims 1 to 3, characterized in that the recombinant protein is selected from Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 2, Der p 2.0101, Der p 2.0102, Der p 2.0103, Der p 2.0104, Der p 2.0105, Der p 2.0106, Der p 2.0107,

Der p 2.0108, Der p 2.0109, Der p 2.0110, Der p 2.0111, Der p 2.0112, Der p 2.0113, Der p 20, Der p 21, Der p 3, Der p 4, Der p 5, Der p 6, Der p 7, Der p 8 and Der p 9.

6. The method as claimed in one of claims 1 to 3, characterized in that the recombinant protein is selected from Blo t 1, Blo t 5, Blo t 9, Blo t 10, Blo t 12 and Blo t 21.

7. The method as claimed in one of claims 1 to 6, characterized in that the agroinfiltration is carried out under vacuum by the Venturi effect.

8. The method as claimed in claim 7, characterized in that the agroinfiltration is carried out either by a step of putting the plant under vacuum for 2 minutes, or by vacuuming, preferably at -0.8 bar for 2 minutes, then breaking the vacuum and returning to atmospheric pressure, preferably for 30 seconds, then putting under vacuum, preferably at -0.8 bar for 2 minutes, followed finally by return to atmospheric pressure.

9. A recombinant protein obtainable by the method as claimed in one of claims 1 to 8.

10. An expression vector comprising the sequence SEQ ID NO: 21 and an insert, said insert comprising at least one nucleic acid sequence coding for a peptide selected from SEQ ID NO: 1 to 20, said nucleic acid sequence being directly fused with a second nucleic acid sequence encoding the protein of interest.

11. The expression vector as claimed in claim 10, characterized in that the protein of interest is an allergen.

12. The expression vector as claimed in claim 10 or 11, characterized in that the protein of interest is selected from the allergens of *Dermatophagoides farinae*, the allergens of *Dermatophagoides pteronyssinus*, the allergens of *Blomia tropicalis*, the allergens of *Euroglyphus manei*, the allergens of *Acarus siro*, cockroach allergens, tree or grass pollen allergens, allergens of animals, allergens of molds, allergens of hevea latex and allergens responsible for food allergies.

CLAIMS

1. A method for producing a recombinant allergen in a plant, notably a tobacco plant, preferably *Nicotiana benthamiana*, comprising the following steps:

a) culture of the plant aeroponically or hydroponically, preferably on mobile floats, and under LED lighting, then

b) agroinfiltration of the plant obtained in a), under vacuum, by agrobacteria comprising a DNA fragment coding for the recombinant allergen, then

c) returning the plants to culturing after step b), in the same conditions as for step a), then

d) extraction and purification of the recombinant allergen from the aerial parts of the plants produced in step c).

2. The method as claimed in claim 1, characterized in that the recombinant allergen is a recombinant mite allergen.

3. The method as claimed in claim 1 or 2, characterized in that the recombinant allergen is selected from the allergens of *Dermatophagoides farinae*, the allergens of *Dermatophagoides pteronyssinus*, the allergens of *Euroglyphus manei*, the allergens of *Acarus siro*, the allergens of *Blomia tropicalis*, cockroach allergens, tree or grass pollen allergens, allergens of animals, allergens of molds, allergens of hevea latex and allergens responsible for food allergies.

4. The method as claimed in any one of claims 1 to 3, characterized in that the recombinant allergen is selected from Der f 10, Der f 11, Der f 13, Der f 14, Der f 15, Der f 16, Der f 17, Der f 18, Der f 2, Der f 2.0101, Der f 2.0102, Der f 2.0103, Der f 2.0104, Der f 2.0105, Der f 2.0106, Der f 2.0107, Der f 2.0108, Der f 2.0109, Der f 2.0110, Der f 2.0111, Der f 2.0112, Der f 2.0113, Der f 2.0114, Der f 2.0115, Der f 2.0116, Der f 2.0117, Der f 20, Der f 3, Der f 4, Der f 5, Der f 6, Der f 7, Der f 8, Der f 9 and Der f HSP70.

5. The method as claimed in any one of claims 1 to 3, characterized in that the recombinant allergen is selected from Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 2, Der p 2.0101, Der p 2.0102, Der p 2.0103, Der p 2.0104, Der p 2.0105, Der p 2.0106, Der p 2.0107,

Der p 2.0108, Der p 2.0109, Der p 2.0110, Der p 2.0111, Der p 2.0112, Der p 2.0113, Der p 20, Der p 21, Der p 3, Der p 4, Der p 5, Der p 6, Der p 7, Der p 8 and Der p 9.

6. The method as claimed in any one of claims 1 to 3, characterized in that the recombinant allergen is selected from Blo t 1, Blo t 5, Blo t 9, Blo t 10, Blo t 12 and Blo t 21.

7. The method as claimed in any one of claims 1 to 6, characterized in that the agroinfiltration is carried out under vacuum by the Venturi effect.

8. The method as claimed in claim 7, characterized in that the agroinfiltration is carried out either by a step of putting the plant under vacuum for 2 minutes, or by vacuuming, preferably at -0.8 bar for 2 minutes, then breaking the vacuum and returning to atmospheric pressure, preferably for 30 seconds, then putting under vacuum, preferably at -0.8 bar for 2 minutes, followed finally by return to atmospheric pressure.

9. The method as claimed in any one of claims 1 to 8, characterized in that the DNA fragment coding for the recombinant allergen is incorporated in an expression vector comprising the sequence SEQ ID NO: 21 and an insert, said insert comprising at least one nucleic acid sequence coding for a peptide selected from SEQ ID NO: 1 to 20, said nucleic acid sequence being directly fused with the DNA fragment coding for the recombinant allergen.

10. The method as claimed in claim 9, characterized in that the allergen is selected from the allergens of *Dermatophagoides farinae*, the allergens of *Dermatophagoides pteronyssinus*, the allergens of *Blomia tropicalis*, the allergens of *Euroglyphus manei*, the allergens of *Acarus siro*, cockroach allergens, tree or grass pollen allergens, allergens of animals, allergens of molds, allergens of hevea latex and allergens responsible for food allergies.