

Agro-Infiltration-Novel Technique for Plant Based Protein Production

Pallerla Saisupriya*¹ and Pidigam Saidaiah²

¹Department of Vegetable Science, College of Horticulture, Sri KondaLaxmanTelangana State Horticultural University, Rajendranagar (500030), Hyderabad, Telangana, India

²Department of Genetics and Plant breeding, College of Horticulture, Sri KondaLaxmanTelangana State Horticultural University, Mojerla (509382), Telangana, India

ARTICLE ID: 003

Abstract

Agro infiltration is a novel method which is used in plant biotechnology to produce a desired protein through induction of transient expression of genes in a plant. It is simple, rapid, reproducible and useful technique. Agro infiltration accommodates transforming plants with large genes encoding complex proteins, such as antibodies. The main advantage is that different genes, either alone or in combination, can be expressed together in a single leaf. It has been widely used in analysis of foreign gene expression, hypersensitive reaction, gene silencing, promoter activity and identification of new disease-resistance genes.

Keywords: Agro infiltration, biotechnology, expression, genes, proteins

Introduction

Plants are one of the most promising pharmaceutical production platforms that are robust, scalable, low-cost and safe. They offer several advantages over current bioreactor-based platforms for protein production. Transgenic plants have emerged as a promising technology to generate recombinant biopharmaceutical proteins and vaccines.

Genetic transformation is a powerful method used in a variety of molecular studies, such as gene function analysis, protein production, protein-protein interaction and promoter activity. There are two kinds of genetic transformation namely stable transformation and transient transformation. Stable transformation is a labour-intensive low-throughput process. Comparatively, transient transformation is an easy and efficient method. The recent development of deconstructed virus-based vectors has allowed rapid and high-level transient expression of recombinant proteins, and in turn, provided a preferred plant based production platform. Agro infiltration will greatly facilitate the realization of plant transient expression

systems as a premier platform for commercial production of pharmaceutical proteins. Agro infiltration is introduction of a suspension of Agro bacterium containing target gene into a plant in order to add a specific gene.

Procedure of Agro infiltration

Introduction of gene of interest to a strain of *Agrobacterium tumefaciens*. The strain is grown in a liquid culture and the resulting bacteria are washed and suspended into a suitable buffer solution which is infiltrated into plant tissues. Inside the leaf the Agro bacterium remains in the intercellular space and it transfers the gene of interest as part of the Ti plasmid-derived T-DNA into the plant cells. The bacteria creates a mechanism that burrows a hole and transfers the new T-DNA strand into the plant cell, which moves into the nucleus of the plant and integrates into the plant's chromosome. Then gene is transiently expressed through synthesis of RNA in all transformed cells. The plant can be monitored for a possible effect in the phenotype, subjected to experimental conditions and used for purification of the protein of interest. The majority of the plant cells in the infiltrated region express the transgene and the expression typically reaches its highest level in 2–3 days after infiltration. In most of the cases, the transient expression of transgene has been reported to be evaluated 4-5 days after infiltration (Wroblewski *et al.*, 2005).

Types of Agro Infiltration:

There are two types of agro infiltration

1. Syringe Agro infiltration
2. Vacuum Agro infiltration

Syringe Agro Infiltration:

It is the most popular method of agro infiltration involves the use of a needleless syringe to introduce Agro bacterium into plant leaves. In this method, the tip of the syringe is pressed against the underside of a leaf while simultaneously applying gentle counter pressure to the other side of the leaf. The Agro bacterium suspension is then injected into the airspaces inside the leaf through stomata, or by making a tiny incision to the underside of the leaf.

Advantages of Syringe Agro Infiltration

As syringe infiltration is easier to perform, it is more frequently utilized for protein production at a small scale. It is simple and requires only small volumes of Agro bacterium culture. It has the flexibility to either infiltrate the whole leaf with one target gene or use spot

infiltration to introduce genes of multiple targets on one leaf. The main advantage is that different genes, either alone or in combination, can be expressed together in a single leaf. Syringe agro infiltration has been applied successfully in a variety of plant species, including tomato, lettuce and pea.

Vacuum Agro Infiltration

In a beaker containing the agro bacterium buffer solution, leaves or whole plants are submerged and then beaker is placed in a vacuum chamber. The vacuum is then applied by forcing air out of the intercellular spaces within the leaves through stomata. As the vacuum is released, the pressure difference forces the Agro bacterium suspension into the leaves through the stomata into the mesophyll tissue.

Advantages of Vacuum Agro Infiltration:

The main advantage of vacuum infiltration is that whole leaves and even entire plants can be infiltrated at once, hence there is high scalability potential. It has ability to extend gene and protein function analysis to plant species. It is more robust and can infiltrate large no. of plants in short period of time.

Applications:

- It is used to study plant pathogen interactions.
- Used for production of a biotic stress tolerant plants.
- For the plant gene functional analysis with transient silencing assay.
- This method is used for large scale production of recombinant proteins by offering advantages in terms of yield, speed and cost.
- Vaccines can be produced against hepatitis B, HIV and influenza virus
- By using this method antibodies can be produced in the plant.
- Rapid production of therapeutic agents in plant systems (Chen *et al.*, 2013)

Advantages of Agro Infiltration

- Generation of numerous transgenic plants in a huge number of crop plants.
- Highly scalable, robust, safe, and can produce complex proteins.
- High speed and more consistent technique.
- High levels of the transgene expression in comparison to that of stable transgenic plants.
- Yields of the recombinant protein are generally higher.

- More interestingly, the transient expression) is often 1000 fold higher than the stable expression of the integrated transgene.
- Less requirement of time to analyse the effect of the transgene.

Disadvantages of Agro Infiltration

- The scalability of transient expression systems remained a challenge.
- Since the production scale could potentially be limited, further optimization of expression vectors was also desirable.
- There is a need to search for new expression vectors to further enhance the accumulation of recombinant protein.

Agro Infiltration for Expression of Pharmaceutical Proteins and vaccine production

Transgenic plants have emerged as a promising technology to generate recombinant biopharmaceutical proteins and vaccines (Goldstein and Thomas, 2004). In plants, there is an immense potential for mass production of recombinant proteins. Plants provide a low-cost system for protein production because they are easily transformed and moreover the transformed protein can maintain an appropriate structure. Also, the plant with a transient protein is ready for the evaluation within a few days. Overall, agro infiltration has become a method of popular choice for assigning a gene function. This method is well established in several fruit-bearing plants like tomato, melon and cucumber. Today, transient transformation using *Agrobacterium tumefaciens* by far the preferred method of protein production as it provides safe, high-level and very rapid transgene expression in comparison to transgenic plant. Agro infiltration is an Effective and scalable Strategy of gene delivery for Production of Pharmaceutical proteins. The short timeline and higher levels of recombinant protein accumulation make transient expression an attractive platform for pharmaceutical production in plants. In addition, the concept of encapsulating proteins/antigens in the leaves instead of purifying them for immunization may benefit vaccine production in the developing countries where cold chain facilities are lacking and emerge as a commercially viable approach for urgent vaccine development.

Conclusion

Agro - infiltration is an effective method for gene transfer into the plant. Useful tool to boost research in molecular farming. It is a very good tool to study protein over expression in the plant. The combination of syringe and vacuum infiltration provides researchers,



biotechnologists and educators agro infiltration is a simple, efficient, robust and scalable methodology for transient expression of recombinant proteins in plants. It is user-friendly method. It is useful method to study the gene function of a gene at the specific organ of the plant. It is employed extensively on commercial scale for recombinant protein production.

References

- Chen, Q., Lai, H., Hurtado, J., Stahnke, J., Leuzinger, K. and Dent, M. (2013).Agroinfiltration as an effective and scalable strategy of gene delivery for production of pharmaceutical proteins.*Advanced Techniques in Biology and Medicine*.1: 103. doi:10.4172/atbm.1000103.
- D. A. Goldstein and J. A. Thomas.(2004). Biopharmaceuticals derived from genetically modified plants.*Quarterly Journal of Medicine*.97 (11): 705-716.
- Wroblewski, T., Tomczak, A. and Michelmore, R. (2005).Optimization of Agrobacterium - mediated transient assays of gene expression in lettuce, tomato and Arabidopsis. *Plant Biotechnology Journal*. 3: 259-273