

## سیستم‌های بیان پروتئین مبتنی بر گیاه برای تولید پروتئین و پپتید ضد میکروبی

آزاده نیک نژاد

دریافت: ۱۳۹۷/۰۵/۱۵ / ویرایش: ۱۳۹۷/۰۶/۲۰ / پذیرش: ۱۳۹۷/۰۶/۳۱ / انتشار: ۱۳۹۷/۰۹/۳۰

گروه سلولی و مولکولی، دانشکده علوم زیستی، دانشگاه خوارزمی، تهران، ایران

مسئول مکاتبات: niknejad@khu.ac.ir

**چکیده.** گیاهان یک سیستم بیانی برای تولید پروتئین‌های هترولوگ، به خصوص پروتئین‌های نوترکیب درمانی هستند. سال‌های اخیر، اکثر پروتئین‌های نوترکیب در باکتری‌ها یا سلول‌های پستاندار تولید می‌شوند. اگر چه گاهی سلول‌های حشرات، مخمر و قارچ برای تولید پروتئین‌های نوترکیب استفاده می‌شود. محصول سیستم گیاهی می‌تواند سالم، اقتصادی و راحت باشد و این امکان وجود دارد به طور گسترده در کشاورزی و صنایع، به ویژه در علوم زیستی و صنعت داروسازی استفاده شود. گیاهان سیستم‌های بیانی پروتئینی مناسب در تولید با کیفیت، هزینه کم و تغییرات پساترجمه‌ای صحیح هستند. استفاده از گیاه تراریخته در تولید واکسن‌ها، آنتی‌بادی‌ها و پروتئین‌های دارویی در سال‌های اخیر تبدیل به یک نقطه عطف در مهندسی ژنتیک گیاهی شده است. استراتژی‌های کارآمد برای تولید پروتئین‌های نوترکیب اهمیت بیشتری را به دست می‌آورند، به عنوان برنامه‌های کاربردی و مهم نیاز به مقدار زیادی از پروتئین‌های نوترکیب با کیفیت بالا برای استفاده در صنعت است. در این مقاله، مشکلات رایج در تولید پروتئین‌های نوترکیب و پپتید ضد میکروبی در سیستم‌های بیانی پروتئین مبتنی بر گیاه مورد بررسی قرار گرفته و استراتژی‌های راه حل آنها مورد بحث قرار گرفته است.

واژه‌های کلیدی. آنتی‌بادی، پروتئین‌های دارویی، پروتئین‌های نوترکیب، گیاهان تراریخته

## Plant-based expression systems for protein and antimicrobial peptide production

Azadeh Niknejad

Received: 06.08.2018 / Revised: 11.09.2018 / Accepted: 22.09.2018 / Published: 21.12.2018

Department of Cell and Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran  
Correspondent author: niknejad@khu.ac.ir

**Abstract.** Molecular farming technology offers a unique advantage that almost any protein can be produced economically and safely under very controlled conditions. Besides traditional production systems, such as bacteria, yeasts, insects and mammal cell lines, plants can now be used to produce eukaryotic recombinant proteins, especially therapeutic ones. Their advantages as hosts for protein production include correct post-translational modifications, low-cost maintenance and no risk of contamination by human pathogens. The system is widely applied in agriculture and industry, especially in life science and pharmaceutical industry. The application of transgenic plants in the production of vaccines, antibodies and pharmaceutical proteins has been playing a key role in plant genetic engineering in recent years. The production of recombinant proteins plays a critical role in the production of high amounts of high-quality proteins. In this review, common problems in the production of recombinant proteins and antimicrobial peptides in plant-based expression systems are discussed and strategies for their solution are suggested. Viral vector-mediated transient gene expression in plants enables rapid production of pharmaceutical proteins such as vaccine antigens and antibodies. To conclude, plant-based systems have the potential to bring unique efficacy-enhancing features to increase the utility and effectiveness of vaccines and therapeutics.

**Keywords.** antibodies, pharmaceutical proteins, therapeutic proteins, transgenic plant

## INTRODUCTION

Among various expression systems such as bacteria, yeast, mammalian cells and insects, plants

are becoming an attractive system for the production of recombinant proteins for pharmaceuticals, industrial proteins, monoclonal

antibodies and vaccine antigens, due to a large number of advantages including safe usage, rapid scale-up, long-term storage and low-production cost (Daniell *et al.*, 2001, 2009b; Fischer and Emans, 2000; Fischer *et al.*, 2003, 2004, 2013; Horn *et al.*, 2004; Howard and Hood, 2005; Klimyuk *et al.*, 2008; Ma *et al.*, 2003, 2005; Rybicki, 2009; Schmidt, 2004 Thomas *et al.*, 2011). In addition, plant cells have the ability to perform post-translational modifications (PTM) of proteins, which are typical of eukaryotic organisms (Gomord and Faye, 2004; Mett *et al.*, 2008; Vitale and Pedrazzini 2005). PTM affects the proteins activities and functions such as folding, stability, solubility as well as dynamic interactions with other molecules. The major type of PTM for plant-made recombinant proteins is glycosylation (Stulemeijer and Joosten, 2008; Webster and Thomas, 2012). PTM is an action following translation for additional covalent modifications with changing tertiary and quaternary structures of proteins.

A wide variety of promoters have been used for protein expression in plants, including constitutive, inducible and tissue-specific, native and synthetic promoters (Egelkroust *et al.*, 2012). The most

widely-used promoter is the cauliflower mosaic virus 35S promoter (CaMV35S), which promotes high-level of gene expression through the transcriptional terminator of the *Agrobacterium* nopaline synthase gene (*nos*) (Barampuram and Zhang, 2011; Lee *et al.*, 2008). Several molecular events, such as gene transcription, mRNA translation and protein accumulation, affect overall success of protein expression (Table 1).

The recombinant proteins accumulation in plant system depends on several factors including a choice of suitable host tissue and subcellular location, understanding the nature of the foreign proteins and determine their possible effect on the host plant as well as the post-translational modifications and the degree of protein purification (Egelkroust *et al.*, 2012; Fischer *et al.*, 2004; Schiermeyer *et al.*, 2004).

Recently the phenomenon of recombinant protein expression and its corresponding impact have sparked a heated debate. Although contested by many, the matter of plant system is highly beneficial. Therefore, there are several options for protein expression in plant systems (Table 2) that will be discussed in this review.

**Table 1.** Summary of factors affecting gene transcription, mRNA translation and protein accumulation Source: Modified from Egelkroust *et al.* (2012)

Factors affecting transcription rate	Factors affecting translation rate
Promoter selection	Post-transcriptional gene silencing
Transcription factor binding (TFB) sites	Codon usage
Upstream regulatory sequences (URS)	mRNA stability
Intron-mediated sequences	Recruitment and retention of translation-related protein
Transcriptional gene silencing (TGS)	<b>Factors affecting protein accumulation</b>
Pre-mRNA stability	Protein stabilisation
Transgene trait stacking	Protein localization
The impact of the site of integration	Protein modification
The number of gene copies	Cellular milieu

**Table 2.** List of required options for plant expression systems (Egelkroun *et al.*, 2012)

options for plant expression system
<b><i>Choice of host plant</i></b>
dicot or monocot food or non-food
<b><i>Type of transformation method</i></b>
biological (viral, bacterial) physical (biolistic, electroporation)
<b><i>Expression parameters</i></b>
stable or transient constitutive or tissue-specific
<b><i>The intracellular location</i></b>
cytoplasm or apoplast Organelle Plastid

### Host plants

Protein expression platforms have been developed for a variety of host systems including seed crops (e.g., corn, canola, soybeans and rice) leafy crops (e.g., tobacco, alfalfa and lettuce), plant cell cultures (e.g., tobacco, carrot and rice), hairy root cultures and aquatic plants (e.g., *Lemna minor*), in addition to moss and green algae (Wilken and Nikolov, 2012). One of the major plant systems used for the production of recombinant proteins is tobacco. Because of high leaf biomass yields, it can be cropped several times a year (Basaran and Rodriguez-Cerezo, 2008; Twyman *et al.*, 2003; Twyman, 2004; Wilken and Nikolov, 2012).

#### *Nicotiana tabacum* L. (Tobacco)

The genus *Nicotiana* belongs to the family Solanaceae, which includes 76 species, originating in North America, South America, Australia and Africa (Chase *et al.*, 2003; Knapp *et al.*, 2004). *Nicotiana tabacum* is an amphidiploid/allotetraploid species with 48 chromosomes, generated from hybridisation between *N. sylvestris* and either *N. tomentosiformis* Goodspeed or *N. otophpra* Grisebach, all of which originate in South America (Kitamura *et al.*, 2001; Lewis, 2011). *Nicotiana tabacum*, as a model plant in research, provides many practical advantages for large-scale production of recombinant proteins, including high biomass yield, low maintenance and

high speed production (Twyman, 2004). It is the non-feed crop which carries a reduced risk of transgenic material contaminating food chains and contains a high amount of nicotine and other alkaloids that are necessary to be removed (Stoger *et al.*, 2005; Twyman *et al.*, 2003; Twyman, 2004). Ma *et al.* (1995, 1998) reported the first successful expression of the *secretory monoclonal antibody* in transgenic *N. tabacum*. The two tobacco cell lines, BY-2 (bright yellow 2) and NT-1 (*N. tabacum* 1), are readily available for *Agrobacterium*-mediated genetic transformation and have been optimised for rapid growth (Doran, 2013).

#### *Nicotiana benthamiana* Domin

The non-cultivated tobacco species, *Nicotiana benthamiana*, is a unique Australian native plant that may have resulted from hybridisation between *N. suaveolens* and *N. debneyi* (Goodspeed, 1954; Goodin *et al.*, 2008). It is an amphidiploid species with 38 chromosomes (Goodspeed, 1954) that used as a host in the production of monoclonal antibodies and vaccines. It is a suitable host species to produce recombinant proteins using viral vectors such as tobacco mosaic virus (TMV) (Twyman, 2004; Yang *et al.*, 2004). It can be transformed with high efficiency and maintained easily, due to its short stature, short regeneration time and high seed production. It is also useful for the transient expression of genes (Goodin *et al.*, 2008).

## Transformation methods and expression systems

Genetic transformation can be achieved by physical or biological methods. Physical methods are usually used to transfer non-viral vectors by using a gene gun (biolistics) and chemical polyethylene glycol (PEG) transformation or electroporation of protoplasts (Barampuram and Zhang, 2011; Rao *et al.*, 2009). The biological method of plant cell genetic transformation by *Agrobacterium* involves the transfer and integration of a large tumor-inducing (Ti) or rhizogenic (Ri) plasmid resident in *Agrobacterium* into the plant nuclear genome. Although several species of *Agrobacterium* are known, two species are predominantly used for transformation: *Agrobacterium tumefaciens* (T-DNA transfer for generation of transgenic plants) and *Agrobacterium rhizogenes* (Ri-plasmid transfers necessary DNA to generate transgenic hairy roots) (Gelvin, 2003; Gleba *et al.*, 2014; Rao *et al.*, 2009).

The production of recombinant proteins in plants, also known as molecular farming, is classified into two expression systems, i.e., stable and transient expressions. Stable expression involves integration of foreign DNA into the plant genome and transfer to the next generations (Sharma *et al.*, 2005). The storage organs such as seeds have been used for the stable expression of proteins of interest (Scheller and Conrad, 2004). The chloroplast and nuclear stable transformations require more than two months, on the one hand, and several months, on the other. (Patiño-Rodríguez *et al.*, 2013).

Transient expression systems include *Agrobacterium*-mediated and viral-based transformation, wherein the foreign gene is not integrated in the genome and being expressed only for a few days after being introduced into the plant cell/tissue.

The strategy relies on *A. tumefaciens*, which contains transgenes; the bacteria are infiltrated directly into the organs of interest by injection or by using a vacuum chamber (agroinfiltration) (Klimyuk *et al.*, 2014; Leckie and Stewart, 2011; Leuzinger *et al.*, 2013; Vaghchhipawala *et al.*, 2011). Transient expression is generally used to verify the activity of the expression constructs and generates small amounts of recombinant protein for functional analyses (Twyman *et al.*, 2003). Many results have been obtained so far with viral backbones such as those of the RNA viruses, Tobacco mosaic virus (TMV), Potato virus X (PVX) and Cowpea mosaic virus (CPMV) (Gleba *et al.*, 2014; Marillonnet *et al.*, 2005). Transient expression, using viral vectors, is one of the best and most rapid methods to obtain a high yield of protein expression (Egelkroun *et al.*, 2012).

## Expression systems

The production of recombinant proteins in plants, also known as molecular farming, is classified into two expression systems including stable and transient expressions.

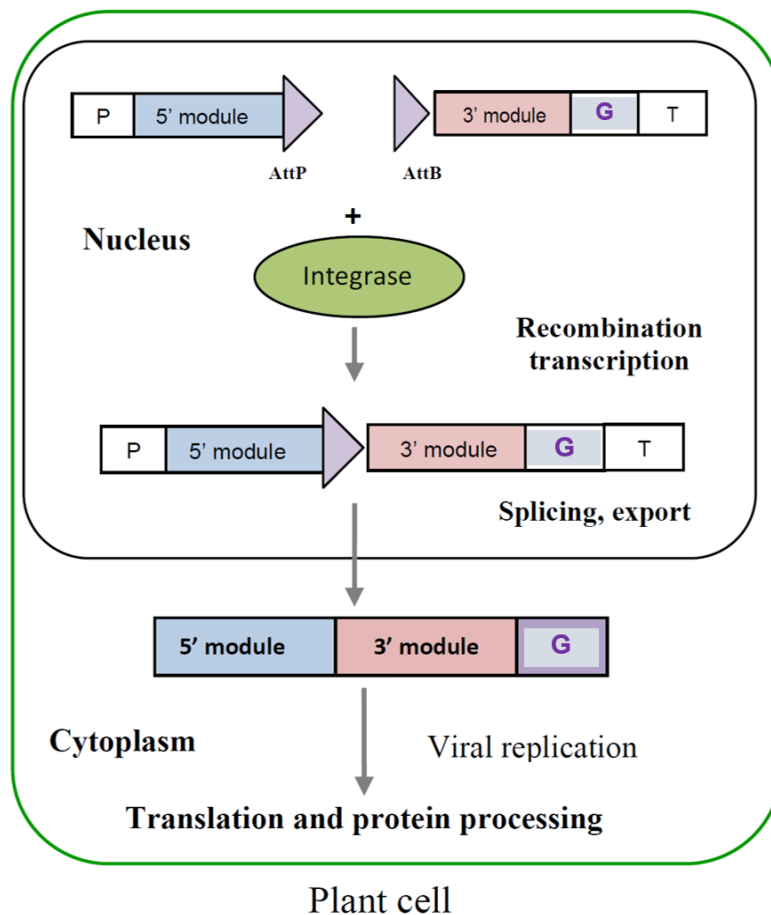
### Stable expression

Stable expression involves the production of transgenic plants or transgenic cell cultures. The foreign DNA is integrated into the plant genome and is transferred to the following generations (Sharma *et al.*, 2005). The storage organs of crop plants such as seeds have been used for the stable expression of the proteins of interest (Fischer *et al.*, 2004).

### Transient expression using viral vectors

Transient viral-based systems are amongst the best available systems and have the advantage of serving as a rapid method for large-scale production of proteins such as antibodies and vaccine antigens (Gleba *et al.*, 2007; Pogue *et al.*, 2002). The viral vector system is based on two strategies: 'full virus' vectors used to express long polypeptides (at least 140 amino acids) and 'deconstructed' virus vectors, which rely on *A. tumefaciens* to deliver DNA copies of one or more viral RNA replicons to plant cells (Gleba *et al.*, 2007; Gleba and Giritich, 2012). One of the main systems of deconstructed virus vectors is the 'magnICON<sup>®</sup>' from ICON genetics, based on the tobacco mosaic virus (TMV), which, instead of supplying whole RNA or linear DNA, it is divided in different modules captured between the left and right border of T-plasmid of *A. tumefaciens* (Gleba *et al.*, 2004; Gleba *et al.*, 2014; Marillonnet *et al.*, 2004).

The gene of interest is cloned in a 3' vector module. Various 5' modules are then used for targeting the expressed protein to different parts of the plant cell (cytosol, chloroplast or apoplast). An integrase module is used for the recombination of 3' and 5' modules *in planta*. These modules recombine within the plant cell, the resulting DNA is transcribed and the recombination sites (integrase) are spliced out in order to create an RNA replicon to produce the desired protein (Marillonnet *et al.*, 2004). Three *A. tumefaciens* cultures deliver different vector modules containing different viral components, targeting gene sequences. These pro-vector 5' and 3' modules assemble within the plant cell in the form of a functional viral vector (Marillonnet *et al.*, 2004). After being delivered by agroinfiltration, they are assembled inside the cell with the help of a site-specific recombinase to create an RNA replicon to produce the desired protein (Fig. 1).



**Fig. 1.** Schematic showing the assembly of the three viral vector modules and production of protein. The 5' and 3' modules with integrase in the plant cell allow recombining via the AttP and AttB sites. The intron is then spliced out. Viral replication occurs and translation and interest protein processing occurs. P: ACT2 promoter, T: *nos* terminator, G: Gene of interest. Source: Modified from Marillonnet *et al.* (2004).

The advantage of this system is the high-yield production of the desired protein because of viral amplification (Marillonnet *et al.*, 2004). Targeting the proteins into different subcellular compartments can alter protein stability (Gils *et al.*, 2005) e.g., the improvement of stability by chloroplasts (Doran, 2006). The green fluorescence protein (GFP) was expressed in different subcellular compartments of *N. benthamiana* using this system and produced the recombinant protein at up to 80% of total soluble protein (Marillonnet *et al.*, 2004, 2005). A considerable amount of research has been published on the use of the magnICON<sup>®</sup> system (Gleba *et al.*, 2004, 2005, 2007; Gils *et al.*, 2005; Giritch *et al.*, 2006; Huang *et al.*, 2010; Marillonnet *et al.*, 2004, 2005; Niknejad *et al.*, 2016; Santi *et al.*, 2006, 2008; Werner *et al.*, 2011).

#### Subcellular location in a plant system for protein accumulation

The targeting of foreign proteins to the most appropriate subcellular compartment is important for protein accumulation, stability, folding and post-translational modification (Twyman, 2004). The choice depends on the structural characteristics of the protein and the use of appropriate signals (Benchabane *et al.*, 2008; Faye *et al.*, 2005). Several subcellular compartments including the cytosol, endoplasmic reticulum (ER), apoplast, vacuole and chloroplast have been considered (Benchabane *et al.*, 2008; Ma *et al.*, 2003).

#### Cytosol

Recombinant proteins are retained in the cytosol when no targeting signals are used. Several studies have revealed that recombinant proteins remain stable within the cytosol (De Jaeger *et al.*, 1999; Michaud *et al.*, 1998; Marusic *et al.*, 2007). However, the cytosol may not be the best location

for several reasons: high levels of protease and ubiquitin activity, the negative redox potential that may affect the correct folding of proteins via formation of disulphide bonds, and a lack of important co- and post-translational modifications, such as glycosylation, which may affect folding, assembly and the tertiary structural stability (Benchabane *et al.*, 2008; Faye *et al.*, 2005).

### Endoplasmic Reticulum (ER)

Targeting proteins to the ER can lead to high yields of recombinant proteins *in planta* (Conrad and Fiedler, 1998; Wandelt *et al.*, 1992). Entry of recombinant proteins into the endoplasmic reticulum (ER) can be retained by adding (SEK/H/K)DEL retention sequences to the C-terminal (Benchabane *et al.*, 2008; Michaud *et al.*, 1998; Mainieri *et al.*, 2004), in most cases a (K/H)DEL sequence (Benchabane *et al.*, 2008; Ma *et al.*, 2003). The presence of chaperones in the ER helps to di-sulphide bonds formation and correct folding of the recombinant protein (Benchabane *et al.*, 2008; Faye *et al.*, 2005).

### Apoplast

Proteins can be targeted into the apoplast via the secretory pathway (Benchabane *et al.*, 2008). The plant apoplast includes the cell wall matrix and intercellular spaces in a wide range of physiological functions (Witzel *et al.*, 2011). The lack of KDEL in endosperm-expressed protein results in targeting to the apoplast, where the recombinant protein mostly accumulates in the space under the cell wall (Christou *et al.*, 2004).

### Vacuole

The vacuole is one of the alternative destinations for recombinant proteins produced in plants (Benchabane *et al.*, 2008). Additionally, it serves several key roles in planta e.g., control of cell turgidity, levels of macromolecules, accumulation of toxic secondary metabolites and storage of high-energy compounds in vegetative tissues (Benchabane *et al.*, 2008; Twyman, 2004).

### Chloroplast

The chloroplast is considered to be one of the cellular destinations in a plant expression system through the addition of an appropriate targeting signal peptide (Hyunjong *et al.*, 2006). The recombinant proteins may be moved to the chloroplast. Chloroplast transformation offers uniform expression rates, a high copy number of the transformed genes (due to hundreds of plastids in a cell), co-expression of multiple genes from the

same construct, minimal transgene escape in the environment due to the maternal inheritance of chloroplast DNA and minimal gene silencing (Daniell *et al.*, 2004).

Post-translational modifications, such as multimerisation and disulphide bridge formation, can occur in the stroma (Daniell, 2006; Parachin *et al.*, 2012). Thus, the chloroplast can be considered to be a suitable compartment with no need for the cell secretory pathway or modifications such as glycosylation, although, some endogenous proteases can affect the stability and accumulation of recombinant proteins (Adam and Clarke, 2002). However, this may not be relevant issue for proteins expressed at very high yield (Daniell, 2006).

### Plant systems for heterologous production of antimicrobial peptide

Anti-microbial peptides or proteins (AMPs) identified from prokaryotes and eukaryotes are usually less than 100 amino acid residues long and often have a high frequency of positively charged amino acids (Silva *et al.*, 2011). They are strongly cationic (pI: 8.9-10.7) having a net charge of 2+ to 7+, mainly due to the presence of arginine, lysine and histidine residues, heat-stable (100°C, 15 minutes), and additionally, they may have 50% hydrophobic amino acids and do not have any lysing/killing effects on eukaryotic cells (Hancock and Chapple 1999; Li *et al.*, 2012). AMPs have shown to serve as an important mechanism of natural resistance and also as a metabolically rapid inexpensive first line defence against pathogens (Brokaert *et al.*, 1997; Egorov *et al.*, 2005).

The antimicrobial activity of such peptides was observed to involve ionic interactions between their basic residues and the negatively charged bacterial membrane constituents, such as phospholipid head groups (Jenssen *et al.*, 2006; Melo *et al.*, 2009; Shai, 2002). On the other hand, the outermost layer of the eukaryotic membranes is composed of lipids with no net charge and zwitterionic head groups positioned within the inner layers; membrane-binding is controlled by hydrophobic rather than ionic interactions (Lee *et al.*, 2011a).

During the past 15 years, a wide range of AMPs have been shown to play essential roles in plant defense systems. Their sizes range from 2 to 9 kDa and many of them have a cysteine-rich structure with 6 or 8 cysteine residues involved in disulphide bridges; e.g., thionins, defensins, non-specific lipid transfer proteins (nsLTPs), hevein - and knottin-like peptides, MBP1, IbAMP and snakins (Broekaert *et*

*al.*, 1997; Benko-Iseppon *et al.*, 2010; Egorov *et al.*, 2005; García-Olmedo *et al.*, 1998; Nawrot *et al.*, 2014; Odintsova *et al.*, 2009). Some of the cysteine-rich AMPs, such as defensins, thionins and LTPs, are observed in wheat plants (Egorov *et al.*,

2005). The hevein-like peptide with a 10 cysteine motif isolated from *Triticum kiharae* has shown antifungal and antibacterial activity (Dubovskii *et al.*, 2011; Odintsova *et al.*, 2009).

**Table 3.** AMPs heterologous produced in *Nicotiana tabacum*

AMP transformed	Source	Objective	Resistance against	References
LFchimera	chimerical peptide	Production peptide and antimicrobial activity	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Erwinia amylovora</i> , <i>Ralstonia solanacearum</i>	Chahardoli <i>et al.</i> (2018)
protegrin-1 (PG-1)	Synthesized peptide	Production peptide and antimicrobial activity	<i>Klebsiella pneumoniae</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Mycobacterium bovis</i> , <i>Candida albicans</i>	Patiño-Rodríguez <i>et al.</i> (2013)
Dermaseptin Lysozyme C4V3	<i>Phyllomedusa sauvagii</i> , <i>Gallus gallus</i> , Synthetic	Production and isolation of the peptide and vaccine	Anti-HIV activity, induction of mammalian immune response	Rubio-Infante <i>et al.</i> (2012)
EtMIC2	<i>Eimeria tenella</i>	Peptide production	<i>Eimeria tenella</i> (chicken coccidiosis)	Sathish <i>et al.</i> (2011)
Retrocyclin-101 and Protegrin-1	Artificial AMP based on rhesus monkey ( <i>Macaca mulatta</i> ) circular minidefensins	Chloroplast genome transformation aiming peptide production	<i>Erwinia carotovora</i> , tobacco mosaic virus	Lee <i>et al.</i> (2011b)
NmDEF02	<i>Nicotiana megalosiphon</i>	Proof of concept for crop improvement	<i>Phytophthora parasitica</i> var. <i>nicotianae</i> , <i>Peronospora hyoscyami</i> f.sp. <i>tabacina</i> , <i>Alternaria solani</i> , <i>Phytophthora infestans</i>	Portieles <i>et al.</i> (2010)
Trichokonins	<i>Trichoderma pseudokoningii</i>	Proof of concept for crop improvement	Tobacco mosaic virus	Luo <i>et al.</i> (2010)
Pal and Cpl-1	Phages infecting <i>S.pneumoniae</i>	Chloroplast genome transformation aiming peptide production	<i>Streptococcus pneumoniae</i>	Oey <i>et al.</i> (2009)
MsrA2 (N-methionine-dermaseptin B1)	<i>Phyllomedusa sauvagei</i> and <i>Phyllomedusa bicolor</i>	Gene stacking for crop improvement	<i>Fusarium solani</i> , <i>Fusarium oxysporum</i> , <i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Sclerotinia sclerotiorum</i> , <i>Pythium aphanidermatum</i> , <i>Pectobacterium carotovorum</i>	Yevtushenko & Misra (2007)

Antimicrobial peptides/proteins (AMPs) are unique biologically active molecules that comprise defense systems against numerous pathogens such as bacteria, fungi, parasites and/or viruses. Heterologous expression systems, including bacteria and fungi as host cells, have been used for the production of AMPs with different sizes, folds and complexities (Parachin *et al.*, 2012). Plant systems have also been used as suitable sources for the production of AMPs and can be directly used for crop improvement without purifying the peptide or proteins (Desai *et al.*, 2010; Giddings *et al.*, 2000). The heterologous production of radish Rs-AFP2 defensin in tobacco protected the transgenic tobacco from *Alternaria longipes* (Terras *et al.*, 1995), whereas the alfalfa anti-fungal peptide (alfAPF) defensin reduced the infection in transgenic potato plants against the fungal pathogen *Verticillium dahliae* (Gao *et al.*, 2000).

Due to their antimicrobial activity and complex structures, several AMPs cannot be produced in microbial systems. For example, retrocyclin-101 (RC101) and Protegrin-1 (PG1) can be used as therapeutic agents against bacterial and/or viral infections; none has yet been produced in microbial systems, but both have been expressed in the tobacco chloroplast with a yield of around 20% of total soluble protein and retention of antiviral activity (Lee *et al.*, 2011b). The large-scale production of AMPs can be applied in numerous industries such as biotechnology, pharmaceuticals, cosmetics and food industry. However, the limiting factor is the high production costs of some AMPs due to their molecular mass and protein folding characteristics.

The AMP can be degraded by endopeptidases, which also limits their bioactivity. Thus, selecting the right system is essential for large-scale production and functional recombinant AMPs. Plant-based expression systems are promising platforms for further engineering and production of AMPs (Parachin *et al.*, 2012). All AMPs heterologously produced in *N. tabacum* are listed in Table 3.

## CONCLUSION

In conclusion, plants can act as 'biofactories' for the production of recombinant proteins, to reduce the production costs due to the low maintenance cost and high quality of the therapeutic proteins (Davies, 2010; Gomord and Faye, 2004; Gleba, 2007; Ling *et al.*, 2010; Mett *et al.*, 2008; Melnik and Stoger, 2013; Niknejad

*et al.*, 2016). Moreover, several remarkable advances make plant expression system an attractive high-tech platform for plant-made protein pharmaceuticals (PMPs).

## ACKNOWLEDGMENTS

The author acknowledges Prof. Mrinal Bhawe and Dr. Daine Webster, Swinburne University of Technology, Melbourne, Australia.

## REFERENCES

- Adam, Z. and Clarke, A.K.** 2002. Cutting edge of chloroplast proteolysis. – *Trends Plant Sci.* 7: 451-456.
- Barampuram, S. and Zhang, Z.J.** 2011. Recent advances in plant transformation. – *Methods Mol. Biol. (Clifton, N.J.)* 701: 1-35.
- Benchabane, M. Goulet, C. Rivard, D. Faye, L. Gomord, V. and Michaud, D.** 2008. Preventing unintended proteolysis in plant protein biofactories. – *Plant Biotech. J.* 6: 633-648.
- Benko-Iseppon, AM. Galdino, SL. Calsa Jr, T. Kido, EA. Tossi, A. Belarmino, LC. and Crovella, S.** 2010. Overview on plant antimicrobial peptides. – *Curr. Protein Pept. Sci.* 11: 181-188.
- Broekaert, W.F. Cammue, B.P.A. De Bolle, M.F.C. Thevissen, K. De Samblanx, G.W. and Osborn, R.W.** 1997. Antimicrobial peptides from plants. – *Crit. Rev. Plant Sci.* 16: 297-323.
- Chase, M.W. Knapp, S. Cox, A.V. Clarkson, J.J. Butsko, Y. Joseph, J. Savolainen, V. and Parokony, A.S.** 2003. Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). – *Ann. Bot.* 92: 107-127.
- Chahardoli, M. Fazeli, A. Niazi, A. and Ghabooli, M.** 2018. Recombinant expression of LFchimera antimicrobial peptide in a plant-based expression system and its antimicrobial activity against clinical and phytopathogenic bacteria. – *Biotech. Equipment* 32:3, 714-723.
- Christou, P. Stoger, E. and Twyman, R.M.** 2004. Monocot expression systems for molecular farming. In: Fischer R and Schillberg S (eds.) *Molecular Farming*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. pp 55-67.
- Conrad, U. and Fiedler, U.** 1998. Compartment-specific accumulation of recombinant immunoglobulins in plant cells: An essential tool for antibody production and immunomodulation of physiological functions and pathogen activity. – *Plant Mol. Biol.* 38: 101-109.
- Daniell, H. Streatfield, S.J. and Wycoff, K.** 2001. Medical molecular farming: Production of antibodies, biopharmaceuticals and edible vaccines in plants. – *Trends Plant Sci.* 6: 219-226.
- Daniell, H. Cohill, P.R. Kumar, S. and Dufourmantel, N.** 2004. Chloroplast genetic



- engineering. In: Daniell H and Chase CD (eds.) Molecular Biology and Biotechnology of Plant Organelles. Springer, Netherlands. pp: 443-490.
- Daniell, H.** 2006. Production of biopharmaceuticals and vaccines in plants via the chloroplast genome. – *Biotech. J.* 1: 1071-1079.
- Davies, H.M.** 2010. Review article: Commercialization of whole-plant systems for biomanufacturing of protein products: evolution and prospects. – *Plant Biotech. J* 8: 845-861.
- De Jaeger, G. Buys, E. Eeckhout, D. De Wilde, C. Jacobs, A. Kapila, J. Angenon, G. Van Montagu, M. Gerats, T. and Depicker, A.** 1999. High level accumulation of single-chain variable fragments in the cytosol of transgenic *Petunia hybrida*. – *Euro. J. Biochem.* 259: 426-434.
- Desai, P.N. Shrivastava, N. and Padh, H.** 2010. Production of heterologous proteins in plants: Strategies for optimal expression. – *Biotech. Adv.* 28: 427-435.
- Dubovskii, P.V. Vassilevski, A.A. Slavokhotova, A.A. Odintsova, T.I. Grishin, E.V. Egorov, T.A. and Arseniev, A.S.** 2011. Solution structure of a defense peptide from wheat with a 10-cysteine motif. – *Biochem. Biophys. Res. Commun.* 411: 14-18.
- Doran, P.M.** 2006. Foreign protein degradation and instability in plants and plant tissue cultures. – *Trends Biotechn.* 24: 426-432.
- Doran, P.M.** 2013. Therapeutically important proteins from in vitro plant tissue culture systems. – *Curr. Med. Chem.* 20: 1047-1055.
- Egelkrou, E. Rajan, V. and Howard, J.A.** 2012. Overproduction of recombinant proteins in plants. – *Plant Sci.* 184: 83-101.
- Egorov, T.A. Odintsova, T.I. Pukhalsky, V.A. and Grishin, E.V.** 2005. Diversity of wheat antimicrobial peptides. – *Peptides* 26: 2064-2073.
- Faye, L. Boulaflois, A. Benchabane, M. Gomord, V. and Michaud, D.** 2005. Protein modifications in the plant secretory pathway: Current status and practical implications in molecular pharming. – *Vaccine* 23: 1770-1778.
- Fischer, R. and Emans, N.** 2000. Molecular farming of pharmaceutical proteins. – *Transgenic Res.* 9: 279-299.
- Fischer, R. Schillberg, S. Buyel, J.F. and Twyman, R.M.** 2013. Commercial aspects of pharmaceutical protein production in plants. – *Curr. Pharm. Des.* 19: 5471-5477.
- Fischer, R. Stoger, E. Schillberg, S. Christou, P. and Twyman, R.M.** 2004. Plant-based production of biopharmaceuticals. – *Curr. Opin. in Plant Biol.* 7: 152-158.
- Fischer, R. Twyman, R.M. and Schillberg, S.** 2003. Production of antibodies in plants and their use for global health. – *Vaccine* 21: 820-825.
- Gao, A.G. Hakimi, S.M. Mittanck, C.A. Wu, Y. Woerner, B.M. Stark, D.M. Shah, D.M. Liang, J. and Rommens, C.M.T.** 2000. Fungal pathogen protection in potato by expression of a plant defensin peptide. – *Nat. Biotech.* 18: 1307-1310.
- García-Olmedo, F. Molina, A. Alamillo, J.M. and Rodríguez-Palenzuela, P.** 1998. Plant defense peptides. – *Peptide Sci.* 47: 479-491.
- Giddings, G. Allison, G. Brooks, D. and Carter, A.** 2000. Transgenic plants as factories for biopharmaceuticals. – *Nat. Biotech.* 18: 1151-1155.
- Giritch, A. Marillonnet, S. Engler, C. Van Eldik, G. Botterman, J. Klimyuk, V. and Gleba, Y.** 2006. Rapid high-yield expression of full-size IgG antibodies in plants coinfecting with noncompeting viral vectors. *Proc. Natl. Acad. Sci. USA.* 103: 14701-14706.
- Gils, M. Kandzia, R. Marillonnet, S. Klimyuk, V. and Gleba, Y.** 2005. High-yield production of authentic human growth hormone using a plant virus-based expression system. – *Plant Biotech. J.* 3: 613-620.
- Gleba, Y. Klimyuk, V. and Marillonnet, S.** 2007. Viral vectors for the expression of proteins in plants. – *Curr. Opin. Biotech.* 18: 134-141.
- Gleba, Y. Tusé, D. and Giritch, A.** 2014. Plant viral vectors for delivery by *Agrobacterium*. In: Palmer K and Gleba Y (eds.) *Current Topics in Microbiology and Immunology; Plant Viral Vectors*. Springer, Berlin Heidelberg, Germany. pp 155-192.
- Gleba, Y. Klimyuk, V. and Marillonnet, S.** 2005. Magnification - A new platform for expressing recombinant vaccines in plants. – *Vaccine* 23: 2042-2048.
- Gleba, Y. Marillonnet, S. and Klimyuk, V.** 2004. Engineering viral expression vectors for plants: The 'full virus' and the 'deconstructed virus' strategies. – *Curr. Opin. Plant Biol.* 7: 182-188.
- Gleba, Y. and Giritch, A.** 2012. Vaccines, antibodies, and pharmaceutical proteins. In: Altman A and Hasegawa PM (eds.) *Plant Biotechnology and Agriculture*. Elsevier Inc, USA. pp 465-479.
- Gomord, V. and Faye, L.** 2004. Posttranslational modification of therapeutic proteins in plants. – *Curr. Opin. Plant Biol.* 7: 171-181.
- Goodspeed, TH.** 1954. The genus *Nicotiana*. *J. Am. Pharm. Assoc.* 45: 193-193
- Goodin, MM. Zaitlin, D. Naidu, RA. and Lommel, SA.** 2008. *Nicotiana benthamiana*: Its history and future as a model for plant-pathogen interactions. – *Mol. Plant Microbe. Interact.* 21: 1015-1026.
- Hancock, R.E.W. and Chapple, D.S.** 1999. Peptide antibiotics. *Antimicrob. Agents Chemother.* 43: 1317-1323.
- Horn, M.E. Woodard, S.L. and Howard, J.A.** 2004. Plant molecular farming: Systems and products. – *Plant Cell Rep.* 22: 711-720.
- Howard, J.A. and Hood, E.** 2005. Bioindustrial and biopharmaceutical products produced in plants. – *Adv. Agron.* 85: 91-124.
- Huang, F.C. Studart-Witkowski, C. and Schwab, W.** 2010. Overexpression of hydroperoxide lyase gene in *Nicotiana benthamiana* using a viral vector system. – *Plant Biotech. J* 8: 783-795.
- Hyunjong, B. Lee, D.S. and Hwang, I.** 2006. Dual targeting of xylanase to chloroplasts and peroxisomes as a means to increase protein accumulation in plant cells. – *J. Exp. Bot.* 57: 161-169.

- Jenssen, H. Hamill, P. and Hancock, R.E.W.** 2006. Peptide antimicrobial agents. – *Clin. Microbiol. Rev.* 19: 491-511.
- Kitamura, S. Inoue, M. Shikazono, N. and Tanaka, A.** 2001. Relationships among *Nicotiana* species revealed by the 5S rDNA spacer sequence and fluorescence in situ hybridization. – *Theor. Appl. Genet.* 103: 678-686.
- Knapp, S. Chase, M.W. and Clarkson, J.J.** 2004. Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae). – *Taxon* 53: 73-82.
- Klimyuk, V. Pogue, G. Herz, S. Butler, J. and Haydon, H.** 2014. Production of recombinant antigens and antibodies in *Nicotiana benthamiana* using 'Magniflection' technology: GMP-compliant facilities for small- and large-scale manufacturing. In: Palmer K and Gleba Y (eds.) *Current Topics in Microbiology and Immunology; Plant Viral Vectors*. – Springer, Berlin Heidelberg, Germany. pp 127-154.
- Klimyuk, V. Marillonnet, S. Knäblein, J. Mccaman, M. and Gleba, Y.** 2008. Production of recombinant proteins in plants. – In: Knaeblein J. (eds.) *Modern Biopharmaceuticals*. – Wiley-VCH Verlag GmbH & Co, Weinheim, Germany. 3: pp 893-917.
- Lee, S.H. Kim, S.J. Lee, Y.S. Song, M.D. Kim, I.H. and Won, H.S.** 2011a. De novo generation of short antimicrobial peptides with simple amino acid composition. – *Regul. Pept.* 166: 36-41.
- Lee, S. Li, B. Jin, S. and Daniell, H.** 2011b. Expression and characterization of antimicrobial peptides Retrocyclin-101 and Protegrin-1 in chloroplasts to control viral and bacterial infections. – *Plant Biotech. J.* 9: 100-115.
- Lewis, R.** 2011. *Nicotiana*. In: Kole C (ed.) *Wild Crop Relatives: Genomic and Breeding Resources*. – Springer Berlin Heidelberg, Germany. pp 185-208.
- Leckie, B.M. and Stewart J, CN.** 2011. Agroinfiltration as a technique for rapid assays for evaluating candidate insect resistance transgenes in plants. – *Plant Cell Rep.* 30: 325-334.
- Leuzinger, K. Dent, M. Hurtado, J. Stahnke, J. Lai, H. Zhou, X. and Chen, Q.** 2013. Efficient agroinfiltration of plants for high-level transient expression of recombinant proteins. – *J. Vis. Exp.* 77: 50521.
- Li, Y. Xiang, Q. Zhang, Q. Huang, Y. and Su, Z.** 2012. Overview on the recent study of antimicrobial peptides: Origins, functions, relative mechanisms and application. – *Peptides* 37: 207-215.
- Ling, H.Y. Pelosi, A. and Walmsley, A.M.** 2010. Current status of plant-made vaccines for veterinary purposes. – *Expert Rev. Vaccines* 9: 971-982.
- Luo, Y. Zhang, D.D. Dong, X.W. Zhao, P.B. Chen, L.L. Song, X.Y. Wang, X.J. Chen, X.L. Shi, M. and Zhang, Y.Z.** 2010. Antimicrobial peptides induce defense responses and systemic resistance in tobacco against tobacco mosaic virus. – *FEMS. Microbiol. Lett.* 313: 120-126.
- Ma, J.K.C. Barros, E. Bock, R. Christou, P. Dale, P.J. Dix, P.J. Fischer, R. Irwin, J. Mahoney, R. Pezzotti, M. Schillberg, S. Sparrow, P. Stoger, E. and Twyman, R.M.** 2005. Molecular farming for new drugs and vaccines. Current perspectives on the production of pharmaceuticals in transgenic plants. – *EMBO. Rep.* 6: 593-599.
- Ma, J.K.C. Hiatt, A. Hein, M. Vine, N.D. Wang, F. Stabila, P. Van Dolleweerd, C. Mostov, K. and Lehner, T.** 1995. Generation and assembly of secretory antibodies in plants. – *Science* 268: 716-719.
- Ma, J.K.C. Hikmat, B.Y. Wycoff, K. Vine, N.D. Chargelegue, D. Yu, L. Hein, M.B. and Lehner, T.** 1998. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. – *Nat. Med.* 4: 601-606.
- Ma, J.K.C. Drake, P.M.W. and Christou, P.** 2003. The production of recombinant pharmaceutical proteins in plants. – *Nat. Rev. Genet.* 4: 794-805.
- Mainieri, D. Rossi, M. Archinti, M. Bellucci, M. De Marchis, F. Vavassori, S. Pompa, A. Arcioni, S. and Vitale, A.** 2004. Zeolin. A new recombinant storage protein constructed using maize  $\gamma$ -zein and bean phaseolin. – *Plant Physiol.* 136: 3447-3456.
- Marillonnet, S. Thoeringer, C. Kandzia, R. Klimyuk, V. and Gleba, Y.** 2005. Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. – *Nat. Biotech.* 23: 718-723.
- Marillonnet, S. Gritsch, A. Gils, M. Kandzia, R. Klimyuk, V. and Gleba, Y.** 2004. In planta engineering of viral RNA replicons: Efficient assembly by recombination of DNA modules delivered by *Agrobacterium*. *Proc. Natl. Acad. of Sci. USA.* 101: 6852-6857.
- Marusic, C. Nuttall, J. Buriani, G. Lico, C. Lombardi, R. Baschieri, S. Benvenuto, E. and Frigerio, L.** 2007. Expression, intracellular targeting and purification of HIV Nef variants in tobacco cells. – *BMC. Biotech.* 7: 1-12.
- Melnik, S. and Stoger, E.** 2013. Green factories for biopharmaceuticals. – *Curr. Med. Chem.* 20: 1038-1046.
- Melo, M.N. Ferre, R. and Castanho, M.R.B.** 2009. Antimicrobial peptides: Linking partition, activity and high membrane-bound concentrations. – *Nat. Rev. Microbiol.* 7: 245-250.
- Mett, V. Farrance, CE. Green, B.J. and Yusibov, V.** 2008. Plants as biofactories. *Biologicals* 36: 354-358.
- Michaud, D. Vrain, T. Gomord, V. and Faye, L.** 1998. Stability of recombinant proteins in plants. In: Cunningham C and Porter AR (eds.) *Recombinant Proteins from Plants*. Humana Press. New York, USA. pp 177-188.
- Nawrot, R. Barylski, J. Nowicki, G. Broniarczyk, J. Buchwald, W. and Goździcka-Józefiak, A.** 2014. Plant antimicrobial peptides. – *Folia Microbiologica* 59: 181-196.
- Niknejad, A. Webster, D. and Bhawe, M.** 2016. Production of bioactive wheat puroindoline proteins in *Nicotiana benthamiana* using a virus-based transient expression system. – *Protein Exp. Purif.* 125:43-52.

- Odintsova, T.I. Vassilevski, A.A. Slavokhotova, A.A. Musolyamov, A.K. Finkina, E.I. Khadeeva, N.V. Rogozhin, E.A. Korostyleva, T.V. Pukhalsky, V.A. Grishin, E.V. and Egorov, T.A.** 2009. A novel antifungal hevein-type peptide from *Triticum kiharae* seeds with a unique 10-cysteine motif. – FEBS. J. 276: 4266-4275.
- Oey, M. Lohse, M. Scharff, L.B. Kreikemeyer, B. and Bock, R.** 2009. Plastid production of protein antibiotics against pneumonia via a new strategy for high-level expression of antimicrobial proteins. Proc. Natl. Acad. Sci. USA. 106: 6579-6584.
- Parachin, N.S. Mulder, K.C. Viana, A.B. Dias, S.C. and Franco, O.L.** 2012. Expression systems for heterologous production of antimicrobial peptides. – Peptides 38: 446-456.
- Patiño-Rodríguez, O. Ortega-Berlanga, B. Llamas-González, Y.Y. Flores-Valdez, M.A. Herrera-Díaz, A. Montes-De-Oca-Luna, R. Korban, S.S. and Alpuche-Solís, Á.G.** 2013. Transient expression and characterization of the antimicrobial peptide protegrin-1 in *Nicotiana tabacum* for control of bacterial and fungal mammalian pathogens. – PCTOC. 115: 99-106.
- Pogue, G.P. Lindbo, J.A. Garger, S.J. and Fitzmaurice, W.P.** 2002. Making an ally from an enemy: Plant virology and the new agriculture. – Ann. Rev. Phytopathol. 40: 45-74.
- Portieles, R. Ayra, C. Gonzalez, E. Gallo, A. Rodriguez, R. Chacón, O. López, Y. Rodriguez, M. Castillo, J. Pujol, M. Enriquez, G. Borroto, C. Trujillo, L. Thomma, B.P.H.J. and Borrás-Hidalgo, O.** 2010. *NmDef02*, a novel antimicrobial gene isolated from *Nicotiana megalosiphon* confers high-level pathogen resistance under greenhouse and field conditions. – Plant Biotech. J. 8: 678-690.
- Rubio-Infante, N. Govea-Alonso, D.O. Alpuche-Solís, A.G. García-Hernández, A.L. Soria-Guerra, R.E. Paz-Maldonado, L.M.T. Ilhuicatzí-Alvarado, D. Varona-Santos, J.T. Verdín-Terán, L. Korban, S.S. Moreno-Fierros, L. and Rosales-Mendoza, S.** 2012. A chloroplast-derived C4V3 polypeptide from the human immunodeficiency virus (HIV) is orally immunogenic in mice. – Plant Mol. Biol. 78: 337-349.
- Rybicki, E.P.** 2009. Plant-produced vaccines: promise and reality. – Drug Discov. Today 14: 16-24.
- Rao, A.Q. Bakhsh, A. Kiani, S. Shahzad, K. Shahid, A.A. Husnain, T. and Riazuddin, S.** 2009. The myth of plant transformation. – Biotech. Adv. 27: 753-763.
- Santi, L. Batchelor, L. Huang, Z. Hjelm, B. Kilbourne, J. Arntzen, C.J. Chen, Q. and Mason, H.S.** 2008. An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. – Vaccine 26: 1846-1854.
- Silva, O.N. Mulder, K.C.L. Barbosa, A.E.D. Otero-Gonzalez, A.J. Lopez-Abarrategui, C. Rezende, T.M.B. Dias, S.C. and Franco, O.L.** 2011. Exploring the pharmacological potential of promiscuous host-defense peptides: From natural screenings to biotechnological applications. – Front. Microbiol. 2: 1-14.
- Sathish, K. Sriraman, R. Subramanian, B.M. Rao, N.H. Balaji, K. Narasu, M.L. and Srinivasan, V.A.** 2011. Plant expressed EtMIC2 is an effective immunogen in conferring protection against chicken coccidiosis. – Vaccine 29: 9201-9208.
- Santi, L. Giritich, A. Roy, C.J. Marillonnet, S. Klimyuk, V. Gleba, Y. Webb, R. Arntzen, C.J. and Mason, H.S.** 2006. Protection conferred by recombinant *Yersinia pestis* antigens produced by a rapid and highly scalable plant expression system. Proc. Natl. Acad. Sci. USA. 103: 861-866.
- Schmidt, F.R.** 2004. Recombinant expression systems in the pharmaceutical industry. – Appl. Microbiol. Biotechnol. 65: 363-372.
- Scheller, J. and Conrad, U.** 2004. Production of spider silk proteins in transgenic tobacco and potato. In: Fischer R and Schillberg S (eds.) Molecular Farming. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. pp: 171-181.
- Schiermeyer, A. Dorfmueller, S. and Schinke, H.** 2004. Production of pharmaceutical proteins in plants and plant cell suspension cultures. In: Fischer R and Stefan S (eds.) Molecular Farming. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. pp: 91-112.
- Shai, Y.** 2002. Mode of action of membrane active antimicrobial peptides. Biopolymers. – Peptide Sci. 66: 236-248.
- Sharma, K.K. Bhatnagar-Mathur, P. and Thorpe, T.A.** 2005. Genetic transformation technology: Status and problems. – In Vitro Cell. Dev. Biol. Plant 41: 102-112.
- Stulemeijer, I.J.E. and Joosten, M.H.J.** 2008. Post-translational modification of host proteins in pathogen-triggered defence signalling in plants. – Mol. Plant Path. 9: 545-560.
- Terras, F.R.G. Eggermont, K. Kovaleva, V. Raikhel, N.V. Osborn, R.W. Kester, A. Rees, S.B. Torrekens, S. Van Leuven, F. Vanderleyden, J. Cammue, B.P.A. and Broekaert, W.F.** 1995. Small cysteine-rich antifungal proteins from radish: Their role in host defense. – Plant Cell 7: 573-588.
- Thomas, D.R. Penney, C.A. Majumder, A. and Walmsley, A.M.** 2011. Evolution of plant-made pharmaceuticals. – Int. J. Mol. Sci. 12: 3220-3236.
- Twyman, R.M.** 2004. Host plants, systems and expression strategies for molecular farming. In: Fischer R and Schillberg S (eds.) Molecular Farming. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. pp: 191-216.
- Twyman, R.M. Stoger, E. Schillberg, S. Christou, P. and Fischer, R.** 2003. Molecular farming in plants: Host systems and expression technology. – Trends Biotech. 21: 570-578.
- Vaghchhipawala, Z. Rojas, C.M. Senthil-Kumar, M. and Mysore, K.S.** 2011. Agroinoculation and agroinfiltration: simple tools for complex gene function analyses. – Methods Mol. Biol. 678: 65-76.
- Vitale, A. and Pedrazzini, E.** 2005. Recombinant pharmaceuticals from plants: The plant

endomembrane system as bioreactor. – Mol. Interv. 5: 216-225.

**Wandelt, C.I. Khan, M.R.I. Craig, S. Schroeder, H.E. Spencer, D. and Higgins, T.J.V.** 1992. Vicilin with carboxy-terminal KDEL is retained in the endoplasmic reticulum and accumulates to high levels in the leaves of transgenic plants. –Plant J. 2: 181-192.

**Webster, D.E and Thomas, M.C.** 2012. Post-translational modification of plant-made foreign proteins; glycosylation and beyond. – Biotech. Adv. 30: 410-418.

**Werner, S. Breus, O. Symonenko, Y. Marillonnet, S. and Gleba, Y.** 2011. High-level recombinant protein expression in transgenic plants by using a double-inducible viral vector. Proc. Natl. Acad. Sci. USA. 108: 14061-14066.

**Wilken, L.R. and Nikolov, Z.L.** 2012. Recovery and purification of plant-made recombinant proteins. – Biotech. Adv. 30: 419-433.

**Witzel, K. Shahzad, M. Matros, A. Mock, H.P. and Mühling, K.H.** 2011. Comparative evaluation of

extraction methods for apoplastic proteins from maize leaves. – Plant Methods 7: 48-58.

**Yang, S.J. Carter, S.A. Cole, A.B. Cheng, N.H. and Nelson, R.S.** 2004. A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by *Nicotiana benthamiana*. Proc. Natl. Acad. Sci. USA. 101: 6297-6302.

**Yevtushenko, D.P. and Misra, S.** 2007. Comparison of pathogen-induced expression and efficacy of two amphibian antimicrobial peptides, MsrA2 and temporin A, for engineering wide-spectrum disease resistance in tobacco. – Plant Biotech. J. 5: 720-734.

\*\*\*\*\*

#### How to cite this article:

**Niknejad, A.** 2018. Plant-based expression systems for protein and antimicrobial peptide production. – Nova Biol. Reperta 5(3): 262-273.