Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 78 (3) 407–415 (2013) UDC 631.1.017+633:66.0:577.1:615.9.000.57
JSCS–4425 *Survey*

SURVEY

Plant molecular farming: opportunities and challenges

RUMYANA VALKOVA, ELENA APOSTOLOVA and SAMIR NAIMOV*

University of Plovdiv, Department of Plant Physiology and Molecular Biology, Plovdiv 4000, Bulgaria

(Received 5 November, revised 16 December 2012)

Abstract: Modern human life is impossible without products derived from classical, contemporary biotechnology. However, large-scale production of biotechnology wares opens a discussion about the economic impact, waste management, biosafety, and bioethical issues. Plant molecular farming offers a relatively inexpensive option for the yielding of many valuable products and demonstrates a number of advantages over classical technologies, but also raises the questions of further development perspectives, hazard identification and risk assessment. This review is focused on these two questions: opportunities offered and challenges faced by modern plant molecular farming systems.

Keywords: molecular farming; biohazard; plant biotechnologies.

CONTENTS

1. INTRODUCTION

During the last decade, plant molecular farming has become a widely used pipeline for the production of large variety of economically important components, among them bio-pharmaceuticals, enzymes, polymers, *etc*. Recently, several plant-derived biopharmaceutical proteins reached the late stages of commercial development. These products include antibodies, vaccines, human blood products, hormones and growth regulators.[1] For such products, plants offer practical and safety advantages as well as lower production costs compared with traditional systems based on microbial, animal cells or transgenic animals. With

* Corresponding author. E-mail: naimov0@uni-plovdiv.bg
doi: 10.2298/JSC121105158V

ever-increasing number of products in development, plant molecular farming is becoming even more competitive. In this review on the subject, recent technological developments in molecular farming are considered. An attempt has been made to give a broad overview on the exploited production systems and on several economic and biosafety issues.

2. BRIEF HISTORY OF MOLECULAR FARMING

The idea of producing valuable molecules using plants is as old as the first plant transformation. Since then a concept of how and which product can be efficiently produced in plants has rapidly evolved and developed. The first successes of genetic engineering could be defined as the first examples of molecular cloning – among them are human somatostatin, human somatotropin, and human insulin. The general idea that plant cells could be transformed was developed in early 1974.[2] Ten years later, the first successful agrobacterium-mediated plant transformation (Fig. 1) was reported by de Block *et al.*[3] At the very beginning, this technology was dedicated to the improvement the agricultural characteristics of crop plants, such as yield,[4] lipid content modification[5] and optimization of the amino acid composition,[6] or to provide agents for plant protection, such as biological insecticides,[7] engineered herbicides resistance[8] and pathogen resistance.[9] Since then, progress has been made in several different directions, including plant transformation systems and plant production lines. Since the initial commercial and scientific interest was focused on the production of proteins or secondary metabolites for internal uses in the transgenic plants, the main efforts

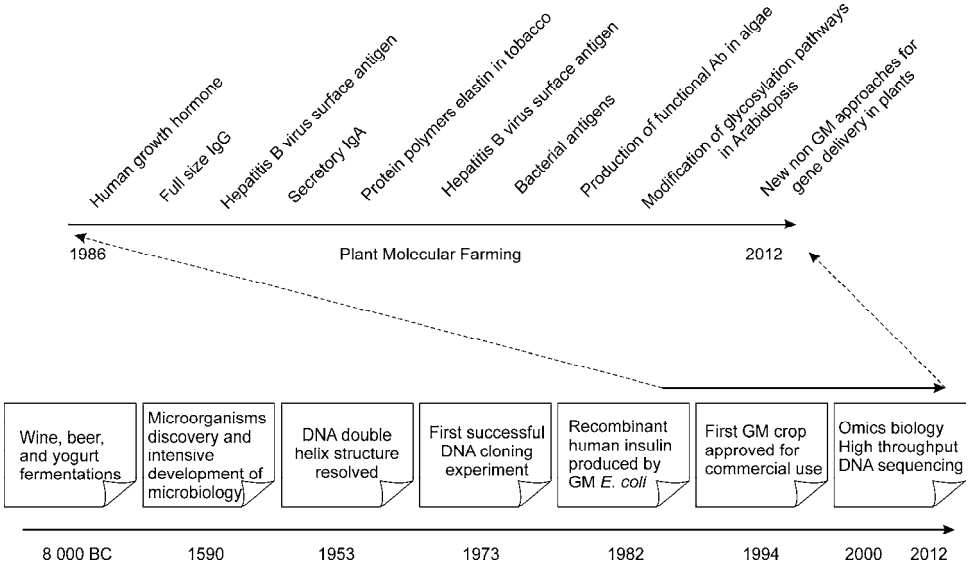

Fig. 1. A concise timetable of the development of molecular biotechnology and molecular plant farming.

were focused on improving transformation efficiency and broadening the host plant range.[10] In the latter respect, plant transformation systems were developed very rapidly. As a result, a number of agrobacterium-mediated transformation, direct gene delivery techniques[11] and pollen path[12] based systems have been deployed in different research or commercial plant genetic modification programs. Lately, the strategies for stable transformation of plants were more intensively substituted with the transient expression approach, which requires less time and allows some difficulties related to plant regeneration and somaclonal variations to be avoided. Most recently used transient expression systems are based on plant viruses "delivered" into the plant cell by *Agrobacterium* infiltration. The last decade of plant farming development can be truly called "a decade of plantibodies[13,14] and biofuel".[15] The intensive research in the field of biofuels and the production of human proteins in plants was initiated in both academic and research institutions and private companies.

3. ADVANTAGES OF THE PLANT MOLECULAR FARMING APPROACH

Expression of potentially valuable pharmaceuticals in plant-based systems has a number of advantages over the traditional biotechnological pipelines. Molecular farming provides payoffs with high technological, economical, social, and ecological impacts. As shown in Fig. 2, plant molecular farming has a remarkable potential for saving time and labor requirements and improving productivity and scalability. One of the important technological positives of this approach is related to the large variety of production systems available for this purpose. For instance, fully contained or open field systems can be easily implemented with some minor adjustments. The fully contained production pipelines do indeed require implementation of bioreactors with properties close to those of the microbiological fermenters. Furthermore, some of the contained production systems demand sufficient light in order to maintain the main plant functions, such as photosynthesis. Light has to be evenly distributed in the fermentation vessel, which in many cases is difficult to achieve or greatly increases the production costs. Therefore, this type of production is usually restricted to locations where the sunlight may be used as an energy source and the design of bioreactors is strongly influenced by the technical solutions implemented for algae cultivation. Alternatively, a genetically modified hairy root plant cultures may be used in dark vessels. However, this system is limited mainly to the production of secondary metabolites. Hairy roots induction is usually achieved by infecting plant tissues with the natural plant pathogen, *Agrobacterium rhizogenes* that causes so-called hairy root disease. The neoplastic roots produced by *A. rhizogenes* infection are characterized by high growth rate and genetic stability. These genetically transformed root cultures can produce high levels of secondary metabolites or amounts comparable to that of intact plants. Hairy root cultures promise

production of valuable secondary metabolites in many plants. The main constraint for the commercial exploitation of hairy root cultures is their scale-up, as a specially designed bioreactor that permits the growth of interconnected tissues unevenly distributed throughout the vessel would have to be developed. Since the hairy root cultures require a fully contained and controlled environment, the maintenance cost become equivalent to or higher than those required for conventional fermentation methods. Another *Agrobacterium* strain, *A. tumefaciens*, is recognized as applicable for plant molecular farming. *A. tumefaciens* is a natural plant pathogen as is *A. rhizogenes* but instead of forming hairy roots, it induces neoplastic growth of formations called "galls". Molecular farming employs genetically modified strains of *A. tumefaciens*. The genetic modification of this bacterial strain causes asymptomatic infection, which is coupled with the introduction of an additional gene set, coding the expression of a particular economically important product. Depending on the implemented experimental procedure, two types of results can be achieved: 1) stable plant transformation and 2) transient expression of a particular gene. Both methods differ in many aspects. The first method requires usage of a natural tumor-inducing plasmid, split in

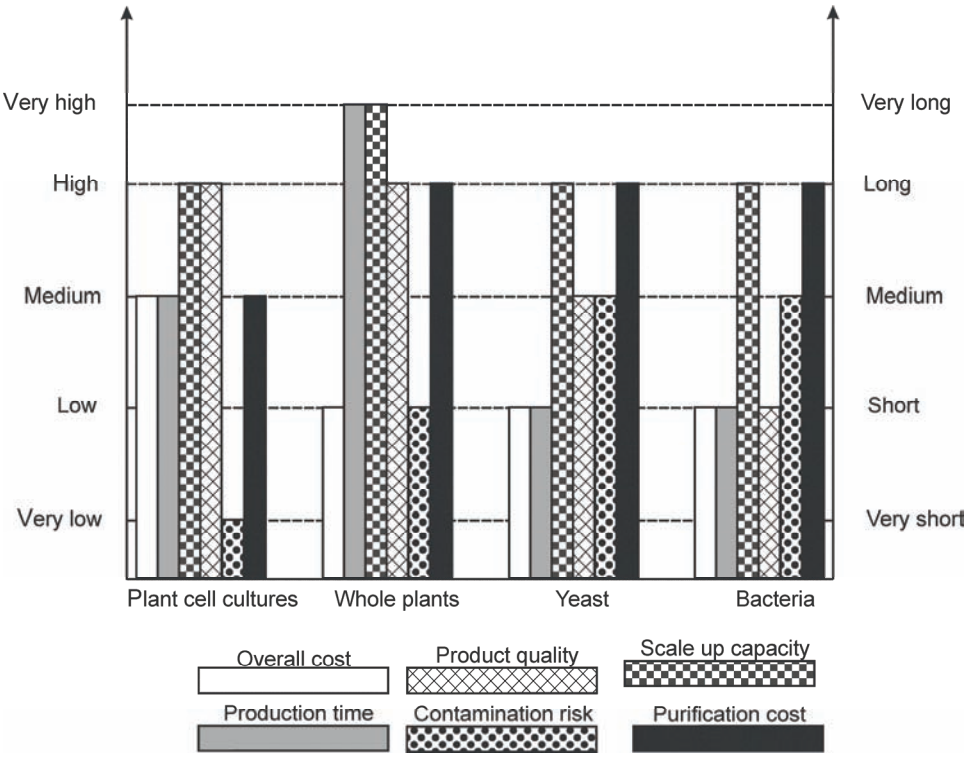

Fig. 2. A comparative analysis of molecular plant farming *vs*. traditional biotechnological production by Xu *et al*.[16] (with modifications).

binary vector systems, modified in such a way that only the desired part of the plasmid is incorporated into the plant genome. A stable transformation results in inheritable expression of the whole gene set, which usually confers expression of two genes – the gene of interest and a marker gene. The latter enables the positive or negative selection of the transgenic cells. The stable plant transformation also offers an additional opportunity for the translocation of the protein product into different cell compartments[17] and therefore, modification of the natural plant synthetic pathways becomes possible. In this respect, the system allows the production of not only recombinant proteins, but also the enhancement of the production of different secondary metabolites. Regarding the experimental procedure, stable plant transformation is more time and labor consuming but offers more sustainable expression in the next generations. Alternatively, transient expression offers a short-term high-level expression of a single gene encoding for the protein of interest. In this case, the expression cassette is usually cloned into the full length or partial plant virus genome, for instance tobacco mosaic virus, potato virus X (reviewed by Wagner *et al.*[18]), tobacco rattle virus (TRV),[19] *etc*., containing the gene of interest under control of a viral or other suitable promoter. The function of the viral genome in this scenario is to ensure the replication and subsequent transcription of its exogenously supplemented section. Depending on the employed viral component, a systemic or local gene expression may be observed. In most cases, the capacity of transient expression systems is limited to the production of recombinant proteins for a short-term period.

Another very promising alternative for genetic manipulation is the chloroplast transformation. It is accepted that the first plastids arose from endosymbiosis between a photosynthetic bacterium and a non-photosynthetic host[20] and therefore the chloroplast matrix environment is more bacteria-like, which provides better protein storage conditions for some proteins. The chloroplast genome is semi-autonomous: a large part of the chloroplast proteins are encoded by its own genome but, simultaneously, a small part of the genetic information is translocated to the nucleus of the plant cell – for example the large subunit of Rubisco is encoded by the chloroplast genome but the Rubisco small subunit encoding gene is located into to the plant nuclear genome.[21] Upon translation in the cytosol, the small subunit is subsequently transported into the chloroplasts.

Since the chloroplast transformation is mediated by homologous recombination of the transgene with the chloroplast genome, identification of spacer regions for integration of transgenes and the regulatory sequences is essential for experiment design. As the integration into the chloroplast genome is site-specific, the concerns of a position effect, frequently observed in nuclear transgenic lines,[22] are eliminated. As a result of the lack of transgene silencing, high levels of accumulation of transcripts have been reported.[23]

On the other hand, in most angiosperm plant species, plastid genes are maternally inherited[24] and therefore, transgenes in these plastids are not disseminated by pollen. An important advantage of plastid transformation is the ability to accumulate large amounts of foreign protein. Expression levels of up to 46 % of the total soluble protein were reported by De Cosa *et al.* and protein crystal formation was demonstrated.[25] Until now, chloroplast transformation has been implemented for the production of many valuable therapeutic proteins, such as Human interferon gamma (HIF-γ), Human somatostatin (hST), vaccines against antrax,[26] tetanus,[27] cholera,[28] *etc*. Chloroplast transformation has also been involved in production of a number of biomaterials such as monellin,[29] elastin-derived polymers,[30] *etc*.

In most cases, plant molecular farming systems require a certain degree of biosafety measures. After appropriate risk assessment, transgenic plants may be cultivated under regular field conditions according to the risk management programs developed for certain crops. In fact, only the cost of plant material processing is additional to the standard farming costs.

4. BIOSAFETY ISSUES RELATED TO MOLECULAR FARMING IN PLANTS

Considering that in most cases of molecular farming in plants or plant-derived cell/tissue cultures one is dealing with different degrees of genetically modified organisms, biosafety is gaining significant importance. The first two questions that require adequate answers are: 1) what is the hazard, respectively, how to identify the hazard and 2) what is the biological risk of implementation of such technology. Once the hazard is identified, exposure to the genetically modified event has to be determined. The risk is calculated using the formula:

$$\text{Risk} = \text{Hazard} \times \text{Exposure}$$

Hazard identification requires assessment of the potential gene transfer events, which are divided into two main classes: vertical and horizontal gene transfer. Vertical gene transfer is the movement of genetic material between at least partially sexually compatible plants. In this case, crops for molecular farming should be chosen with the minimization of gene flow in mind. This is the most prevalent form of transgene pollution and occurs predominantly *via* the dispersal of transgenic pollen, resulting in the formation of hybrid seeds with a transgenic male parent.[31] Hybrid seeds could also be generated with the transgenic plant as the female parent if the transgenic crops were fertilized by wild type pollen. In this case, transgene pollution would occur *via* seed dispersal, during growth, harvesting or transport.

Horizontal gene transfer represents genetic material exchange between sexually incompatible species that may belong to very different taxonomic groups. It is common in prokaryotes, resulting in the transfer of plasmid-borne resistance between different bacteria species. There are only few examples of natural gene transfer

between bacteria and higher eukaryotes. Since agrobacterium represents a special case where gene transfer occurs naturally from bacteria to plants, there is a perceived risk for horizontal gene transfer. In most cases, gene transfer from transgenic plants to soil bacteria or microorganisms in the digestive systems of herbivores is a subject of concern. The eventually transferred traits could have unpredictable effects on the relationships between different organisms, *i.e.*, render harmless bacteria pathogenic or make pathogenic species more difficult to control, *etc*.

Numerous potential solutions to the problem of transgene pollution caused by either horizontal or vertical gene transfer have already been developed.

When discussing biosafety issues related to genetically modified plants, it is necessary to recognize contamination with GM material during transport as an important hazard factor, especially in the case of post-harvest processing of the plant material (seeds, foliage, fruits, *etc*.). The risk of contamination becomes significant when genetically modified and conventionally produced plant materials or products are processed in the same installations or transported in the same vehicles. As complete cleaning is not possible in most cases, 0.8 % GM contamination in goods has been accepted as a threshold (this may differ in different countries).

5. CONCLUDING REMARKS

Research over the past 20 years has significantly increased knowledge in the field of molecular biology and especially the understanding of gene expression in higher plants, and technological development enabled plant derived cells or tissue to be cultured using different platforms. This has enabled a move from pure laboratory studies in model species to the exploration of a variety of different plants for the production of recombinant proteins and a number of secondary metabolites. Broad ranges of technical, pharmaceutical and industrial proteins have been produced in plants, some on a commercial basis. The main efforts were focused on overcoming the technical limitations of molecular farming, particularly by increasing low yields in some expression systems. However, there are several further challenges concerning the issues of environmental impact, biosafety and risk assessment, which reflect the release and agricultural-scale cultivation of transgenic plants, as well as the safety of the plant-derived products themselves.

И З В О Д

МОЛЕКУЛАРНИ АСПЕКТИ ГАЈЕЊА БИЉАКА – МОГУЋНОСТИ И ИЗАЗОВИ

RUMYANA VALKOVA, ELENA APOSTOLOVA и SAMIR NAIMOV

University of Plovdiv, Department of Plant Physiology and Molecular Biology, Plovdiv 4000, Bulgaria

Модеран живот људи је немогуће замислити без производа проистеклих из класичне или модерне биотехнологије. Биотехнолошка производња великих размера отвара питања економског значаја, руковања отпадом, биолошке безбедности и биоетике. Га-

јење биљака на молекуларној основи нуди релативно јефтину могућност производње великог броја значајних производа и има одређене предности у односу на класичну производњу, али повлачи са собом и процену перспективе даљег развоја, идентификацију опасности и ризика овакве технологије. У овом ревијском раду описане су могућности и изазови примене система модерног молекуларног гајења биљака.

(Примљено 5. новембра, ревидирано 16. децембра 2012)

REFERENCES

1. M. Paul, C. van Dolleweerd, P. M. Drake, R. Reljic, H. Thangaraj, T. Barbi, E. Stylianou, I. Pepponi, L. Both, V. Hehle, L. Madeira, V. Inchakalody, S. Ho, T. Guerra, J. K. Ma, *Hum. Vaccin.* **7** (2011) 375
2. C. V. Lundeen, *Methods Enzymol*. **31** (1974) 553
3. M. De Block, L. Herrera-Estrella, M. van Montagu, J. Schell, P. Zambryski, *EMBO J*. **3** (1984) 1681
4. H. Xing, C. Lawrence, O. Chambers, H. M. Davies, N. Everett, Q. Li, *Planta* **223** (2006) 1024
5. J. Zou, V. Katavic, E. M. Giblin, D. L. Barton, S. L. MacKenzie, W. A. Keller, X. Hu, D. C. Taylor, *Plant Cell* **9** (1997) 909
6. M. Zeh, A. P. Casazza, O. Kreft, U. Roessner, K. Bieberich, L. Willmitzer, R. Hoefgen, H. Hesse, *Plant Physiol*. **127** (2001) 792
7. T. H. Schuler, G. M. Poppy, B. R. Kerry, I. Denholm, *Trends Biotechnol*. **16** (1998) 168
8. B. J. Mazur, S. C. Falco, *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **40** (1989) 441
9. D. B. Collinge, H. J. L. Jørgensen, O. S. Lund, M. F. Lyngkjær, *Annu. Rev. Phytopathol*. **48** (2010) 269
10. P. J. Hooykaas, *Plant Mol. Biol.* **13** (1989) 327
11. C. Newell, *Mol. Biotechnol*. **16** (2000) 53
12. X. Gao, G. Wang, Q. Su, Y. Wang, L. An, *Biotechnol. Lett*. **29** (2007) 1781
13. S. Schillberg, R. Fischer, N. Emans, *Cell. Mol. Life Sci*. **60** (2003) 433
14. J. Xu, X. Ge, M. C. Dolan, *Biotech. Adv*. **29** (2011) 278
15. M. B. Sticklen, *Nature Rev. Genet*. **9** (2008) 433
16. J. Xu, X. Ge, M. C. Dolan, *Biotechnol. Adv*. 29 (2011) 278
17. N. S. Outchkourov, J. Peters, J. de Jong, W. Rademakers, M. A. Jongsma, *Planta* **216** (2003) 1003
18. B. Wagner, H. Fuchs, F. Adhami, Y. Ma, O. Scheiner, H. Breiteneder, *Methods* **32** (2004) 227
19. S. Bachan, S. P. Dinesh-Kumar, *Methods Mol. Biol*. **894** (2012) 83
20. M. W. Gray, *Curr. Opin. Genetics Dev*. **3** (1993) 884
21. S. Rodermel, *Photosynth. Res*. **59** (1999) 105
22. H. Daniell, *Nat. Biotechnol*. **20** (2002) 581
23. S.-B. Lee, H.-B. Kwon, S.-J. Kwon, S.-C. Park, M.-J. Jeong, S.-E. Han, M.-O. Byun, H. Daniell, *Mol. Breeding* **11** (2003) 1
24. H. Daniell, C. Chase, *Molecular Biology and Biotechnology of Plant Organelles*, Springer, Dordrecht, 2004, p. 93
25. B. D. Cosa, W. Moar, S.-B. Lee, M. Miller, H. Daniell, *Nat. Biotechnol*. **19** (2001) 71
26. J. Watson, V. Koya, S. H. Leppla, H. Daniell, *Vaccine* **22** (2004) 4374
27. J. S. Tregoning, P. Nixon, H. Kuroda, Z. Svab, S. Clare, F. Bowe, N. Fairweather, J. Ytterberg, K. J. von Wijk, G. Dougan, P. Maliga, *Nucleic Acids Res*. **31** (2003) 1174

28. H. Daniell, S.-B. Lee, T. Panchal, P. O. Wiebe, *J. Mol. Biol*. **311** (2001) 1001
29. K.-H. Roh, K.-S. Shin, Y.-H. Lee, S.-C. Seo, H. G. Park, H Daniell, S.-B. Lee, *J. Plant Biol*. **49** (2006) 34
30. C. Guda, S.-B. Lee, H. Daniell, *Plant Cell Rep*. **19** (2000) 257
31. K. S. Eastham, J. B. Sweet, *Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer*, Environment Issue Report 28, European Environment Agency, Copenhagen, 2002.