PLANTS AS BIOREACTORS FOR INDUSTRIAL MOLECULES

EDITED BY

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Dr. Santosh Kumar Upadhyay is currently working as an Assistant Professor at the Department of Botany, Panjab University, Chandigarh, India. Prior to this, Dr. Upadhyay was DST-INSPIRE faculty at the National Agri-Food Biotechnology Institute, Mohali, Punjab, India. He did his doctoral work at the CSIR-National Botanical Research Institute, Lucknow and received his Ph.D. in Biotechnology from UP Technical University, Lucknow, India. He has been working in the field of Plant Biotechnology for more than 14 years. His present research focuses in the area of functional genomics. He is involved in the bio-prospecting and characterization of various insect toxic proteins from plant biodiversity and defence and stress signaling genes in bread wheat. His research group at PU has characterized numerous important gene families and long noncoding RNAs related to the abiotic and biotic stress tolerance and signaling in bread wheat. He has also established the method for genome editing in bread wheat using CRISPR-Cas system and developed a tool SSinder for CRISPR target site prediction. His research contribution led to the publication of more than 70 research papers in leading journals of international repute. Further, there are more than five national and international patents, 40 book chapters, and 11 books in his credit. In recognition of his substantial research record, he has been awarded NAAS Young scientist award (2017–2018) and NAAS-Associate (2018) from the National Academy of Agricultural Sciences, India, INSA Medal for Young Scientist (2013) from the Indian National Science Academy, India, NASI-Young Scientist Platinum Jubilee Award (2012) from the National Academy of Sciences, India, and Altech Young Scientist Award (2011). He has also been the recipient of the prestigious DST-INSPIRE Faculty Fellowship (2012), and SERB-Early Career Research Award (2016) from the Ministry of Science and Technology, Government of India. Dr. Upadhyay also serves as a member of the editorial board and reviewer of several peer-reviewed international journals.

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Preface

In the past two decades, the application of plants as bioreactors for the production of various industrial molecules has evolved into an important research area with numerous new opportunities. Low production cost, easy to scale up, good-quality produce, and easy downstream processing have attracted the rapid growth of plants as bioreactors in recent years. Genetic and gene engineering methods are helpful in further improving the yield and quality of the product in various plants. Several industrial products have been produced in plant bioreactors, such as pharmaceutical proteins, vaccines, medical diagnostics proteins, industrial proteins, antibodies, attenuated viral particles, and nutritional supplements, including carbohydrates, minerals, vitamins, etc. Since the product quality, concentration, and yield, etc., are essential for commercial products, various strategies such as tissue-specific expression, enhanced transcript stability, translation optimization, and subcellular accumulation are developed and further improved to increase proteins and other products yields in transgenic plants. Several plant-derived products have also been reached in the market.

The high-value biomolecules in the biosphere happen to be an excellent attraction for research and development. The scientific community's primary objectives are the technological developments to exploit biotic and abiotic components of the ecosystem for societal benefits in a sustainable manner. It is desirable to develop cost-effective biological systems to produce biomolecules vital in various sectors. Advancement in biotechnological research has enabled the engineering of various plants to produce biomolecules such as proteins, carbohydrates, and lipids, with crucial effects on health and agriculture. These biological systems have been proved to be economical devices for expressing natural molecules of pharmaceutical and nutraceutical significance and, therefore, called bioreactors. Recent biosynthetic technologies have paved the way to develop expression platforms for pilot scale biosynthesis of the metabolites of medical and agricultural importance. The leading advantages of plant bioreactors have emerged the opportunities for the development of edible vaccines and molecular farming of pharmaceutical proteins, insecticidal proteins, antioxidant molecules, secondary metabolites, bioavailable micronutrients, functional food products, etc.

The present book covers the holistic knowledge about plants as bioreactors from a general introduction to the applications in numerous fields, including pharmaceuticals, nutraceuticals, secondary metabolites, carotenoids, flavonoids, biopesticides, biofuels, etc. This book will act as a repository to get comprehensive information on the application of plants as a bioreactor in one place. This information would be significantly valuable for graduate students, academicians, researchers, and the general public.

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1

Plants as Bioreactors: An Overview

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1.1 Introduction

Plants are the primary producers of the ecosystem on which all other living organisms rely (Malmstrom 2010). They provide essential human diet components, such as carbohydrates, proteins, vitamins, and minerals, and are also a significant source of various phytochemicals (Kumar et al. 2021; Upadhyay and Singh 2021). Phytochemicals are naturally produced by plants as a result of their metabolic pathways and are also referred to as secondary metabolites (Anulika et al. 2016). For a long time, several pharmaceutical companies have used secondary metabolites to treat a variety of diseases. Furthermore, these chemicals are used in various industries, including cosmetics, herbicides, insecticides, flavors, and perfumes (Singh et al. 2017; Dixit et al. 2021a). On the other hand, traditional farming results in variation in the quality and quantity of raw materials due to different environmental conditions in different geographical areas (Seufert et al. 2012). Furthermore, many plants that produce commercially useful metabolites are difficult to cultivate outside their native environments, and as a result, they are overexploited, leading to their extinction (D'Amelia et al. 2021). A survey revealed surprising results that one-fifth of the 50 000 medicinal plants used today belong to threatened species (Pan et al. 2014). To safeguard these plants, several technologies such as plant-cell bioreactors and tissue culture have been used (Hussain et al. 2012). But with the discovery of recombinant-DNA (rDNA) technology, the entire research attention shifted to the development of transgenic crops. Now rDNA technology is mainly focused on improving and modifying proteins of commercial importance (Khan et al. 2016). Transgenic crops are considered bioreactors for the development of commercially important proteins. To develop transgenic crops, we can employ a variety of strategies. The most frequent and widely used strategy is Agrobacterium-mediated transformation, which includes the transformation of the desired gene into Agrobacterium cells, and then these cells are used to infect plants to transfer the essential genes to the plant

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genome. Electroporation, particle bombardment, and polyethylene glycol-mediated gene absorption are some more ways of direct gene transfer (Basso et al. 2020). The recombinant proteins produced in transgenic plants are then directed to different organelles for regular eukaryotic post-translational modifications (Hofbauer and Stoger 2013). A strong promoter sequence that boosts the expression of the desired product is necessary for excessive production of foreign protein (Gopal and Kumar 2013). Transgenic plants used as a bioreactor to create necessary plant products are a simpler, more appealing, and less expensive technique than microbiological systems (Yao et al. 2015). One big advantage is that plants frequently bloom throughout the year, making it simple to deliver the product to market on time. Plants that produce more biomass have the potential to produce more genetically modified commodities. We can also keep modified proteins in seeds for an extended period without losing their biological capabilities (Gunn et al. 2012). Furthermore, due to their natural and higher biochemical potential, plants are regarded as the best source of alkaloids, carbohydrates, fatty acids, proteins, phenolic compounds, and steroids (Sharma and Sharma 2009). However, for transgenics to be successful, contamination of food crops and their products as a result of gene integration and expression must be addressed (Bawa and Anilakumar 2013). As a result, strict regulatory issues must be addressed to establish transgenics for the development of recombinant proteins (Bawa and Anilakumar 2013). Plant bioreactors have recently achieved major advances in several fields. There is a myriad of products that are produced by plant bioreactors, such as medical, nutritional, industrial, and biodegradable plastics (Sharma and Sharma 2009). The main focus, however, is on the development of therapeutic proteins that can be taken orally or applied topically following extraction and purification (Daniell et al. 2009). The fermentors and bioreactors can be replaced with greenhouses with a proper plant growth chamber to reduce the upstream facility. In addition, plant tissues can be processed for oral delivery of food proteins, which would also reduce downstream processing (Fischer and Buyel 2020). Despite more than 20 years of research and reports about the efficacy of plant-produced products, very few products have gone all the way through the production and regulatory hurdles (Whitelam et al. 1993). In this chapter, we will discuss the factors that are essential for the optimization of the production of recombinant proteins in plants and different products developed by this approach.

1.2 Factors Controlling the Production of Recombinant Protein

1.2.1 Choice of the Host Species

The host plant species must be carefully chosen to ensure the efficient production of recombinant protein. Before selecting the host, we must consider several factors such as host species, biomass yield, and cost of the scale-up process (Sharma and Sharma 2009). To achieve great success, we must first investigate the species- or tissue-specific factors that influence recombinant protein accumulation and quality (Whitelam et al. 1993). There are several model plant species, such as alfa–alfa, Arabidopsis, banana, lettuce, pea, potato, purple false brome, maize, rice, soybean, tobacco, and tomato, which we can choose for recombinant protein production (Richter et al. 2000). Plants can produce a