



Sustainable Production of Industrial Enzymes Using Plant-Based Expression Systems: A Comprehensive Review

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Received August 29, 2025; Accepted January 29, 2026; Online Published September 30, 2026

Abstract

The demand for enzymes continues to rise across various industrial sectors, including biofuels, paper, textiles, food, and environmental remediation. Although conventional microbial and mammalian cell-based production platforms have shown promise for producing industrially important enzymes, they are often associated with sustainability challenges such as high costs, biosafety concerns, and complex purification steps. Consequently, plants have been investigated as a feasible alternative for recombinant enzyme production in the development of sustainable systems. Moreover, compared with traditional microbial and mammalian systems, plant-based platforms offer low-cost cultivation, scalability, and safety benefits. This review provides a comprehensive analysis of plant-based systems for enzyme production, highlighting commonly used host organisms, expression techniques, and the importance of genetic elements such as promoters, signal peptides, and expression vectors. It also focuses specifically on major classes of industrial enzymes that are efficiently produced in plants, including cell-wall-degrading enzymes, lignin-modifying enzymes, enzymes used in food and detergent production, and those employed in pharmaceutical and diagnostic applications. Finally, the review concludes by highlighting advances in synthetic biology and the advantages of transient expression strategies over transgenic methods for industrial enzyme production. Overall, this review aims to promote further research and commercial application toward more sustainable and efficient enzyme production approaches.

Keywords: Molecular Farming, Plant-based Expression Systems, Industrial Enzymes, Recombinant Enzyme Production, Transient Expression, Sustainable Biomanufacturing

Citation: Akbar Aly AB, Shanmugaraj B, Senthil Murugan NT, Ramalingam S. Sustainable Production of Industrial Enzymes Using Plant-Based Expression Systems: A Comprehensive Review. J Appl Biotechnol Rep. 2026;13(3):2103-2115. doi:10.30491/jabr.2025.506447.1845

Introduction

Enzymes contribute to the enrichment and maintenance of diverse life forms on Earth, including humans. As biological catalysts, they are used in various industries, such as biofuels, food, pharmaceuticals, agriculture, leather, paper, and textiles.¹ Enzymes serve as environmentally friendly and sustainable alternatives to chemicals in industrial applications. Enzymatic reactions are highly specific and generate fewer by-products, which are generally less toxic. Moreover, the immobilization and recycling of enzymes are both economically viable and environmentally beneficial, and enzymes can be inactivated when necessary.² Recombinant DNA technology addresses supply consistency issues by enabling high-yield production of target enzymes in optimized hosts such as *Escherichia coli*, *Pichia pastoris*, and various plant systems. Studies indicate that more than 60% of industrial enzymes used in the detergent and food

industries are now manufactured via recombinant methods.³ Furthermore, enzymes are utilized in various fields, including technical industries, food processing, biofuel production, and organic synthesis within the pharmaceutical and cosmetic industries.⁴ For instance, the detergent industry employs enzymes to break down fats, starch-based stains, and proteins. Cellulases play a significant role in the textile industry, where they are used to enhance colour vibrancy and provide a softer texture to fabrics. In the pulp and paper sector, a range of enzymes, including amylases, cellulases, mannanases, and xylanases, are widely used. These enzymes decrease the viscosity of processing mixtures, improve product softness, increase brightness by breaking down residual glucomannan, and simplify the pulp bleaching process. Overall, these enzymatic treatments help improve quality and efficiency in both industries.⁵ Similarly, enzymes

such as lipases and lactases play a crucial role in cheese manufacturing and the production of lactose-free dairy products, facilitating essential biochemical transformations. In the baking industry, amylases and related carbohydrate hydrolases improve dough stability and handling, thereby optimizing the baking process. In juice processing, these enzymes help reduce viscosity and clarify the liquid, ensuring that the final product meets quality standards.^{4,6} Additionally, cellulases, hemicellulases, and xylanases facilitate the breakdown of lignocellulose in bioethanol production.⁷ However, the chemical synthesis of these enzymes remains too costly compared with recombinant production.^{8,9} Among the various hosts successfully used to produce these industrially important enzymes, plant hosts offer sustainable and low-cost production.¹⁰

Numerous plant-based expression systems have been utilized for the large-scale manufacturing of essential proteins and metabolites used in the pharmaceutical,

nutraceutical, and cosmetics sectors (Figure 1). Plant platforms are cost-effective, capable of synthesizing complex proteins with post-translational modifications (PTMs), and scalable.^{11,12} They facilitate the development of vaccines, antibodies, antimicrobial peptides, hormones, growth factors, and industrial enzymes.¹³ Moreover, plant molecular farming adheres to the principles of green chemistry and the circular bioeconomy, positioning it as a viable strategy for sustainable biomanufacturing. The integration of protein engineering with synthetic biology represents a significant advancement in the production of plant-based enzymes. This approach enables the production of enzymes with enhanced durability, improved thermostability, and better substrate specificity. In particular, the expression of these enzymes within plant tissues converts plants into functional bioreactors, enabling the direct use of raw plant biomass containing active enzymes. This reduces downstream processing and lowers costs.¹⁴

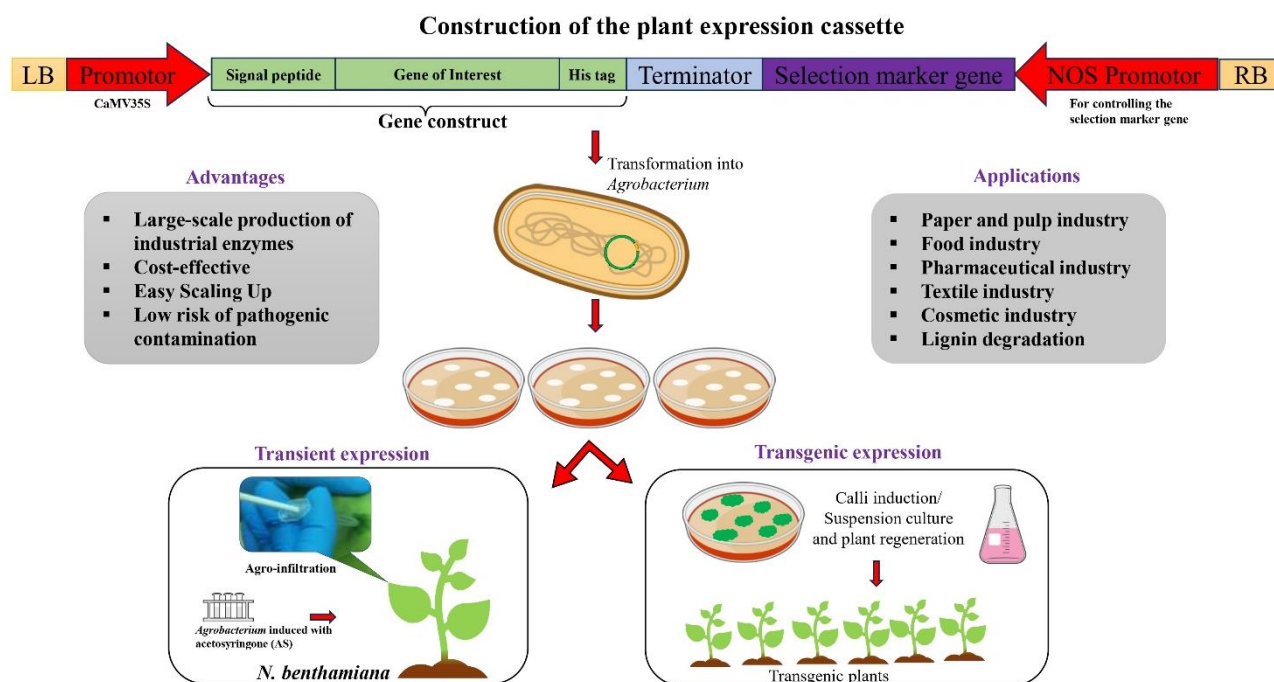


Figure 1. Overview of Plant-Based Enzyme Production Strategies. Illustrations adapted from NIAID NIH BioArt Source (bioart.niaid.nih.gov/bioart/).

This review provides a comprehensive analysis of recent advances in plant-based systems for enzyme production, with particular focus on enzymes that have significant industrial value and have been successfully expressed in plant systems. It covers both transient expression methods (agroinfiltration in *Nicotiana benthamiana*) and stable transgenic approaches across various plant hosts used to produce industrial enzymes, with selected examples. Furthermore, this review discusses the various industrial uses of plant-derived enzymes, including applications in biofuels, pharmaceuticals, food and feed, textiles, and

environmental mitigation, and provides insight into future directions for this sustainable and scalable approach to global enzyme production. A comprehensive literature review was conducted to identify relevant studies on the sustainable production of industrial enzymes via plant-based expression systems. Databases including PubMed, Web of Science, Scopus, and Google Scholar were searched for articles published from 1987 to 2025. The search strategy combined terms such as "plant-based expression," "industrial enzymes," "recombinant protein production in plants," "molecular farming," and "sustainable enzyme production."

Only peer-reviewed original research articles, reviews, and relevant book chapters published in English were included.

Plant Systems for Recombinant Enzyme Production

The use of plants as bioreactors for protein production has attracted interest from both academia and industry, particularly following the initial proof of concept. Research institutions and industry have continued to further develop plant-based systems, leading to significant recent advances.¹⁵⁻¹⁷ Most biologics for human use are currently produced using microbial or mammalian cell culture techniques; however, these traditional systems incur high production costs due to the need for specialized infrastructure, including fermenters, complex purification procedures, cold-chain preservation, and logistics.¹⁸⁻²⁰ Nevertheless, protein yields in plants are often lower than those in microbial systems such as *Escherichia coli* or *Pichia pastoris*. These systems excel in rapid growth and high expression levels but lack the complex PTMs required by some industrial enzymes and exhibit hyperglycosylation and improper folding.²¹⁻²³ According to Chen et al. (2016), these constraints underscore the need to investigate alternative and more cost-effective production platforms.²⁴ However, such technologies must be safe, scalable, and economical for recombinant enzyme production.²⁵ Plant-based expression platforms, including stable transgenic lines, transient agroinfiltration, and cell cultures, have become flexible and affordable alternatives to conventional microbial and mammalian systems. They rely on simple, low-cost growth media and, importantly, do not require sterile fermenters, thereby reducing infrastructure costs and biosafety concerns.^{25,26} *Nicotiana benthamiana* is a widely used plant system owing to its large biomass, rapid protein production via transient expression, and efficient transformation methods. Furthermore, its intrinsic biosafety benefits arise from the absence of human pathogens and endotoxins in plant tissues. Overall, these features make plant systems particularly appealing for producing industrial enzymes, vaccines, and other biopharmaceuticals,^{27,28} often delivering competitive yields at much lower operational costs than fermenter-based systems.²⁵ Therefore, *N. benthamiana* is particularly well suited for rapid and large-scale enzyme production.²⁹

Several biosafety and regulatory issues limit the widespread commercialization of plant-based proteins. Accordingly, to maintain the quality and safety of plant-derived proteins, they must be produced under controlled conditions that comply with Good Manufacturing Practices (GMP).^{30,31} Regulatory frameworks for genetically engineered plants primarily involve agencies such as the US FDA, USDA, and EPA. These agencies assess risks associated with environmental release, human health, and crop contamination.³² Furthermore, the lack of regulatory harmonization remains a global challenge, complicating

market access and product registration across jurisdictions.³³ In addition, public skepticism regarding genetically modified (GM) plants remains a major limitation to public acceptance, particularly in Europe and Asia. Factors such as cultural values, media portrayals, and distrust of major biotechnology companies also influence these concerns.³⁴ Effective commercialization of GM plants depends on factors such as transparent communication with the public, comprehensive safety data, and harmonized regulatory policies to maximize the potential of plant-based protein production.

Host Plants

Plant expression platforms include whole plants, hairy roots, moss, suspension cells, microalgae, and duckweed. Each platform has its own advantages and limitations. The choice of host plant depends on market demand, the type of recombinant protein, and production cost.³⁵ Whole-plant expression is the primary method in bioproduction and involves either transient or stable transformation, with genetic material inserted into the nuclear or chloroplast genome. Stable transformation provides heritable transgenes for seed stock and scalable protein production, but it is expensive and time-consuming to establish and analyze.^{36,37} In contrast, transient plant molecular farming offers the primary advantage of rapid production within four weeks. *Nicotiana benthamiana* and *Nicotiana tabacum* are the most commonly used host plants for these processes.¹¹ In addition to widely used tobacco plants, various other plant species have been shown to be effective for enzyme production, especially when long-term protein stability or oral delivery is required. Cereal crops such as *Triticum aestivum* (wheat), *Oryza sativa* (rice), *Hordeum vulgare* (barley), and *Zea mays* (maize) are considered valuable because of their capacity to stably accumulate recombinant proteins in seeds.^{38,39} Moreover, maize is used for recombinant enzyme expression owing to its efficient transformation protocols and substantial biomass yield.⁴⁰ However, the potential for gene flow via cross-pollination raises biosafety concerns. In contrast, self-pollinating crops such as rice and barley provide better containment and are regarded as safer for genetic modification.⁴¹ Furthermore, leguminous plants such as *Pisum sativum* (pea) are cost-effective and high in protein, although their biomass yield is limited. Additionally, plants such as *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) serve as flexible hosts for enzyme expression in edible tissues and various cellular compartments, thereby expanding production options.⁴²

Beyond classic hosts such as tobacco and cereals, new non-traditional plant platforms show great promise for the biosynthesis of recombinant enzymes owing to their rapid biomass growth, low nutrient requirements, and low biosafety risk. Aquatic microplants such as duckweed (*Wolffia arrhiza*) are attracting attention; however, their genetic transformation

remains technically challenging.⁴³ Meanwhile, the bryophyte *Physcomitrella patens* offers a significant advantage by simplifying the production of complex therapeutic enzymes because of its high-efficiency homologous DNA integration and advanced glyco-engineering capabilities. However, it has been reported that recombinant α -galactosidase from mammalian cells shows higher therapeutic activity than that from moss-based systems.⁴⁴

Importance of Promoters and Signal Peptides for Subcellular Targeting

Recombinant genes are efficiently expressed using plant transformation systems. These constructs consist of specific selection markers, signal peptides, and promoters. One of the most commonly used vectors for *Agrobacterium*-mediated transformation is pBI121, which contains the constitutive CaMV 35S promoter. The CaMV 35S promoter, derived from *Cauliflower mosaic virus*, is a widely used tool in plant biotechnology. As a constitutive promoter, it enables gene expression across various plant tissues and developmental stages.⁴⁵ Moreover, some viral vectors derived from *Potato virus X* (PVX), *Tobacco mosaic virus* (TMV) [MagnICON system], and *Cowpea mosaic virus* (CPMV) are used for transient expression because of their efficient large-scale protein production.⁴⁶ For instance, researchers constructed transgenic rice plants overexpressing the rice-derived *EXGI* (exo-glucanase) and *ENGI* (endo-1,3-beta-glucanase) genes to improve saccharification efficiency. They used modified binary vectors designated pBUH4 (*EXGI*) and pBUH3 (*ENGI*). These vectors express the enzyme genes under the control of the maize ubiquitin promoter (P_{ubi}) and the nopaline synthase terminator. Transgenic plants produced using pBUH4 showed considerably higher cellulase activity and produced more glucose and reducing sugars from mature rice stems than controls. These results indicate an increase in biomass conversion potential, with expression levels of 6.5–7.5% in two different rice varieties. However, the *ENGI* enzyme produced using pBUH3 showed no expression, that is, no shoot regeneration, suggesting that overexpression of *ENGI* is harmful to rice cells. Indeed, constitutive production of the enzyme caused various morphological defects, including leaf splitting, surface cracking, aberrant vascular lacunae, and reproductive sterility. Despite these developmental defects, the pBUH4 vector was considered effective for high-level enzyme expression and improved saccharification, making it a suitable choice for efficient production of carbon-neutral biofuels.⁴⁷

To achieve effective recombinant gene expression in plants, it is essential to employ both a robust expression vector and a potent promoter. Consistent transgene expression relies on a well-designed genetic construct combined with strong regulatory elements.⁴⁸ Constitutive promoters are frequently utilized to ensure uniform gene

activity across various tissues and developmental stages. Nevertheless, native promoters often exhibit limited strength and specificity, prompting the development and use of synthetic promoters. Advances in synthetic biology allow the design of promoters that drive constitutive, inducible, or tissue-targeted expression, thereby addressing the limitations of natural promoters.^{49,50} Another study reported the use of the *ENGI* gene, which was fused to the β -glucuronidase (GUS) reporter gene and placed under the control of the CaMV 35S promoter. The construct was then introduced into higher plants via *Agrobacterium*-mediated transformation. The construct showed high and broad enzyme expression, as determined by GUS assays. These results confirmed that the CaMV 35S promoter has strong activity and enables effective control of *ENGI* expression in transgenic plants.⁵¹

Recent studies have demonstrated that various promoter systems can effectively enhance recombinant enzyme synthesis in plant expression platforms. For example, bovine lysozyme (BvLz) was efficiently produced in *Saccharum* spp. (sugarcane) by combining multiple strong promoters to drive transgenic expression. Culm-specific and constitutive promoters, such as the sugarcane dirigent (SCDir) and maize ubiquitin (Ubi1) promoters, were combined to increase transcriptional activity. This approach boosted recombinant protein accumulation in culms by 20-fold, demonstrating the effectiveness of stacked culm-specific and constitutive promoters in increasing expression levels.⁵² Furthermore, in *O. sativa*, expression of manganese superoxide dismutase (MnSOD) under the regulation of the oxidative stress-responsive SWPA2 promoter markedly improved tolerance to drought and oxidative stress, exemplifying the value of inducible promoters in engineering stress-adaptive crops.⁵³

Signal peptides play an essential role in directing recombinant proteins to specific subcellular locations, thereby enhancing structural stability and proper folding. Signal peptides from barley α -amylase (Amy32b SP) and tobacco PR1b (PR1b SP) transport proteins to the apoplast.^{54,55} These signal peptides have been successfully used in various plant-based expression systems to ensure proper protein localization and secretion, which is required for high yields of functionally active recombinant proteins. For instance, researchers expressed the thermostable xylanase from *Clostridium thermocellum* in transgenic tobacco via *Agrobacterium*-mediated transformation and targeted it to the apoplast using the PR1b signal peptide. The enzyme accumulated at high levels without disrupting plant development and was readily isolated. These findings demonstrate the effectiveness of apoplast targeting for safe and efficient protein production.⁵⁶

Industrial Enzymes Produced in Plants

As noted above, plants have emerged as attractive biofactories for producing a wide variety of industrially

important enzymes. These include hydrolases such as cellulases, xylanases, and glucanases, which play crucial roles in the efficient production of carbon-neutral biofuels.⁵⁷⁻⁵⁹ Cellobiohydrolases and endoglucanases have also been successfully expressed in maize and tobacco, facilitating the breakdown of lignin-rich biomass.⁶⁰ Furthermore, to support lignin modification and environmental remediation, enzymes such as laccases and peroxidases are expressed in *Arabidopsis*, where their genes are actively transcribed into mRNA in developing xylem tissues.⁶¹ In addition, the production of thermostable proteases, such as subtilisin in chloroplast-transformed tobacco, extends their use in detergent and food processing.⁶² The selective use of various enzyme classes in plant expression platforms offers a sustainable pathway for producing industrially significant biocatalysts. Moreover, enzymes from a wide range of classes have been expressed in plants such as *Zea mays*, *Nicotiana tabacum*, *N. benthamiana*, *Oryza sativa*, and *Arabidopsis thaliana*.

Cell Wall Degrading Enzymes

Fermentative processes are the primary source of industrial enzymes such as xylanase and cellulase. For low-value applications such as converting lignocellulosic material into fuel ethanol, the cost of producing cellulose-degrading enzymes through fermentation is prohibitive. However, field-grown crops can compete with other production methods in terms of cost-effective biomass generation. Biomass production is inexpensive, and with minimal infrastructure, it can be produced in large quantities. Enzymes can also be economically produced in genetically modified plants for applications in the food, feed, and processing industries.⁶³ Therefore, researchers are exploring transgenic plants as an alternative approach for producing cell-wall-degrading enzymes, with the aim of reducing manufacturing costs.^{56,64-66} In addition, the cell-wall-degrading enzymes produced in plant expression systems are listed in Table 1.

Table 1. Cell Wall-Degrading Enzymes Expressed in Various Plant Expression Systems and Their Applications

Specific enzyme	Expression type	Host plant	Expression level	Subcellular target	Application area	Ref
(1,3-1,4)-beta-glucanase	Transgenic expression	<i>Hordeum vulgare</i>	NA	Protoplasts	Industrial processes of malting and brewing	64
(1,3-1,4)-beta-glucanase	Transgenic expression	<i>N. tabacum</i>	NA	Apoplast		79
Cellulases E2 and E3	Transgenic expression	<i>N. tabacum</i>	0.1% and 0.02% of total soluble protein (TSP)	Cytosol	Used in the conversion of lignocellulosic biomass to ethanol	80
t-Egl (truncated cellulase)	Transgenic expression	<i>N. tabacum</i>	0.1–0.5% of TSP	Cytosol	Food industry and paper industries	81,82
XylanaseA (<i>XynA</i>)	Transgenic expression	<i>Oryza sativa</i>	NA	Cytoplasm	Ruminants feed industry	83
Xylanase (XYLII)	Transgenic expression	<i>Arabidopsis thaliana</i>	30–50 mg of TSP	Peroxisome and chloroplast	Used in environment-related technologies	84
CBH I (cellobiohydrolase I)	Transgenic expression	<i>Zea mays</i>	Cell wall: NA ER: 3.5–4.5% of TSP	Cell wall, ER, and vacuole	Lignocellulosic biomass used in biofuel production	60
Endo-β-1,4-glucanase	Transgenic expression	<i>Zea mays</i>	Vacuole: 8–9% of TSP Cell wall: 3.7–5.1 % of TSP ER: 3.2–3.8% of TSP Vacuole: NA			
Cel6A (Cellobiohydrolase II)	Transgenic expression	<i>N. tabacum</i>	2 to 4% of TSP	Chloroplast	Conversion of lignocellulosic biomass to ethanol	85
Cel6B	Transgenic expression				Lignocellulosic biomass used in biofuel production	
BglB	Transgenic expression	<i>N. tabacum</i> , <i>Arabidopsis thaliana</i>	4.5% - cytosol and 5.8% - chloroplast	Chloroplast and cytosol	Biofuel production	86
Endoglucanase Cel5A	Transgenic expression	<i>N. tabacum</i>	Up to 5.2% of TSP	Chloroplast	Biofuels, textile industry, pulp and paper industry, food industry	87
<i>XynA</i> (Endo-β-1,4-xylanase)	Transient expression and Transgenic expression	<i>Arabidopsis thaliana</i> and <i>N. tabacum</i>	NA	Apoplast	Biofuel production	88
Zera-xylanase	Transient expression	<i>N. benthamiana</i>	9% of TSP	ER	Pulp and paper industry, food and feed processing	89

β -Mannanase	Transgenic expression	<i>N. tabacum</i>	25 units per gram of leaf (fresh weight)	Chloroplast	Biofuel, paper, oil, pharmaceutical, detergent industry	⁹⁰
CBHI (Cellobiohydrolase I)	Transgenic expression	<i>Saccharum officinarum</i>	NA	ER	Biofuel production	⁹¹
Endoglucanases (<i>ce/D</i>)	Transgenic expression	<i>N. tabacum</i>	72.20% of TSP	Chloroplast	Production of fermentable sugars	⁹²
Exoglucanase (<i>ce/O</i>)	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast	from lignocellulosic biomass, biofuel production, and production of cellulosic ethanol	
pectate lyases (<i>pe/B</i>)	Transgenic expression	<i>N. tabacum</i>	81.84% of TSP	Chloroplast		
pectate lyases (<i>pe/D</i>)	Transgenic expression	<i>N. tabacum</i>	79.43% of TSP	Chloroplast		
Cutinase	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
Swollenin	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
Xylanase (<i>xyn2</i>)	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
Acetyl xylan esterase	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
Beta glucosidase	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
lipase (<i>lipY</i>)	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast		
EXG1	Transgenic expression	<i>Oryza sativa</i>	NA	NA	Lignocellulosic biomass used in biofuel production	⁴⁷
β -glucosidase (<i>BEG1</i>)	Transgenic expression	<i>Oryza sativa</i>	NA	NA	Biofuel production	
Endo-1,3- β -glucanase (<i>ENG1</i>)	Transgenic expression	<i>Oryza sativa</i>	NA	Cytosol	Biofuel production	
sso1354 (cellulase gene)	Transgenic expression	<i>N. tabacum</i>	NA	Apoplast	Biomass conversion	⁹³
Lipase (LipA)	Transient expression	<i>N. benthamiana</i>	9% of TSP	NA	Biofuels, detergents, food processing, textile industry	⁹⁴
Endoglucanase (TrCel5A)	Transgenic expression	<i>N. tabacum</i>	NA	Apoplast	Processing of lignocellulose to fermentable sugars	⁹⁵
Endo- β -1,4-glucanase	Transient expression	<i>Helianthus annuus</i>	NA	Apoplast	Biofuel production, food and beverage industry	⁹⁶
Cellulase (Cel5A)	Transient expression	<i>N. tabacum</i>	NA	ER	Biofuel production, paper industry	⁹⁷
β -glucosidase	Transgenic expression	<i>N. tabacum</i>	75 % of TSP	Chloroplast	Biofuel production	⁹⁸
Endo- β -1,4-xylanase	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast	Biofuel production	
Endoglucanase	Transgenic expression	<i>N. tabacum</i>	up to 2 % of TSP	Chloroplast	Biofuel production	

NA- Not available

Lignin-Modifying Enzymes (LMEs)

Lignin-modifying enzymes (LMEs), such as laccases, manganese peroxidases, lignin peroxidases, and versatile peroxidases, are essential oxidative enzymes used for bioremediation, biomass valorization, and sustainable bioprocessing.⁶⁷ These enzymes are crucial for degrading complex lignin structures by breaking down phenolic and non-phenolic units through oxidative radical formation. Although native production of LMEs in white-rot fungi is limited by slow growth and complex culture conditions, such as the requirement for specific nutrient-limited media,

attaining high yields of LMEs can be challenging. Therefore, plants such as *Nicotiana benthamiana* have become attractive alternative hosts for recombinant LME expression because of their scalability and eukaryotic post-translational modification (PTM) machinery.^{67,68} Several fungal LMEs have been successfully expressed in *N. benthamiana* by targeting them to the apoplast using the *Sinopodophyllum hexandrum* signal peptide. This approach facilitates the recovery of active peroxidases and ensures their proper secretion, thereby preventing intracellular degradation.⁶⁹ These strategies provide new opportunities for sustainable biomass conversion

Table 2. Lignin-Modifying Enzymes (LMEs) Expressed in Plant Expression Systems

Specific enzyme	Expression type	Host plant	Expression level	Subcellular target	Ref
Fungal Laccase	Transgenic expression	<i>Zea mays</i>	0.1% of dry weight	NA	99
Lignin peroxidase	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	69
Versatile peroxidases	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	
Manganese peroxidases	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	
Laccase	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	
Cellobiose dehydrogenase (CDH)	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	
Pyranose oxidase (POX)	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	
Aryl-alcohol oxidase (AAO)	Transient expression	<i>N. benthamiana</i>	NA	Apoplast	

NA- Not available

Table 3. Food and Detergent Enzymes Expressed in Various Plant Expression Systems and Their Applications

Specific enzyme	Expression type	Host plant	Expression level	Subcellular target	Application area	Ref
α -Amylase	Transgenic Expression	<i>N. tabacum</i>	0.3% of TSP	Apoplast	Starch processing and Alcohol production	70
Xylanase	Transgenic expression	<i>N. tabacum</i>	NA	Apoplast	Paper industry and agriculture	56
Phytase	Transgenic expression	<i>N. tabacum</i>	14.4% of TSP	NA	Food and feed processing industries	63
Phytase	Transgenic expression	<i>Glycine max</i>	NA	NA	Food industries	100,101
Endoglucanase	Transgenic expression	<i>N. tabacum</i>	0.1% of TSP	Cytosol	Used in the conversion of lignocellulosic biomass to ethanol and other important industrial chemicals	80
Endoglucanase	Transgenic expression	<i>Medicago trunculata</i>	0.01% of TSP	Cytosol		
Cellobiohydrolase	Transgenic expression	<i>Medicago trunculata</i>	0.001–0.002% of TSP	Cytosol		
Cellobiohydrolase	Transgenic expression	<i>N. tabacum</i>	0.02% of TSP	Cytosol		
α -Amylase	Transgenic expression	<i>N. benthamiana</i>	5% of TSP	NA	Starch processing and Alcohol production	102
Xylanase	Transient expression	<i>N. tabacum</i>	3% of TSP	Apoplast	Food and feed, paper and pulp, textile, and pharmaceuticals industry	103,104
Endo- β -1,4-glucanase	Transgenic expression	<i>N. tabacum</i>	1.35% of TSP	Chloroplast	Biofuel Production	105
		<i>Solanum tuberosum</i>	2.6% of TSP	Chloroplast		106
		<i>N. tabacum</i>	1.6% of TSP	Apoplast, Chloroplast, and Cytosol		107
		<i>N. tabacum</i>	NA	Chloroplast		108
XynA (Endo- β -1,4-xylanase)	Transient expression in <i>N. tabacum</i>	<i>Arabidopsis thaliana</i> , <i>N. tabacum</i>	NA	Apoplast	Food and feed processing industries	88
	Transgenic expression in <i>Arabidopsis</i>					
Endo- β -1,4-glucanase	Transgenic expression	<i>N. tabacum</i>	0.25% of TSP	Apoplast	Biofuel Production	109
		<i>Zea mays</i>	2.1% of TSP	Apoplast		110
		<i>Lemna minor</i> 8627	0.24% of TSP	Cytosol		111,112
		<i>Oryza sativa</i>	4.9% of TSP	Apoplast		113
		<i>Zea mays</i>	1.13% of TSP	Apoplast		114
		<i>Zea mays</i>	ER: 6.1 ± 4.8 %, cell wall: 0.5 %, and vacuole: 5.6 ± 2.7 % of TSP	ER, cell wall and vacuole		60
		<i>Zea mays</i>	1.13% of TSP	Apoplast		114
		<i>N. tabacum</i> , <i>Zea mays</i>	NA	Apoplast		115
		<i>Oryza sativa</i>	6.1% of TSP	Apoplast		116
Endoglucanase	Transgenic expression	<i>Saccharum officinarum</i>	NA	ER	Ethanol production	91
Zera-xylanase	Transient expression	<i>N. tabacum</i>	9% of TSP	ER	Paper and pulp industry	89
Endo- β -1,4-glucanase	Transgenic expression	<i>Oryza sativa</i>	NA	Endosperm	Biofuel Production	117
GDSL-like Lipase	Transient expression	<i>N. benthamiana</i>	50 μ g /g of leaves	NA	Food industries	118,119
Endo- β -1,4-glucanase	Transgenic expression	<i>Arabidopsis thaliana</i>	26% of TSP	Apoplast	Biofuel Production	120

Cp-Eg1	Transgenic expression	<i>N. tabacum</i>	8.85 g FW/ plant in 24 weeks	Chloroplast	Food industry and textile industry	121
Cp-CelD	Transgenic expression	<i>N. tabacum</i>	129.6 g FW/plant in 9 weeks	Chloroplast		
Cp-lipase	Transgenic expression	<i>N. tabacum</i>	61.9–106.0 g FW/plant during 6–15 weeks	Chloroplast		
Cp-mannanase	Transgenic expression	<i>N. tabacum</i>	3.2 to 9.8 g FW/plant as their age increased from 9.5 to 24.5 weeks	Chloroplast		
Bovine Lysozyme (BvLz)	Transgenic expression	<i>Saccharum officinarum</i>	Up to 11.5% of TSP	NA	Food, cosmetic, and agricultural Industries	52

NA- Not available

and biotechnological applications using lignocellulosic feedstocks. Table 2 summarizes some of the LMEs produced in plant systems.

Food and Detergent Enzymes

In addition, a few studies have demonstrated that tobacco and potato plants can be genetically engineered to produce industrially relevant enzymes such as amylases and proteases in their active forms.^{70,71} These recombinant enzymes were

shown to accumulate at high levels in storage tissues, maintain correct folding, and exhibit catalytic activity equivalent to that of their microbial counterparts. These studies highlighted the potential of plant-based systems for producing starch-degrading enzymes for use in food processing, detergents, and the paper industry, as well as proteases for detergents and baking, representing a significant advance toward sustainable agricultural biotechnology.⁷² Some food and detergent enzymes produced in plant systems are summarized in Table 3.

Table 4. Pharmaceutical and Diagnostic Enzymes Expressed in Various Plant Expression Systems and Their Applications

Specific enzyme	Expression type	Host plant	Expression level	Subcellular target	Application area	Ref
CYP79A1 CYP71E1	Transgenic expression Transgenic expression	<i>N. tabacum</i> , <i>Arabidopsis thaliana</i>	NA	ER	Pharmaceutical industry	122,123
Human acetylcholinesterase	Transgenic expression	<i>Solanum lycopersicum</i>	NA	NA	Nerve agent protection, diagnostic and biosensors	124
Human glucocerebrosidase (GCD)	Transgenic expression	<i>Daucus carota</i>	NA	Vacuole	Enzyme replacement therapy for gaucher's disease	75
Human butyrylcholinesterase (BChE)	Transgenic expression	<i>N. benthamiana</i>	NA	ER	Plant-derived bioscavenger to protect animals against an organophosphorous pesticide challenge	125
β-Mannanase	Transgenic expression	<i>N. tabacum</i>	NA	Chloroplast	Biofuel production, paper, oil, pharmaceutical, coffee, and detergent industry	90
Human β-secretase 1 (BACE1)	Transgenic expression	<i>Solanum lycopersicum</i>	136 ± 7 ng /mg of TSP	NA	Pharmaceutical drug development, diagnostic	126
ATX (Autotaxin)	Transgenic expression	<i>Oryza sativa</i>	NA	Cytosol	Pharmaceutical industry	127,128
Human Gastric Lipase (hGL)	Transient expression	<i>N. benthamiana</i>	NA	NA	Therapeutic protein production	129
Human acetylcholinesterase	Transient expression	<i>N. benthamiana</i>	NA	ER	Pharmaceutical and diagnostic sectors	76
Human extracellular superoxide dismutase (hEC-SOD)	Transient expression	<i>N. benthamiana</i>	313.7 µg/g leaf FW	Cytosol	Therapeutic protein in skin disease and arthritis	130

NA- Not available

Pharmaceutical /Diagnostic Enzymes

In addition to food and detergent enzymes, plant-based systems play a vital role in producing enzymes for pharmaceutical and diagnostic applications, which are also

important in the healthcare industry.^{73,74} Elelyso, the first FDA-approved pharmaceutical enzyme produced in plant cells, contains glucocerebrosidase (GCase), which is used to treat Gaucher disease and was successfully synthesized in

carrot (*Daucus carota*) cell suspension cultures.⁷⁵ This milestone demonstrated the viability of using plant systems to produce high-value, glycosylated, human-like proteins suitable for therapeutic applications. Other notable examples include human acetylcholinesterase, which is expressed in *Nicotiana benthamiana* and serves as a bioscavenger and diagnostic agent for exposure to nerve agents.⁷⁶ These advances demonstrate that plant-based platforms are well suited for producing pharmaceutical and diagnostic enzymes because of their ability to synthesize complex recombinant proteins with post-translational modifications that are safe for human use. Moreover, plant-based production is inexpensive and unlikely to be contaminated by human pathogens. Some pharmaceutical and diagnostic enzymes produced in plant systems are summarized in Table 4.

Conclusion

The plant-based production of industrial enzymes offers a promising, sustainable, and scalable platform for biomanufacturing. Recent advances in synthetic biology, including genome-editing approaches and high-throughput transformation methods, are expected to significantly improve the expression and stability of recombinant enzymes in plants. Although promising developments have been made, significant scientific gaps remain. Recombinant enzyme yields in plants are still lower than those obtained through microbial fermentation,⁷⁷ requiring continuous improvements in vector design, promoter optimization, and host selection. Therefore, future research should focus on optimizing yield and solubility in plants for industrial use, including rapid biomass accumulation, inducible expression systems, and glycoengineering to replicate human or fungal PTMs. Furthermore, downstream processing in plant-based enzyme production faces major challenges due to the complex nature of plant material, which contains numerous host proteins and cell debris. This necessitates extensive purification steps, such as filtration and chromatography, which increase both time and cost. Although upstream cultivation in plants is relatively inexpensive, downstream processing often accounts for the majority of total expenses. Overcoming these challenges is crucial to make plant-derived enzymes commercially competitive with microbial and mammalian systems. Improvements in purification technology and integrated processing will be vital for addressing these obstacles and achieving economic viability. The emergence of cell-free plant-based systems and chloroplast-targeted expression methods also provides promising opportunities for enhancing yield and biosafety. With regard to transgenic plants, more time may be required to evaluate and improve the expression of a specific enzyme in various cellular compartments. In addition, obtaining approval for GM plants for commercial enzyme production may also take longer. In contrast, transient expression platforms offer advantages

over transgenic approaches, including rapid production of target enzymes or therapeutic proteins.⁷⁸ However, for full commercial application, it is essential to harmonize regulations, secure public acceptance, and invest in GMP-compliant plant facilities. In conclusion, the convergence of plant biotechnology, industrial demand, and sustainability goals makes plant-based systems a key future focus for enzyme manufacturing.

Authors' Contributions

Conceptualization: BS and RS; literature review: AB, NT, BS, RS; writing—original draft preparation: AB, NT; writing—review and editing: AB, NT, BS, and RS; supervision: BS and RS. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgment

The author (B.S.) gratefully acknowledges the support received from Karpagam Academy of Higher Education, Coimbatore, and DST-FIST (SR/FST/LS-I/2018/187, dated 20.12.2018).

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