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Review

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Engineering Plant Enzymes for Enhanced Agricultural Productivity and Stress Tolerance

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Abstract: Biocatalysts or enzymes are natural, eco-friendly proteins that are essential for biochemical changes in all living beings. However, native enzymes are not reusable, unstable at extreme pH and temperature and are also costly for use in agriculture and industries. Engineering of enzymes to address these limitations involves exploiting structure–function relationships to create robust biocatalytic variants that are catalytically more efficient, have altered substrate specificity and will operate for longer. Some of these novel techniques including rational design, directed evolution, random mutagenesis, DNA shuffling, homology modeling and high throughput screening based on cell surface display allow the modification of existing enzymes to create valuable and cost-effective agricultural products. The engineering of plant enzymes to make them more tolerant to heat or acid, or to extract from biomass, has been less studied, but plant enzymes are known to be engineered to have enhanced properties for use in crops, disease resistance and yield. The design of plant biocatalytic systems, with higher abiotic stress resistance and productivity, has significant potential. The chapter presents a detailed description of the sophisticated techniques of protein engineering that have been developed to obtain catalytically superior and more thermostable and environmentally tough proteins. We explore some of the enzymes of plants–ascorbate peroxidase, papain, carbonic anhydrase and some glycoside hydrolases–that show how molecular optimization directly impacts crop stress adaptation and yields. Lastly, we highlight new, unconventional plant-derived enzymes that could be a valuable resource for scientific research in sustainable biotechnology.

Keywords: enzyme engineering; plant biocatalysts; abiotic stress tolerance; directed evolution; crop productivity

1. Introduction

The global human population is increasing rapidly, and various reports indicate that it will reach 9.7 billion by 2050 due to factors such as high birth rates [1,2]. The world population is projected to grow by 34% in the coming years, which will significantly affect global food demand and international food prices. Reports indicate that agricultural food grain production must increase by 70–100% from current levels to feed the growing population and address potential social and economic challenges that may arise [3–6]. The agricultural sector is expanding rapidly, and it is necessary to stay up to date on its environmental impact and develop ways to address it through eco-friendly alternatives that meet the requirements of the modern world. Enzymes are biological catalysts, typically proteins, that bind specific substrate molecules at their active sites and accelerate chemical reactions by stabilizing the transition state, which helps them catalyze various chemical reactions and solve many problems in society [7]. Enzymes are large protein molecules that have evolved over thousands of generations through natural selection to function efficiently in specific biological processes [8]. They are very useful for regulating metabolic processes, maintaining cellular homeostasis, and determining reaction rates. The most significant aspect of enzymes is that they can reduce the activation energy required for reactions, which increases the frequency of chemical reactions [9,10]. Engineering plant enzymes for enhanced agricultural productivity and stress tolerance targets key enzymatic pathways that govern osmoprotection, oxidative defense, ethylene regulation, and photosynthetic efficiency under abiotic stress. Overexpression of the BADH enzyme in the glycine betaine biosynthetic pathway increases tolerance to salinity and drought while protecting the photosynthetic apparatus in multiple crop species [11,12]. Antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase, scavenge reactive oxygen species that accumulate under drought, salinity, and temperature extremes, and their elevated activity is a primary target for genetic engineering of stress-tolerant crops [13,14]. Bacterial ACC deaminase, introduced into crops via rhizobacteria or direct gene transfer, cleaves the ethylene precursor ACC to suppress stress-induced ethylene accumulation, thereby sustaining root growth and yield under salt, drought, and heavy-metal stress [15–17]. Glutathione transferases represent another engineered enzyme class whose catalytic and non-catalytic functions in xenobiotic detoxification and redox modulation confer tolerance to both biotic and abiotic stresses, with rational enzyme engineering proposed to design improved isoenzymes for climate-resilient cultivars [18,19]. Cytokinin oxidase/dehydrogenase (CKX) is a further enzymatic target whose inhibition modulates cytokinin homeostasis, thereby enhancing grain yield, delaying senescence, and improving abiotic stress tolerance in cereals and other crops [20,21]. The SAL1 phosphatase enzyme has emerged as a key negative regulator of drought and salinity signaling, and its disruption enhances stress tolerance by derepressing stress-responsive genes [22,23]. Collectively, these enzyme engineering strategies span osmolyte synthesis, ROS detoxification, hormone crosstalk, and photosystem protection, offering complementary and stackable routes to sustain crop productivity under increasingly variable climatic conditions. The problem is caused by the growing population and environmental issues. In this case, using enzymes as catalysts to make different chemicals has been shown to be better for the environment and more effective than using inorganic catalysts [24,25]. Certain inorganic compounds—particularly toxic metals, excessive salts, and over-applied synthetic fertilizers—can pose risks to human and animal health and disrupt beneficial microbial communities. However, many inorganic nutrients (e.g., Ca, Mg, K, Fe, Zn, PO_4^{3-}) are essential for human and animal physiology, and inorganic fertilizers, when used judiciously, can support crop productivity without universally harming soil microbiomes. The effects depend on the specific compound, concentration, form, and management practices [26]. The global enzyme market was valued at USD 9.087 billion in 2019 and is projected to reach USD 13.8152 billion by 2027, representing a compound annual growth rate (CAGR) of approximately 5.38% during the period from 2019 to 2027. Research indicates that the market for food enzymes has expanded due to the rising demand for bio-based products in processed agricultural foods and the growth of biotechnology [27]. Over time, changes in enzymes lead to the development of new biocatalysts with improved properties and more biological applications. It has been reported that various industrial sectors, including baking, beverages, cleaning agents, food processing, animal feed, leather, agriculture, pharmaceuticals, and textiles, have increasingly adopted enzymes because of their potential to improve process efficiency, product quality, and sustainability. However, the cost, stability, safety, and environmental benefits of enzymes can vary depending on their source, type, application, and operating conditions [28–31].

Amylase, phosphatase, lipase, and cellulase are a few examples of other key enzymes that help in plant growth and development [32]. These enzymes help with the absorption and availability of nutrients by breaking

down complex organic substances. Their actions not only help plants grow but also make the soil healthier and more fertile, leading to more sustainable crop production. Enzymes from microbial and plant sources are widely used in the food industry for specific processing applications. For example, rennet and microbial proteases are primarily used in dairy processing for milk coagulation and cheese production, whereas pectinases are widely employed for the clarification and processing of fruit juices. Other enzymes, including amylases and proteases, are used in baking and brewing to improve dough properties, fermentation, and product quality. However, a significant challenge in enzyme-based catalysis is the lack of effective biocatalysts for crucial chemical reactions essential to business [33,34]. Also, natural biocatalysts tend to perform poorly in industrial settings, either failing to work well with synthetic substrates or breaking down under adverse reaction conditions. Also, in the field, we are still having major issues with improving the biocatalytic efficiency, stability, and robustness of native enzymes. Enzyme engineering has emerged as a powerful solution to these problems. Recent progress in enzyme engineering enables scientists to concurrently target various enzyme properties through a range of genetic and protein engineering techniques [35,36]. These methods generally alter the specificity, activity, selectivity, kinetics, and stability of enzymes, thereby improving their performance in an industrial setting. Enzyme engineering can also improve enzyme performance and make it easier to process later, thereby increasing their productivity. Biocatalysts that are more resistant to high temperatures, extreme pH levels, and organic solvents have been developed using this method [37,38]. Plant enzymes can be engineered via rational design, directed evolution, and genome editing to enhance crop performance under abiotic and biotic stresses and to improve nutrient use efficiency. Scientists modify the plant's genome to enhance its ability to fight harmful microorganisms [39,40]. Such modification makes the plant more resistant to disease. These changes could include adding new resistance genes or modifying existing ones to improve their effectiveness [41]. Microbes have long been the primary source of extremozymes, but plants are increasingly recognized for their ability to aid in the discovery and development of new enzymes [42]. This chapter examines diverse methodologies and techniques in enzyme engineering for the creation of novel enzyme variants relevant to agriculture, featuring plant-derived enzymes, such as extremozymes, that exhibit exceptional stability and activity under extreme conditions.

2. Enzyme Engineering

Enzymes have been used as biocatalysts in many industrial sectors due to their unique properties, as compared to the traditional inorganic catalysts, such as high chemo-, regio- and stereo-selectivity under mild reaction conditions [43,44]. Native enzymes, however, are frequently difficult to use in industry due to difficulties in achieving and maintaining their catalytic stability and efficiency under non-physiological processing conditions. We see that they have lower catalytic efficiency, less tolerance to organic solvents, and that they break down at high temperatures and under high pressure. All of which, in the end, lead to low product yields [45–47]. So, wild-type enzymes need to be modified to function well under non-physiological conditions and to use non-natural substrates to produce biochemicals important to technology.

Enzyme engineering has been proposed as a very promising field for the design of novels, robust enzymes and the improvement of existing ones. These enzymes play a key role in many biotransformation processes. We see that engineered enzymes exhibit better thermal stability, substrate selectivity, and catalytic efficiency across a wider range of substrates [48]. The primary aim of enzyme engineering is to alter the amino acid sequence, thereby making the enzyme more useful for industrial applications [49,50].

The aim of many enzyme engineering approaches is to better understand the enzyme's sequence and structure, which, in turn, allows us to identify parts that may be altered to improve function. This is the best way we have to improve enzymes, thereby increasing their operational stability, physical robustness, and substrate specificity [51]. In biocatalysis, while the formation of the enzyme–substrate complex is a prerequisite for binding, the reaction rate is primarily governed by the enzyme's capacity to stabilize the transition state and lower the activation energy barrier ($\Delta G^{\ddagger}$) [52].

It is critical to manage amino acid residue changes in the catalytic domain of an enzyme during the design and optimization of this domain. These amino acid residues are frequently engaged in interactions with the enzyme and the substrate. In addition, amino acid changes within the active site, and even those close to it, are likely to change the substrate-enzyme interactions by affecting the structure and/or function of the enzyme [53,54].

3. Methods of Enzyme Engineering

Several enzyme-design techniques—including random mutagenesis, site-directed mutagenesis, enzyme immobilization, molecular modeling, DNA shuffling, and peptidomimetics—are used to engineer enzymes [55].

In general, enzyme engineering approaches can be broadly classified into three main categories: directed evolution, rational design, and semi-rational design, as detailed in Figure 1 and Table 1.

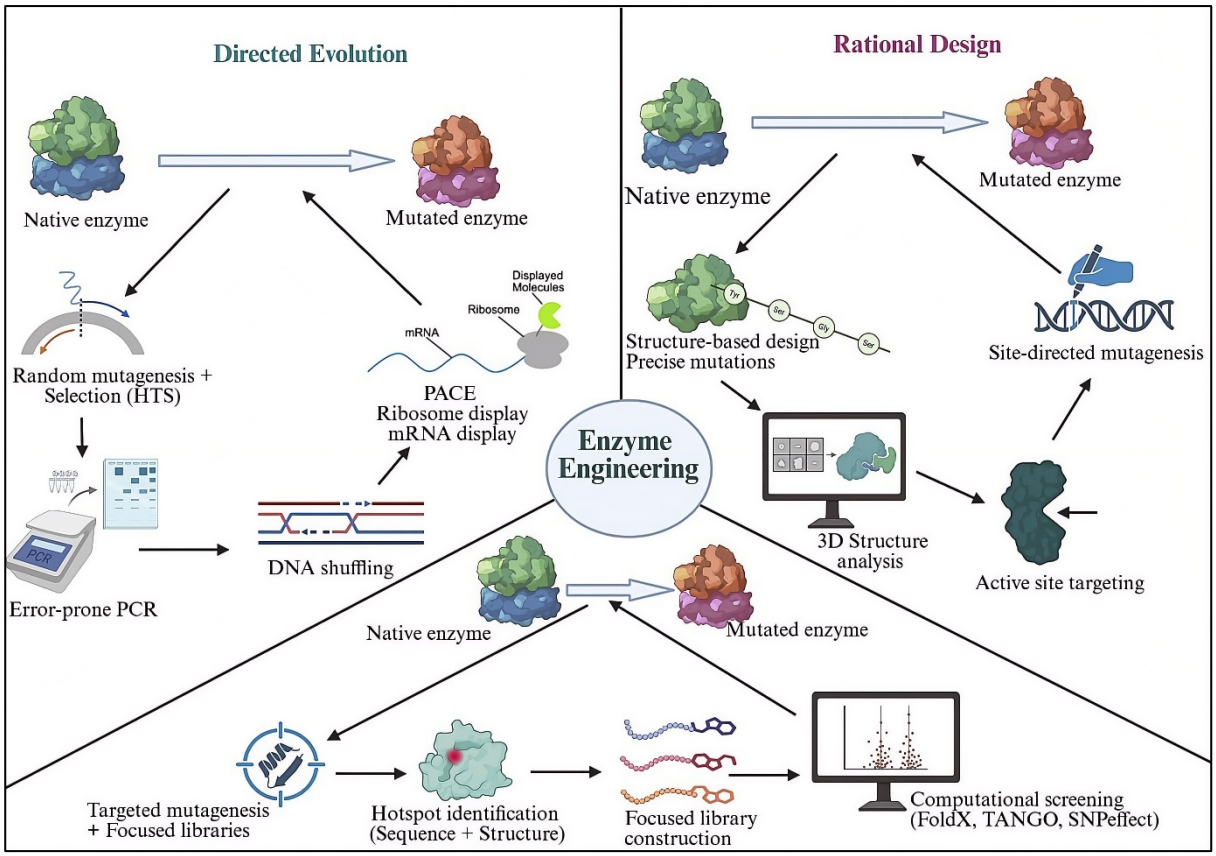

Figure 1. Integrated framework of approaches in enzyme engineering. Directed evolution fosters variability through random mutagenesis and recombination, connecting genotype and phenotype via high-throughput screening (HTS) technologies such as PACE, ribosome display, and mRNA display. Rational design relies on structural and functional knowledge derived from molecular modeling, active-site targeting, and site-specific mutagenesis. In this sense, semi-rational design combines the two approaches by deriving mutational hotspots from sequence and structural data, designing focused libraries, and using iterative computational methods (e.g., FoldX 3.0, TANGO, and SNPeffect 5.0) to rank the variants.

Table 1. Some enzyme engineering methods for the improvement of specific enzyme properties.

S. No.	Enzyme Engineering Methods	Specific Enzyme	Particular Enzyme Properties	References
1.	**Rational design**			
	Site-directed mutagenesis	Xylanase	pH optimum was modified	[56–59]
		Cutinase	2.7-times increase in the half-life of the enzyme	
		Phytase	9.15% increased thermostability	
		Glutathione S-transferase	2.2-times higher Km value	
2.	**Semi-rational design**			
	Semi-rational design	Amylosucrase	8-times higher catalytic efficiency	[60,61]
		Epoxide hydrolase	10-times increased detoxification efficiency	
3.	**Directed evolution**			
	Saturation mutagenesis	Esterase	7.8-times enhanced catalytic constant and thermostability	[62–65]
		Lipase	1.1 S to 594 S maximized enantio-selectivity	
		Xylanase	Increased thermostability	
		Cutinase	2–11-times increased stability	
	DNA shuffling	Phytase	2.5–3.1-times increased specific activity	[66,67]
		Lipases	8.2-times increased specific activity	
	Truncation	Endo-β-glucanase	3-times higher half-life at 65 °C	[68]
	Staggered extension process	Subtilisin E	20–25-times higher half-life at 65 °C	[69]
	Error prone-PCR and DNA shuffling	Novamyl	Temperature 10 °C increased thermal stability at pH 4.5	[70]
	Fusion	Lipases	8.2-times increased specific activity	[71–73]
		Lipoxygenase	2.3–4.5-times increased thermostability at 50 °C	
		α-amylase	3.5-times enhanced catalytic constant	
	Random mutagenesis	Monooxygenase	2.3–3-times higher hydroxylation activity	[74,75]
		Transglutaminase	1.7-times increased specific activity	

3.1. Directed Evolution

Directed evolution is one of the principal methods in enzyme engineering that mimics natural selection by introducing random mutations into the gene encoding a target enzyme and selecting variants that exhibit improved characteristics [76,77]. The first step is to make a variant library using random mutagenesis or recombination. The second step is to use high-throughput screening (HTS) to find better variants based on substrate specificity, thermostability, catalytic activity, or stereoselectivity [78]. This method is especially helpful when we don't know much about how an enzyme's structure and function interact, because it doesn't require complex biochemical or structural data. Directed evolution is one of the best ways to improve enzyme function [79].

Directed evolution employs random mutagenesis and high-throughput screening (HTS) to identify enzyme variants possessing advantageous characteristics. To create large collections of DNA variants, random changes are made to the genes that code for the protein you want. There are many ways to do this, like error-prone PCR, DNA shuffling, and the staggered extension process [80].

Error-prone PCR introduces mutations by amplifying DNA under less-than-ideal conditions, leading to a wide range of mutant populations. However, many of these variants may not work as well [81]. Stemmer [80] first talked about DNA shuffling. It involves breaking up homologous genes and reassembling them without primers to create new genetic combinations. These mutagenesis methods can generate very large libraries, making it hard to identify the best variants. HTS methods can test 10^4–10^5 variants per day using techniques such as fluorescence assays, NMR spectroscopy, chromatography, *in vitro* compartmentalization, microtiter plate screening, and fluorescence-activated cell sorting (FACS) [82].

Systems such as phage-assisted continuous evolution (PACE), ribosome display, and mRNA display connect genotype to phenotype, accelerating adaptation [83]. PACE, for example, subjects the enzymes to mutagenesis and selection cycles within a bacteriophage-based system under controlled temperature conditions [84]. Ribosome display enables the selection of specific peptides or proteins by displaying them on ribosomes and selecting them for their binding or catalytic activity [85]. mRNA display enables the selection of proteins from vast libraries by covalently linking an mRNA molecule to the protein it encodes [84].

3.2. Rational Design

Rational design is a focused, classical approach in enzyme engineering, based on detailed knowledge of protein structure and function. This approach introduces precise, targeted mutations into an enzyme's sequence, yielding predictable results rather than random mutagenesis or directed evolution [86]. Additionally, this approach is particularly effective when the enzyme's 3D structure, active site arrangement, and functional sites are well understood. Rational design is based on the fact that you are able to change certain amino acid residues on purpose, which in turn changes or improves an enzyme's properties, which may include its stability, specificity or catalytic efficiency [87]. This accuracy-based method usually follows a planned-out multi-step process.

Choosing the enzyme to target: The enzyme of choice is determined by its industrial or biomedical value and the availability of structural and functional data. We see a lot of variation in which sites they hit. We use both sequence and structural analysis to identify which parts are likely to mutate [88]. For sequence analysis, we perform multiple alignments of our protein with related proteins, which in turn yields conserved and variable residues. We also use structural analysis, such as crystallographic analysis, to identify active-site residues and binding pockets involved in substrate interactions [89].

Mutagenesis and characterization: Site-directed mutagenesis is applied to substitute, delete, or insert amino acids at identified positions. The resulting mutants are then expressed and evaluated to determine improvements in enzyme performance [90]. Rational design is strongly supported by computational biology, which enhances the accuracy of mutation predictions. The essential software includes molecular dynamics simulations (MDSim360), web-based modeling services, and structural databases that help model enzyme behavior accurately and assess mutational effects [91]. Recent studies have shown that rational design can enhance the activity of various enzymes. For example, two active-site mutations in β-glucosidase from *Trichoderma reesei* were introduced (L167W and P172L), and even with minimal sequence changes, the enzyme showed increased thermal and pH stability, as well as enhanced glucose sensitivity [92]. For this reason, hybrid strategies such as structure-guided consensus design have now combined both sequence- and structure-based information to enhance prediction accuracy with minimal experimental effort [93].

3.3. Semi-Rational Design

Semi-rational design has proven to be a potent method in enzyme engineering, combining the strengths of rational design and directed evolution. This hybrid methodology does not have the major limitations of both

methods: rational design requires substantial structural data, and directed evolution requires substantial labor and time in the screening process [94]. Using information from both sequence and structural analyses, semi-rational design enables targeted mutagenesis, yielding smaller yet better mutant libraries with a greater likelihood of identifying functionally improved mutations. Semi-rational design is a systematic method of enzyme engineering that combines site-directed mutagenesis (rational design) and random mutagenesis (directed evolution). This work focuses on areas of interest, particularly those near or within the active site, where mutations are most likely to increase catalytic activity, stability, or substrate specificity [95,96]. The main features and methodology of semi-rational design include:

1. Target Site Identification: Structural and sequence studies are utilized to help identify regions that are usually linked to helpful mutations. Software such as HotSpot Wizard 3.0 can be used to generate mutability maps that inform residue selection [97].
2. Library Construction: Compared to generating large random libraries, small and smart libraries are generated, which target the residues with the highest probability of interference with enzyme activity [98].
3. Computational Screening: Prediction and elimination of deleterious mutations, before laboratory experimentation, can be done with the aid of software tools like TANGO, FoldX, and SNPeffect [99].

The semi-rational design usually leads to enzymes with improved properties, such as greater stability under extreme conditions, improved stereoselectivity, and higher catalytic efficiency.

4. Engineering Plant Enzyme for Agricultural Enhancement

4.1. Sustainable Crop Production

Peptidases, or proteases, which account for approximately 60% of the global enzyme market, represent a key category in the bioindustry—particularly in the agricultural, pharmaceutical, and detergent sectors, as well as in several other industrial applications [100,101]. Proteolysis refers to the enzymatic cleavage of peptide bonds within a polypeptide chain, resulting in the degradation of proteins into smaller peptides and amino acids [102,103]. The proteases used commercially are environmentally safe because they are non-toxic and non-pathogenic [104]. The development and application of plant-based proteases depend on the availability of agricultural land and specific climatic conditions. Well-known plant-derived proteases include papain (from papaya), ficin (from fig), bromelain (from pineapple), and actinidain (from kiwi), among others [105–107]. For eons, proteases have been used in the food industry. As reported by Choi et al. [108], they have been used in many processes, including baking, cheese production, meat tenderization, and the preparation of soy hydrolysates. Presently, proteases account for a large share of industrial enzymes. Although they have a long history in food science, medicine, and detergent manufacturing, dating back to ancient times, plant-derived proteases have lagged behind their microbial counterparts, mainly due to production costs.

4.2. Nutrient Uptake and Utilization

The health of our crops and the sustainability of modern farming depend on how effectively we obtain and use nutrients. Plants have a complex enzyme-based system that they use to take up, use, and transport important nutrients such as nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), as well as many trace elements [109,110]. But in these native enzymatic pathways, it is often difficult to mobilize nutrients and associate with environmental pollution due to various kinetic bottlenecks and regulatory constraints, which require higher nutrient amounts as compensation [111]. Improve the situation by engineering plant enzymes to enhance nutrient uptake and the plants' ability to use the full range of nutrients that flow through them.

4.2.1. Nitrogen Utilization

Nitrogen deficiency has a significant negative impact on plant growth and development. The key enzymes involved in the reduction of inorganic nitrogen and primary assimilation include nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT) [112,113]. Thus, the genes encoding these key assimilation enzymes have been heavily targeted for transgenic overexpression and targeted mutagenesis for enhanced nitrogen assimilation and primary amino acid production. However, source-sink limitations, complex post-translational feedback regulation, and downstream metabolic bottlenecks mean that overexpression of enzymes at certain points does not necessarily improve plant N use efficiency (NUE) [114]. Meanwhile, the engineering of biological nitrogen fixation (EBNF) approach, which involves incorporating bacterial *nif* gene clusters into non-leguminous plants, is an exploratory synthetic biology method [115]. This is

an interesting solution, but it is still in the early stages of research and has a number of biochemical and cellular issues to address, such as the oxygen sensitivity of nitrogenase, energy supply, and multi-gene regulation.

4.2.2. Phosphorus Uptake and Mobilization

Soil fixation can also make it difficult for plants to obtain phosphorus. The engineering of key enzymes such as phosphoenolpyruvate carboxylase (PEPC), acid phosphatases, and phytases can enhance the solubilization and remobilization of phosphorus [116]. Transgenic plants that carry microbial phytase genes can indeed utilize organic phosphorus more effectively, indicating they require less inorganic phosphate fertilizer.

4.2.3. Sulfur and Micronutrients Uptake

ATP sulfurylase, adenosine 5′-phosphosulfate (APS) reductase and cysteine synthase are important regulatory enzymes of sulfur assimilation [117]. The genes that encode these enzymes can be targeted for overexpression and this contributes to the endogenous production and accumulation of S-amino acids (methionine and cystine) in plant tissues. Likewise, the overexpression of genes encoding ferric chelate reductase and nicotianamine synthase increases the plant's ability to take up Fe, translocation of Fe from the roots to the shoots, and grain loading [118]. This targeted biofortification has the potential to increase the bioavailable essential amino acids and micronutrient levels in edible plant parts, which can then be used to enhance the nutritional value of the plant for human consumption [119].

4.2.4. Associations between Carbon and Nutrients

Enzymes involved in carbon metabolism, including phosphoenolpyruvate carboxylase (PEPC), Rubisco activase, and sucrose synthases, can be manipulated to enhance the ratio of carbon to nutrient uptake. This will facilitate biomass development and enhance nutrient use efficiency [120].

4.3. Improving the Stress Tolerance of Plants

Plants throughout their life cycle face challenges that are biotic (such as insect attacks and disease) or abiotic (such as drought, salinity, high temperatures, and heavy metals). These stresses made it harder for them to grow and be productive [121]. Plants have developed well-structured, sophisticated defense mechanisms and regulatory systems to withstand and adapt to these severe conditions. Enzymes are important for metabolism, signaling, and stress responses in many of these systems [103,122]. People's ideas about how to make plants resistant to different kinds of stress have changed thanks to molecular biology, genetic engineering, and synthetic biology. Specific enzyme engineering has also played a role [123,124]. It can improve stress-resistant metabolic pathways in plants by stabilizing, regulating, or amplifying the activity, stability, or expression of key enzymes [125]. E.g., gray morel aqueous extract (GMAE) has been demonstrated to defend against oxidative stress in cells through enzymes such as glutathione reductase (GR), catalase (CAT), peroxidases (POX), and superoxide dismutase (SOD), which are enzymes associated with cellular stability [126]. The overview of genetically engineered enzyme application in plants with reference to the biotic and abiotic stress is summarized in Tables 2 and 3 below.

Table 2. Engineered plant enzymes and their roles in biotic stress tolerance.

Key Enzymes	Mechanism	Example of Engineered Plant	Pathogen Name	References
Chitinase, β-1,3-glucanase	Hydrolysis of fungal cell wall components; inhibits fungal growth	Rice, Tobacco, and Tomato plants expressing the chitinase gene showed enhanced resistance	*Rhizoctonia solani*	[127,128]
Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione reductase (GR)	Detoxify ROS generated during pathogen infection; maintain redox homeostasis	Transgenic *Arabidopsis* and Tomato with SOD/CAT overexpression showed reduced oxidative damage	*Alternaria solani*	[4,129]
Peroxidase (PO), Laccase, Polyphenol oxidase (PPO)	Promote lignin and phenolic compound deposition; reinforce cell wall barriers	Overexpression of peroxidase genes in Tobacco improved resistance	*Phytophthora nicotianae*	[130]
Glutathione S-transferase (GST), Cytochrome P450s	Detoxify pathogen-derived toxins and xenobiotics	Overexpression of GST in *Arabidopsis* improved tolerance	*Pseudomonas syringae*	[131]
Phenylalanine ammonia-lyase (PAL), Chalcone synthase (CHS), Stilbene synthase (STS)	Synthesis of phytoalexins and phenolic compounds with antimicrobial properties	Soybean and Grapevine expressing *STS* genes showed increased resistance	*Fusarium oxysporum*	[132,133]
Lipoxygenase (LOX), Allene oxide synthase (AOS), Isochorismate synthase (ICS)	Biosynthesis of jasmonic acid and salicylic acid for systemic acquired resistance	Overexpression of *AOS* gene enhanced resistance in *Arabidopsis*	*Botrytis cinerea*	[134,135]

Table 3. Engineered plant enzymes and their roles in abiotic stress tolerance.

Key Enzymes	Mechanism	Example of Engineered Plant	Abiotic Stress	References
Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione reductase (GR)	Detoxification of reactive oxygen species (ROS) produced under drought, salinity, and heat stress	Overexpression of SOD/CAT in tobacco and rice conferred tolerance	Oxidative and drought stress	[136,137]
Pyrroline-5-carboxylate synthetase (P5CS), Trehalose-6-phosphate synthase (TPS), Choline oxidase (codA)	Synthesis of compatible solutes (proline, trehalose, glycine betaine) that maintain osmotic balance	Transgenic Rice expressing *P5CS* gene showed improved	Drought and salt tolerance	[138,139]
Glutathione S-transferase (GST), Aldehyde dehydrogenase (ALDH), Cytochrome P450s	Detoxify harmful by-products and protect cellular macromolecules under abiotic stress	*Arabidopsis* and Maize plants overexpressing *GST* showed better tolerance	Oxidative and salinity stress	[131,140]
Mitogen-activated protein kinases (MAPKs), Calcium-dependent protein kinases (CDPKs), Phospholipase D (PLD)	Regulate stress-induced signal transduction and gene expression	*Arabidopsis* plants overexpressing *MAPK3* displayed enhanced tolerance	Salinity and dehydration	[141,142]
Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Glutathione peroxidase (GPX)	Maintain ascorbate-glutathione redox cycle; reduce oxidative stress	Overexpression of *GPX* in *Brassica napus* improved tolerance	Cadmium and salt stress	[143]
Heat shock proteins (HSPs), Chaperones, ATPases	Maintain protein folding and prevent denaturation during heat stress	Overexpression of *HSP70* in tomato increased tolerance	High temperature	[138,144]

4.3.1. Antioxidant Enzyme Engineering

Several by-products of normal plant metabolic processes and the effects of different environmental stresses are reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) [145]. At low levels, ROS serve as signaling molecules, which regulate several physiological activities of the body. Nevertheless, their presence in large amounts may cause oxidative stress, which leads to the destruction of proteins, nucleic acids, and cell membranes. Plants have an extensive antioxidant defense mechanism that comprises both enzymatic and non-enzymatic components to counter oxidative stress [146]. GR, ascorbate peroxidase (APX), CAT, superoxide dismutase (SOD), and peroxidase (POX) are enzymes involved in ROS detoxification and the restoration of redox balance in cells (Figure 2) [147]. However, the plant's natural defense may fail under extremely high or prolonged stress. The engineering of antioxidant enzymes involves genetic engineering, transgenic strategies, and protein engineering to enhance their functionality, stability, or expression, thereby maintaining the plant's redox defense [148]. The expression of a single antioxidant enzyme gene or multiple antioxidant enzyme genes, or the integration of genes from stress-resistant organisms, can significantly increase ROS-scavenging activity and result in greater resilience to drought, salinity, heat, cold, and pathogen invasion [149,150]. Indicatively, when the *Tamarix hispida* GST (*ThGSTZ1*) gene is overexpressed, the plant is more competent at clearing ROS, thereby increasing its resistance to drought and salt [150].

4.3.2. Enzymes of Osmolyte Biosynthesis

Osmolytes or compatible solutes are low-weight, organic substances that are dissolved and accumulate in plants when they become stressed [151]. Some of them include proline, glycine betaine, sugars, sucrose and trehalose, sugar alcohols, sorbitol and mannitol, and certain polyamines. Osmolytes are essential for stabilizing proteins and membranes, maintaining osmotic equilibrium in cells, and protecting cellular components against stress-induced damage [152]. The osmoprotectants accumulation is under the control of a specific biosynthetic enzyme regulating every one of the most important stages of the synthesis of osmoprotectants [153]. The examples include proline biosynthesis controlled by Δ 1-pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), glycine betaine biosynthesis controlled by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), and biosynthesis of sugar alcohols controlled by mannitol dehydrogenase (MTD) and sorbitol dehydrogenase. The production of trehalose, in turn, involves the activities of trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) [154]. Osmolyte biosynthetic enzymes, genetic manipulation, and metabolic engineering have proven to have great potential for enhancing stress tolerance in plants (Figure 2). Plant tolerance to drought, salinity, and thermal stress is enhanced many times over by overexpression of functional enzyme genes and stress-inducible promoters that, in turn, enhance osmolyte

accumulation [155,156]. Beyond direct enzyme modifications, engineering upstream transcriptional regulators offers a multi-target approach to stress resilience. For instance, ectopic expression of the tomato AP2/ERF transcription factor TERF1 in rice and tobacco activates complex stress-responsive regulons, leading to elevated osmolyte accumulation, enhanced reactive oxygen species (ROS) scavenging, and improved tolerance to drought and salinity [157]. By coordinating multi-gene downstream networks rather than single enzymatic steps, regulatory factors like TERF1 modulate physiological adaptation on a systemic level.

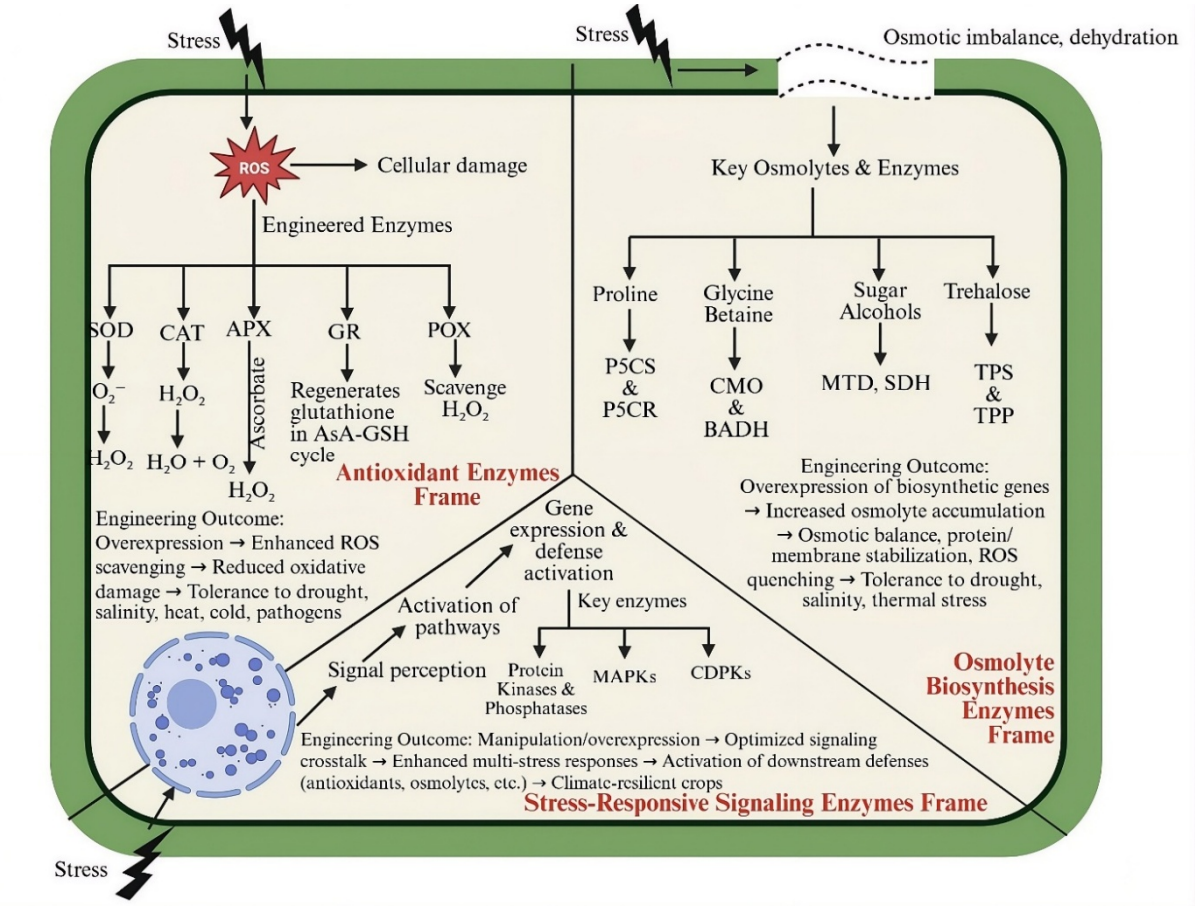

Figure 2. Displays a comprehensive model of enzyme engineering for stress adaptation in plants. Environmental stress increases the production of reactive oxygen species (ROS) and disrupts cellular homeostasis. Changing or increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and peroxidase (POX), improves ROS scavenging and maintains redox balance. Engineered osmolyte-promoting pathways through enzymes like P5CS/P5CR for proline, CMO/BADH for glycine betaine, MTD/SDH for sugar alcohols, and TPS/TPP for trehalose (osmotic adjusters) help proteins and membranes stay stable and protect them from ROS. Also, making stress-responsive signaling enzymes, such as protein kinases, phosphatases, MAPKs, and CDPKs, work more effectively makes it easier for cells to detect signals, activate pathways, and write stress defense genes. All of these things help plants grow that can handle a lot of stress and aren't affected by changes in the weather.

4.3.3. Stress-Responsive Signaling Enzymes

Plants have a complex network of signal transduction pathways that use numerous stress-responsive signaling enzymes to sense and respond to environmental stressors [158]. These enzymes are like molecular switches that help plants live and grow by sensing changes in their environment and triggering the appropriate biochemical and physiological responses. Some of the most important signaling enzymes are protein kinases, phosphatases, mitogen-activated protein kinases (MAPKs), calcium-dependent protein kinases (CDPKs), and adenylate cyclases [159]. These enzymes trigger defense responses, alter how other enzymes function, and control downstream gene expression through phosphorylation-dephosphorylation reactions (Figure 2) [69]. For example, CDPKs integrate calcium signaling with signaling pathways for hormones and ROS, such as abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA). MAPK cascades, on the other hand, send stress signals from receptors to the nucleus. There, they turn on transcription factors that respond to stress [160]. Molecular and genetic methods for

engineering signaling enzymes that respond to stress are effective ways to develop plants that can withstand stress. By altering or overexpressing specific kinases and phosphatases, it is possible to improve cross-talk between signaling pathways, enhance stress network function, and promote integrated defense responses to multiple stimuli. Stress-responsive signaling enzymes are very useful for detecting, sending, and controlling stress responses. By altering how these enzymes function, it may be possible to develop crops that can survive in changing climates.

4.4. Produce Food Enzymes

The enzyme α-amylase breaks down the α-1,4-glycosidic bonds in starch, which includes both amylose and amylopectin units [161]. It is used in many industries, such as paper, pulp, sugar, textiles, food, brewing, starch liquefaction, and alcohol. Gene cloning was used to get a thermostable α-amylase from *Bacillus licheniformis*. The transgenic tobacco plants expressing the *B. licheniformis* α-amylase gene showed a significant increase in enzyme activity, suggesting that the enzyme could be useful in industry. A chimeric construct containing this gene showed much higher expression in tobacco protoplasts, indicating significant potential for biotechnological use [162]. Cereal grains generally lack adequate levels of the essential amino acids lysine and threonine. In plants, the aspartate-family pathway is responsible for synthesizing essential amino acids, including lysine, threonine, methionine, and isoleucine, using aspartate as a common precursor [163]. Plants seem to tightly control the aspartate metabolic pathway at key points [164]. Various plant species have been identified as sources of enzymes, including aspartate kinase, homoserine dehydrogenase, dihydrodipicolinate synthase, and threonine synthase [165–168]. The papaya plant (*Carica papaya*) also produces latex, a natural defense against microbial infections. Papaya latex is a milky liquid that thins under stress but remains unchanged at rest. It has about 15% dry matter, and almost 40% of that is made up of enzymes. Cysteine endopeptidases account for 80% of all enzymes. These endopeptidases are inactive upon secretion but quickly become active [169]. Mamboya and Amri [170] state that papain, one of the main enzymes in papaya latex, is more effective when the fruit is still green. Traditionally, salt precipitation was used to purify papain, but newer methods have greatly increased its yield and activity.

4.5. Enzymes in the Processing of Food

Three sensory characteristics of tenderness, juiciness, and flavor are the most important three palatability characteristics that influence overall consumer satisfaction with meat [171,172]. Of these, tenderness is considered to be one of the most important factors for textural acceptability. The tenderness of meat is a polygenic characteristic mainly controlled by the ratio of the structural integrity of myofibrillar proteins to the quantity and degree of cross-linking of connective tissue (collagen) in relation to the overall resistance of the muscle tissue to mastication. Textural properties such as tenderness indirectly affect the rate and amount of volatiles and tastants released during chewing by different chemical processes, such as the thermal degradation of lipids and the Maillard reaction pathways.

Only five enzymes—papain, ficin, bromelain, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease—have been evaluated and classified by U.S. regulatory agencies as "Generally Recognized as Safe" (GRAS) for meat tenderization. Among natural proteases, actinidin and zingibain have also been found to be useful tenderizers [173,174]. Among these, papain has been found to possess the greatest potential for increasing the tenderness of meat. Plant-derived proteases such as bromelain and ficin possess broad-spectrum activity, effectively hydrolyzing both connective tissue (collagen) and contractile/myofibrillar proteins (e.g., myosin) to facilitate meat tenderization [174]. Furthermore, exogenous plant proteases such as papain and bromelain have been widely investigated to accelerate proteolysis and shorten ripening time in dry-cured ham processing [173,175], as well as to enhance protein hydrolysis in fermented sauces. Enzymes are ubiquitous in the food processing sector and are primarily used to break down, modify, or restructure biomaterials. Novel, cost-efficient, and environmentally friendly enzymatic processes, particularly for fat and oil modification, are becoming more prevalent in industry.

4.6. Enzymes in Brewing

Before the yeast in barley can produce alcohol, the starch must first be converted into fermentable sugars [176]. Unlike winemaking, which goes straight to work on fruit sugars, traditional beer brewing has an extra stage we call malting. During malting, enzymes are introduced that break down starch, converting complex carbohydrates into fermentable sugars. Some enzymes that serve this purpose are present in barley to begin with, like β-amylases, but the majority, we are talking mainly about α-amylases and proteases, only show up during the germination process. Also in 1940, Leo Wallerstein [177] patented the use of proteolytic enzymes for chill-proofing beer, one of the first examples of industrial enzyme application in brewing. To ensure desirable colloidal stability at low temperatures and to prevent haze formation, the brewing industry has also employed ficin as well as crude

bromelain or papain [178–180]. However, papain is now less widely used, particularly in certain European countries that continue to prefer additive-free beers [173].

4.7. Enzymes in Baking

Enzymes can be incorporated individually or as part of complex mixtures, even at very low concentrations, to enhance the quality of baked products, often acting synergistically. Enzymes from three distinct sources are used in baking [181,182].

Natural enzymes are inherently present in flour. Microbial enzymes are produced during the metabolic activity of predominant bacteria. Exogenous enzymes were intentionally added to the dough. Lipolytic enzymes are gaining increasing importance in the baking industry. Recent research shows that phospholipases can serve as an alternative to traditional chemical emulsifiers, as these enzymes hydrolyze polar wheat lipids into highly surface-active lysolipids and free fatty acids, thereby enhancing dough stability and gas retention [183]. Lipases were initially utilized to enhance bread flavor by hydrolyzing ester bonds in triglycerides to release flavor-active free fatty acids and partial glycerides. In baking, proteases are used to hydrolyze gluten, thereby improving dough handling and elasticity. Enzymes with rapid catalytic activity, optimal pH and temperature stability, and no undesirable side activities (such as pentosanase or amylase activity) are ideal for industrial applications [184]. Furthermore, gluten hydrolysates are being investigated for the development of novel high-value food products [185,186]. Bromelain specifically hydrolyzes the wheat glutenin IgE epitope Gln-Gln-Gln-Pro-Pro, making wheat flour hypoallergenic [172]. Hardening and stalling of bread is brought about by the retrogradation and crystallization of starch (which is the main structural component of bread). Enzymes such as lipase and amylase can extend the shelf life of bread and reduce food waste by slowing the rate of starch crystallization. Jegannathan and Nielsen [187] report that this approach can also be financially beneficial by reducing the costs of bread production and distribution through lower grain losses.

4.8. Enzymes as Processing Aids and Biocatalysts in Food Systems

Enzymatic properties enable the synthesis and modification of complex compounds. In addition, their stereospecificity and optical selectivity are valuable in the food industry, where distinct optical configurations correspond to different flavor profiles. Enzymes function primarily as biological catalysts and processing aids to develop desirable flavors or eliminate undesirable off-flavors originating from natural or synthetic sources [188]. For instance, modern processing methods utilize enzymes during the concentration of fruit juices to maintain high sensory quality and aroma stability throughout extended storage [189–191]. Furthermore, enzymes help preserve flavor and color integrity during processing, which explains why purified enzyme preparations are widely adopted as processing aids across various food manufacturing sectors.

5. Future Prospects

In the coming years, we see a convergence of synthetic biology, artificial intelligence, and enzyme engineering, which will expand the field of novel enzyme design, enabling enzymes that perform better in stability, catalytic efficiency, and substrate specificity. Also, we will see the development of what we may term smart metabolic circuits in plants, which will establish self-regulating systems that respond dynamically to environmental stimuli. Also, we will see the integration of enzyme engineering with nanotechnology and bioinformatics, which will, in turn, improve enzyme distribution, monitoring, and functional optimization within plant systems. To that end, it is put forth that the research we do going forward should prioritize:

1. Engineering multi-functional enzymes that can control multiple physiological processes simultaneously.
2. Gene stacking and pathway engineering for cumulative gains in productivity and stress tolerance through metabolic engineering.
3. Biosafety, ecological balance, and social acceptance of genetically engineered crops through open risk assessment and communication.
4. Improving collaborative platforms between molecular biologists, agronomists, and policymakers for fast-tracking translational and field applications.

In conclusion, engineering plant enzymes is a highly effective strategy that can be used to achieve sustainable and climate-resilient agriculture. Future interdisciplinary research, facilitated by responsible innovation and governance, will be critical to ensure that these molecular gains are converted into real-world outcomes for global food security and environmental sustainability.

6. Conclusions

The use of engineered plant enzymes is a ground-breaking strategy to address the growing demands for climate resilience, environmental sustainability, and agricultural output. Enzymes are extremely important in controlling plant growth, energy use, and stress protection. Recent developments in synthetic biology, protein engineering, and molecular biology have enabled the modification of enzymes to improve yield potential, enhance photosynthesis efficiency, help plants absorb more nutrients, and increase stress resilience. By making minor adjustments to enzyme function, crops can grow under unfavorable weather conditions and require fewer chemical fertilizers and pesticides. The combination of enzyme engineering and omics technologies, e.g., transcriptomics, proteomics, metabolomics, and genomics, offers much data on plant metabolic networks and regulatory systems. The system's perception as a complete unit helps reveal the major enzymatic bottlenecks and offers innovative methods for implementing specific adjustments. CRISPR/Cas genome editing technologies have gone even further to revolutionize the domain of enzyme modification because they enable scientists to insert or add modifications at a designated locus without interfering with the genetic integrity of plants.

Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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