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Transgenic Plants in Biopharmaceutical Production

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Abstract
At present there has been a considerable increase in the application of plants biotechnology for production of variety of commercially valuable products for human health care use. Earlier the plants cell or their extract are used for the treatment of human disease and utilized to produced biopharmaceutical protein and peptides as they are simply transformed and provide a cheap source of protein. Many biotechnology companies are engaged in actively developing, field testing and patenting plants expression. So, plant-based production system can be used as an alternative of existing system it is advantageous to use but gained lower social acceptance. Several strategies are involved for yield. Biopharmaceutical also includes a recombinant vaccine, monoclonal antibodies, therapeutic and nutraceutical proteins. The two major challenges are low yield and non - mammalian glycosylation that oppose against full utilisation of plants as alternative Bioreactor to mammalian cell culture. In this review attempt has been made to focus on plants-based production system for recombinants protein production, strategies involved in plants transformation and its potential to produce biopharmaceutical.

Keywords: Biopharmaceutical Protein, Plant Based Vaccines, Antibodies, Nutraceutical

1. Introduction
Recombinant proteins are complex exogenous (‘foreign’) proteins created in expression hosts, and are mostly employed in medical diagnostics and human healthcare as vaccinations, medicines, or monoclonal antibodies [1]. Microbial fermentation and mammalian cell lines have typically been used in commercial protein production, however these methods have drawbacks in terms of cost, scalability, and safety, prompting research into alternatives. Plants have emerged as one of the most potential universal manufacturing platforms for tomorrow's biologics, notwithstanding industry stagnation and conservatism. Plants enabled for the cost effective manufacturing of recombinant proteins on a large scale while removing the possibility of endotoxins or human pathogens contaminating the result Another benefit of using plants to make recombinant proteins is that vaccination candidates can be expressed in edible plant parts, allowing them to be given as unprocessed or partially processed material [2]. Furthermore, stringent regulatory procedures are in place for industrially established mammalian and other cell cultures, which delays industrial acceptance of the new technology or production system. Bacterial expression techniques provide quick synthesis with high product yield, whereas *Saccharomyces cerevisiae* and *Pichia pastoris* (yeast) provide post-translational modifications (PTMs) that are required for the recombinant protease’s functional activity [3].
Production time, High operational expenses, protein yield, possibilities of contamination with pathogenic microbes, limited post translational modification and regulatory approvals are disadvantage of each of production technique. To compete with the existing platform, the new expression system must offer distinct benefits that surpass the constraints of the present ones. Plants have significant advantages over traditional expression platforms, and they demonstrate the system's reliability in producing extremely useful proteins. Plant molecular farming techniques have advanced in the last decade, making plants an appealing manufacturing system that can achieve commercially relevant output levels in a short amount of time [1]. The review also discusses about emerging production platform, several strategies involved in transformation of plants, overview of plants derived recombinant proteins.

2. Emerging production of biopharmaceuticals platforms
2.1 Choosing a species to serve as a host
Since the late 1980s, plants have been used to express recombinant proteins [4]. Since then, the plant expression platform has encountered numerous challenges, with Protalix Biotherapeutics’ first plant-based medicine “Elelyso” being marketed for the treatment of Gaucher’s disease in 2012 [5]. The practise of using plants to produce high-value recombinant proteins such as

antibodies, vaccine antigens, enzymes, growth factors, research or diagnostic reagents, and cosmetic ingredients, ranging from pharmaceutical therapeutics to non-pharmaceutical products [6]. The technology has advanced quickly, and the anticipated limitations of plant molecular farming in its early such as the necessity for high protein expression levels and effective downstream processing, have now been overcome [7].

The expression system in plants are rapid and affordable, optimised growth conditions, free from pathogen and bacterial toxin contaminants, economical and has a post translational modification somewhat similar like mammalian system, only disadvantage being regulatory compliance and limited glycosylation capacity.

Many of the first plant-derived recombinant proteins were made in transgenic tobacco plants and retrieved straight from the leaves. Tobacco's continued appeal stems from the fact that it is a well-established expression host with reliable transformation processes and well-characterized regulatory components for transgenic expression control [2].

Tobacco plant is also well suited for commercial genetic farming due to its large biomass yields and quick scalability. It's also a non-food, non-feed crop, which means it's less likely to contaminate feed and human food chains with transgenic material or recombinant proteins [8]. Tobacco's high amount of nicotine and other harmful alkaloids, which must be entirely eliminated during downstream processing steps, is one disadvantage. Despite the availability of low-alkaloid tobacco varieties, interest has shifted to other leafy crops for pharmaceutical manufacture [9].

Leafy crops including lettuce, alfalfa, and clover have been studied for molecular farming in order to provide oral administration of vaccination antigens without the need for purification or injections. Lettuce is one of these crops, and it has been utilised in clinical studies with a hepatitis B virus subunit vaccine [10].

The benefits of alfalfa include its high biomass yield, the fact that it is a nitrogen-fixing perennial plant. And the glycoproteins synthesised in alfalfa leaves tend to have uniform glycan structures. However, alfalfa is a feed crop, and its leaves contain a lot of oxalic acid, which could make processing difficult. Proteins produced in grain seeds, on the other hand, are shielded from proteolytic destruction and can last for years [11].

Fruit and vegetable transformation procedures for recombinant protein production, such as tomatoes and potatoes, have also been devised [12]. Several cereals have been explored as prospective hosts for recombinant protein production, including rice, wheat, barley, and maize [8]. Maize has a high biomass yield due to its ease of transformation and manipulation *in vitro*, as well as the ease with which transgenic maize production may be scaled up. Some of the plant's expression hosts used for biopharmaceutical production using recombinant pharmaceutical and non-pharmaceutical proteins are Tobacco, alfa alfa, clover, maize, rice, wheat, soybean, barley, potato, carrot, tomato having their advantages and disadvantages.

2. Strategies used for Recombinant Production in Plants

2.1 Stable Nuclear Transformation

The incorporation of a foreign gene or genes of interest into the nuclear genome of the plant, thereby altering its genetic makeup, and leading to the expression of the transgene after integration with the host genome, conferring stably inheritable traits that were not present in the untransformed host plant, is known as stable nuclear transformation. The approach has been used to accumulate protein in cereal seed dry seeds, allowing for long-term storage at room temperature without protein breakdown [13]. Furthermore, because cereal crops are grown almost everywhere, the system has a great scale-up capacity. However, popular acceptability of this technology is hampered by some crops' extended production cycles and the risk of cross-pollination with native species or food crops.

2.2 Stable Plasmid Transformation

Plastid transformation is a valuable alternative to nuclear transformation because it offers a number of benefits that nuclear transformation does not, such as natural biocontainment of transgene flow through out-crossing because plastids are inherited through maternal tissues in most species and pollen does not contain chloroplasts, implying that the transgene may not be transferable, easing public concerns [14]. After numerous generations of plant regeneration from blasted leaf explants, transgenic plants with homoplastomic chloroplast transformation (in which every chloroplast carries the transgene) are selected on selection media containing spectinomycin [15] or in conjunction with streptomycin. They have previously accumulated up to 3–6% of total soluble proteins in tobacco chloroplasts from a bacterial and a human therapeutic protein. Despite its immense potential, plastid transformation is presently limited in its applications, as it has only been accomplished in a few plant species, including tomato, eggplant, lettuce, and soybeans [16]. Only tobacco, which is inedible and heavily regulated due to its high content of harmful alkaloids, allows for routine chloroplast transformation. Protein stability is also expected to alter over time, even when kept refrigerated [13].

2.3 Plants Cell Suspension Cultures

Plant cell suspension culture is a plant-based biopharmaceutical production platform that can be used instead of mammalian cells. The technology ensures sterile *in vitro* settings as well as high levels of confinement [17] and downstream processing and purification that is less expensive and easier. Furthermore, because of the homogeneity in cell size and type, using suspension cultured cells as an expression system could reduce protein and N-glycan heterogeneity [18]. Dow Agroscience's purified injectable Newcastle disease virus (NDV) vaccine for chickens, which has been approved by the USDA Center for Veterinary Biologics, and Protalix's recombinant human glucocerebrosidase for the treatment of Gaucher's disease are two examples of plant suspension culture-produced biopharmaceuticals. Suspension culture is still not the best production platform the plant system has to offer, as the overall yield and usability are somewhat limited by the diminishing level of recombinant protein during the late stationary phase due to increased proteolytic activity [19] and the approach is presently restricted to a small number of well-studied plant cell lines such as tobacco, rice, carrot, or *Arabidopsis* [20].

2.4 Transient Expression System

For plant molecular farming, the transitory production platform is arguably the quickest and most practical option

[21]. The following approaches, which were originally developed for fast validation of expression constructs, are now commonly employed for the manufacture of large amounts of proteins in a matter of weeks [22].

2.5 Agroinfiltration Method

The agroinfiltration method, which involves infiltrating a suspension of recombinant *Agrobacterium tumefaciens* into tobacco leaf tissue, which facilitates the transfer of T-DNA to a very high percentage of cells, where it expresses the transgene at very high levels without stable transformation, as in transgenic crops. This approach has now been refined into a high-yielding transient expression system for clinical-grade biopharmaceutical production [22].

2.6 Virus Infection Method

The ability of plant viruses such as tobacco mosaic virus (TMV) and potato virus X (PVX) to be utilised as vectors to carry foreign genes into plants without integration is crucial to the virus infection strategy [23]. Both platforms infect tobacco plants before transiently expressing a target protein in plant tissue. Meanwhile, using this expression method, they were able to obtain a protein yield of up to 17% of the total protein. The recombinant protein, like other fresh plant-based manufacturing methods, must be handled right away to avoid tissue deterioration and, of course, protein instability. This is the one small disadvantage of the system. Nonetheless, the technology has been used by a pharmaceutical business, Large Scale Biology Corporation, for the manufacturing of idiotype vaccines for the treatment of B-cell non-lymphoma, Hodgkin's which have completed phase I clinical testing [24].

2.7 The Magnifection Technology

The ability of both the viral vector-based expression system and the Agrobacterium-mediated technique to generate high-level co-expression of two or more polypeptides required for the assembly of heterooligomeric proteins is limited [25]. As a result, Icon Genetics developed MagnICON®technology, a new robust transient expression system that involves removing the coat protein (responsible for systemic movement) of noncompeting virus strains and systemic delivery of the resulting viral vectors to the entire plant using Agrobacterium as the delivery vehicle and primary infection [26].This technique boosted not just infectivity but also amplification, resulting in high-level co-expression of many polypeptides that were able to form functional hetero-oligomeric proteins at 100-fold higher levels, including a full-sized IgG [25] and *Yersinia pestis* antigens fusion protein F1–V [27]. Various platforms, such as Icon Genetics, Kentucky BioProcessing, Fraunhofer, and a host of others, are currently developing advanced viral vectors to facilitate ease of manipulation, a wider host range, speed, safety, low cost, and high quality and yield of pharmaceutical proteins required for clinical development.

3. Plant Production System Limitations and Optimization

Despite the tremendous advantages of plant production systems over existing systems [28], there are significant drawbacks that cause them to have a lesser level of social acceptance. Low yield and non-mammalian glycosylation are two main obstacles to fully utilising plants as alternative bioreactors to mammalian cell culture. The difficulty of selecting the best host plant for any particular biomolecule, as well as downstream processing, is being taken seriously.

3.1 Low Yield is a Concern

3.1.1 Transcript Expression Optimization

The use of strong and constitutive promoters, such as the cauliflower mosaic virus 35S RNA promoter (CaMV 35S) and maize ubiquitin-1 promoter (ubi-1), for dicots and monocots, respectively, is the general method for optimising transcript expression [29]. Transgenes (vaccine antigen HBsAg M, murine single chain variable fragment (scFv) G4 and human interferon) are also driven by organ- and tissue-specific promoters in tissues and organs such as the tuber, seed, and fruit [30]. This tissue-specific expression avoids the recombinant protein from accumulating in the vegetative organs, which could impede plant growth and development. Inducible promoters, whose activity are controlled by a chemical or an external stimulus, can also be utilised to avoid the lethality problem [31]. Furthermore, transcription factors can be utilised as promoter boosters to improve transgenic expression levels even more [32]. Furthermore, it was recently shown that the terminator of *Arabidopsis thaliana's* heat-shock gene causes a fourfold increase in transcription of a foreign gene.

Furthermore, by directing the transgene into the plastids, the problem of position impact can be eliminated. The use of certain genetic components, like as the cAMP response elements (CREs), for cotransfer with the transgene in the T-DNA, has been employed to optimise the creation of single-copy transgenics [33]. Furthermore, a novel technology involving the creation of genetically autonomous artificial minichromosomes has been touted as having endless potential and several significant advantages, including gene stability due to the lack of gene silencing and location impact [34].

3.1.2 Increasing the Stability of Protein

For improving recombinant protein stability subcellular targeting is required. Subcellular targeting can improve protein stability and also define the type of associated protein processing. It can be used to optimise downstream isolation and purification processing by adding fusions and affinity tags [29]. An N-terminal signal peptide that is cleaved for protein release into the endoplasmic reticulum can be used to direct proteins to the secretory pathway (ER). When fused with a stabilising partner, full-size immunoglobulins that target the secretory pathway have been found to accumulate at higher levels and be easily recovered [35]. So far, the majority of plant-derived recombinant proteins have been released into the apoplastic area of the cell wall [36], where they have been found to accumulate at 100-fold higher quantities than in the cytosol. Recent research suggests, however, that the tomato cathepsin D inhibitor (SlCDI) could be employed as an in-built protein-stabilizing agent in plants to safeguard cytosol-targeted recombinant proteins [37]. Targeting and maintaining recombinant protein in different subcellular compartments of the secretory pathways, such as the ER, could improve the stability and accumulation of recombinant protein [38].

3.2 The Glycosylation Challenge

Glycosylation is the covalent bonding of sugar moieties to proteins to improve folding, biological activity, solubility, and bioavailability [18]. Glycosylation takes place in the

secretory pathway in the ER and the Golgi apparatus in plants. In terms of N-glycan composition, however, there are variances in glycosylation patterns between plants and mammals. Plants add (1, 3) fucose and (1, 2) xylose residues to their glycoproteins' N-glycan, whereas mammals add (1, 6) fucose moieties, glucose, and sialic acid residues. As a result, when plant-derived therapeutic animal proteins are supplied to humans, to avoid the problem of immunogenicity and allergic reactions that these variations could potentially produce [41], engineering the plant to execute authentic human N-glycosylation is required. To change the Nglycosylation pattern in plants, a variety of techniques have been employed. The fusion of the ER-retention signal KDEL, for example, limited glycosylation of plant-derived antibodies to only high mannose-type N-glycan [42], despite the fact that such antibodies with high mannose N-glycan had previously been shown to be unstable following injection into mice [39]. Also, the knock-in strategy, which involves the expression of a hybrid enzyme created by fusing the CST domain of human-1,4-galactosyltransferase I with the CST domain of Arabidopsis-1,2-xylosyltransferase, was shown to cause high-level galactosylation of Nglycans and a sharp decrease in the level of N-glycans with xylose and fucose moieties [37]. Furthermore, plant-expressed protein sialylation and sialic acid production have been described [43]. As a result, it is obvious that glycosylation engineering can be used to alter plant-made antibodies [40] thereby broadening the possibilities for producing ideally glycosylated proteins with enhanced biological activity for application as human therapies.

3.3 Plants that are Appropriate as Hosts

The selection of an appropriate host for deploying molecular farming technology is critical to the technology's overall success. In reality, despite its subtlety, this is the first line of defence for ensuring recombinant protein production efficiency. As a result, the most important factor to consider when selecting ideal host plants for molecular farming is cost. Total biomass yield, storage properties, ease of transportation, value of the recombinant protein itself, set-up costs, scale-up and maintenance costs, costs of containment, labour availability, land area required, length of production cycle, downstream processing costs, and edibility are all important economic factors [44]. In addition to economic considerations, the ideal host should be adaptable to transformation and regeneration. As a result, the best host plant for a specific recombinant protein must be established empirically based on these emphasised characteristics, as no single optimal system has yet been discovered [44].

Seed-based expression appears to be more ideal in this regard, in that it not only does not interfere with the plant's growth and development, but it also does not require immediate freezing, drying, or processing, as the accumulation of protein in the seed provides protection against proteolytic digestion, allowing long-term storage at room temperature without the protein losing its activity. Seed-based expression platforms are also the most cost-effective in applications that demand a significant amount of recombinant proteins per year [44]. Furthermore, the seed-based approach enables the oral administration of vaccine antigens or pharmaceutical proteins for vaccination, immunotherapy, and disease treatment. Cereal seeds, particularly rice and maize, are gaining popularity in this area. Maize offers a number of advantages, including the largest biomass production of all food crops, ease of transformation, and scale-up [45]. Rice is a self-pollinating plant, so it has a lesser danger of unintended gene flow, which is why Ventria Bioscience has pioneered the manufacture of two rice-derived proteins, human lactoferrin and lysozyme, which have acquired regulatory approvals and are already on the market. Soybean has been studied for its ability to produce a humanised antibody against the herpes simplex virus, as well as bovine casein and human growth hormone [46]. Soybean was also used to produce a functional hypotensive peptide, which helped model mice lower their systolic blood pressure [47].

3.4 Cost of Downstream Processing

The cost of downstream processing of plant-derived recombinant proteins is estimated to be over 80% of total production expenses [48]. As a result, it has received the attention it deserves in terms of developing measures to decrease it to the minimal minimum. However, using liquid tissues like tomatoes as production systems has the potential to minimise this cost because they are easier to extract from than dry tissue like cereals [44]. Furthermore, because tomatoes are produced in greenhouses, they appear promising as a host crop for biosafety concerns about transgenic plant containment. Using SemBioSys Genetics' oleosin fusion technology, the oil bodies in certain oilseed crops such as safflower and rape seeds are currently being used to simplify recombinant protein purification while lowering downstream costs. For example, in the case of accumulating and purifying biologically active human insulin, Apolipoprotein AI (Milano), and human growth hormone, the strategy involves targeting the recombinant protein to the seed of oilseed crops as a fusion with oleosin, thus simplifying the extraction of the fusion proteins from the oil bodies and the release of the recombinant protein from its fusion partner [49].

The advantage of lower manufacturing costs is thought to make it easier for less developed countries to participate in pharmaceutical production in plants. There are various plant-derived recombinant proteins that were formerly supposed to be supplied as edible vaccines that may be eaten directly as fruits (tomatoes, banana) and vegetables (lettuce, carrot), requiring no processing and hence incurring no expenditures. Banana, in particular, was touted as a viable host fruit crop for making edible vaccine, particularly for poor nations, because it is widely grown in these countries and does not require long-distance transit or meeting chilling requirements [50]. Bananas provide additional benefits like as good digestibility and palatability, and as a result, they were previously accepted for newborn vaccination. Potatoes' aptitude for oral vaccine manufacture has also piqued interest, owing to their ability to be consumed raw or with minimal preparation. Because of the specific molecular environment in the tuber, potatoes, like seeds, have the advantage of product stability. However, in order for the crop to be suitable for oral vaccine administration, it needs undergo some processing, such as cooking, in order to eliminate endotoxins and degrade the thermolabile protein products [51]. However, due to ethical concerns, the research focus has shifted to the creation of heat stable oral vaccinations. Is giving way to the manufacture of plant-derived vaccines, which would still be processed, formulated in a repeatable manner, and

administered under monitoring to assure repeatable results [36].

Alternative plant-based expression platforms, such as *Physcomitrella patens*, a moss, and green algae, such as *Chlamydomonas reinhardtii*, are now being sought after as a result of the desire for high containment of transgenic plants these days. Both of these expression methods have the inherent benefit of being able to be grown in bioreactors under controlled circumstances.

4. The Production of Pharmaceuticals in Plants

A number of comprehensive review articles on production systems for molecular farming in plants have recently been published [3, 11, 52, 53]. In 1982, recombinant human insulin from bacteria became the first commercially produced biopharmaceutical, roughly coinciding with the first development of a genetically modified plant in 1984 [54]. Plant expression of human growth hormone fusion protein, interferon, monoclonal antibodies, and serum albumin was quickly followed by a demonstration of the potential of plants for pharmaceutical manufacture. Numerous examples of pharmaceutical manufacturing in plants have occurred since that time, and they are detailed below in three broad therapeutic categories: Antibodies, vaccinations, and other medicines are some of the most common examples.

4.1 Antibodies

Monoclonal antibodies (mAbs) have played an important role in the development of biotechnology as well as as medicinal and diagnostic goods. Mice are used to make traditional therapeutic monoclonal antibodies. The human immune system quickly recognised these proteins as alien, limiting the therapeutic efficacy of these antibodies, especially with repeated administration [55]. Recombinant technologies, on the other hand, have enabled the manufacture of entirely human antibodies by replacing murine antibodies with partially humanised or chimeric antibodies. The latter can be made using yeast or other gene expression array technologies, or it can be created from mice with human immunoglobulin genes [52, 55]. Recombinant technology can also be utilised to 'evolve' an antibody gene in order to develop better affinity binding antibodies [52]. As a result, contemporary recombinant antibodies have lower immunogenicity and higher biological activity than older monoclonal antibodies [55]. The first fully human therapeutic monoclonal antibody (Humira, Adalimumab, Abbott Laboratories) was marketed, and one would expect a low rate of neutralising antibody formation. There are already over a dozen FDA-approved mAbs, with up to 700 therapeutic Abs in development [52]. Plants now have the potential to be a virtually limitless source of mAbs, dubbed 'plantibodies' by some. Antibody expression techniques have long been based on tobacco plants. Potatoes, soybeans, alfalfa, rice, and corn are among the other plants that have been employed. Full-size antibodies, Fab fragments, single-chain antibody fragments, bi-specific scFv fragments, membrane anchored scFv, and chimeric antibodies are all examples of antibody forms. Plant cells may express recombinant secretory IgA, unlike mammalian cell expression systems (sIgA). sIgA is a complex multi-subunit antibody that has been effectively expressed in the tobacco plant and may be beneficial in topical immunotherapy. Humanized antibodies against herpes simplex virus-2 can be produced by transgenic soybeans. GE maize is said to be capable of manufacturing human antibodies at yields of up to a kilogramme per acre, and has been shown to keep antibody function for up to five years in normal storage settings [52]. Antibodies produced from plants can attach to pathogenic organisms, serum or bodily fluid effector proteins (e.g. interleukins), tumour antigens to deliver imaging or anti-tumour medicines, or a cellular receptor site to up- or downregulate receptor function. Plant glycosylation patterns, on the other hand, differ from those found in mammalian systems, and glycosylation is required for antibody-mediated complement activation or the beginning of cellular immune responses [53, 55]. Plantibodies can transport plant glycoproteins or be nonglycosylated as a result of genetically eliminating glycosylation sites, but they can't induce any of these occurrences. This does not appear to be a significant constraint, however, because therapeutic applications of monoclonal antibodies are frequently mediated by protein or receptor molecule binding and inactivation, rather than complement or cell-mediated immunity. Tobacco has been used to produce a chimeric secretory IgG/IgA antibody that is efficient against a surface antigen of Streptococcus mutans and has been shown to be useful against dental caries [56]. Soybeans can produce a humanised antiherpes simplex virus (HSV) that has been shown to suppress the spread of HSV-2 in animals. Antibodies against carcinoembryonic antigen can be produced using rice and wheat expression systems, which could be useful for *in vivo* tumour imaging. Finally, a viral vector for plants has been discovered. Tobacco has been used to make a transiently expressed tumor specific vaccine for the treatment of Lymphoma [58].

Although at least one product has been studied clinically, and numerous have been examined *in vitro* and in animal systems and appear to be equivalent to mammalian-cell-derived counterparts, no 'plantibodies' have yet reached the commercialization stage [59]. Given the high levels of synthesis, low immunogenicity, and apparent usefulness of recombinant human antibodies generated from plants, plants appear to have a lot of potential for future monoclonal antibody manufacture.

4.2 Vaccines

The development of low-cost, edible (i.e., oral) vaccinations has generated a lot of attention [60]. To produce both systemic (Ig-G-mediated) and local membrane (Ig-A-mediated) immunity, traditional edible vaccines, such as those for polio attenuated organisms or semi-purified materials entirenprotein should be used. Plant vaccines can express whole proteins, but DNA encoding only desired antigenic sequences from harmful viruses, bacteria, and parasites has gotten a lot of attention [61]. Plant tissues can be used to produce key immunogenic proteins or antigenic sequences, which can then be consumed as edible subunit vaccines [61]. The mucosal immune system can help induce tolerance to eaten or inhaled antigens, as well as guard against diseases and poisons. Mucosal regions can produce secretory Ig-A (sIg-A) and stimulation of certain immune cells, hence these areas are particularly important in the creation of edible vaccines.

Plant-based vaccines provide a variety of advantages over animal-based vaccinations, including greater safety, stability, adaptability, and efficacy [62]. Plant-based vaccinations can be cultivated locally, saving money on storage and delivery. Relevant antigens are naturally kept in

plant tissue, and oral vaccinations can be given directly in the food product in which they are cultivated, without the need for purification [61, 62]. In many cases, refrigeration does not appear to be required to preserve vaccine efficacy, removing a major hurdle to prior international vaccination efforts [61]. Plants modified to express only a subset of the pathogen's antigenic components may lessen immunotoxicity and other side effects, and plant-derived vaccines are devoid of mammalian viral infection. Finally, multi-component vaccines can be created by inserting numerous genetic elements or cross-breeding transgenic lines that express antigens from a variety of pathogenic species.

However, there are certain drawbacks to using transgenic plants to make vaccines. Given the diversity of protein expression among and within plant species, acquiring a protein concentration sufficient to impart complete immunity is a fundamental restriction of recombinant antigen expression in transgenic plants. To eliminate variability and provide constant, effective vaccination, tight control of expression yields will very certainly be required.

Transgenic potatoes can produce antigens of the enterotoxigenic E. coli heat labile enterotoxin B subunit, which can be used to immunise against diarrhoea-causing viruses and bacteria. Other 'edible vaccinations' are in the works for rabies, veterinary foot and mouth disease, cholera, and autoimmune diabetes. Hepatitis B surface antigen can be expressed in transgenic lupin and lettuce plants. The tobacco plant is being used to generate an "edible vaccination" against the measles virus. A plant-based oral subunit vaccine for the respiratory syncytial virus is being developed using either the apple or the tomato [89]. The plant species used in the manufacture and distribution of an oral vaccination might be chosen to meet certain objectives. Many food plants have been transformed, including alfalfa, apple, asparagus, banana, barley, cabbage, canola, cantaloupe, carrots, cauliflower, cranberry, cucumber, eggplant, flax, grape, kiwi, lettuce, lupins, maize, melon, papaya, pea, peanut, pepper, plum, potato, raspberry, rice, service berry, soybean, squash, strawberry, sugar beet, sugarcane, sunflower [62]. Corn, soybeans, rice, and wheat are among the high-volume, high-acreage plants that may provide benefits. Corn is a suitable choice for vaccine manufacture because it is a large component of the domestic animal's diet. The banana could be the plant of choice for producing vaccines in humans, particularly newborns. Bananas are a popular component of many newborn diets, and they can be eaten raw, avoiding the risk of protein denaturation caused by high temperatures. Unfortunately, producing transgenic bananas is more complex than producing other food crops, and the process takes longer. Because of the lower quantities of hazardous compounds in cereals and other edible plants, they are better for vaccine development that plant species like tobacco. There are various chances to identify and create low-cost plant-derived vaccine components, including edible plant-based vaccines, it is clear.

4.3 Other Therapeutic Agents

Hormones (somatotropin), enzymes, interleukins, interferons (IFN), and human serum albumin are just a few of the various medicinal compounds obtained from plants [2]. Biotherapeutic compounds with similar properties have been produced in mammalian and bacterial cell systems. There is a global demand for HAS, and plant production would provide the benefit of being devoid of human harmful viruses. Human alpha-1-antitrypsin, a protein with therapeutic potential in emphysema and hepatic disorders, can be produced by genetically modified rice plants. Hirudin, a blood anticoagulant initially identified from leeches, may now be produced from oilseed rape, tobacco, and mustard. At least two subtypes of human INF can be encoded by transgenic potato plants, with some of them having the potential to reduce the severity of certain malignancies and disorders. Transgenic tobacco plants can also express erythropoietin. Nearly 20 years ago, erythropoietin, a glycoprotein used to treat anemias, was commercialised from mammalian systems. Human haemoglobin has long been pursued as a blood substitute, and human haemoglobin generated from transgenic tobacco is currently being studied to ensure the molecule's function and oxygen-carrying capacity [62]. Pharmaceutical proteins generated by transgenic plants have typically been modest, averaging less than 1% of total soluble protein. While this is sufficient for the cost-effective production of highly active pharmaceutical molecules, improved technologies for high-level protein expression will almost certainly be required for the practical production of high-volume human replacement proteins like haemoglobin, or blood coagulation factors.

5. Conclusions

Plants have a number of benefits over conventional production systems for large-scale expression of recombinant proteins, but there are still a number of obstacles to overcome in terms of yield and product quality. The many PMF methods, including as nuclear, chloroplast expression, viral transfection, and transient expression systems, each have their own set of characteristics that enable them to address a wide range of product "targets" with fewer production limitations in a short amount of time. In recent years, several scientific and technical obstacles related to the plant platform have been overcome. The regulatory load associated with therapeutic protein production, on the other hand, is a substantial impediment to widespread adoption of the plant system. Because of the reduced costs and greater scalability of plant manufacturing systems, non-pharmaceutical protein commercialisation is simple and faster due to fewer regulatory hurdles.

The benefits of recombinant plant DNA technology in the manufacturing of antibodies, vaccines, various medicines, and even high-volume plasma proteins are becoming clearer. Plant-derived pharmaceuticals are highly likely to play a large part in the future of clinical therapies, given the technologies involved. As a result, the legal framework and constraints imposed on plant-derived products around the world will have a significant impact on the technology's global acceptability. The desire for industrially or pharmaceutically valuable recombinant proteins, together with the plant system's established manufacturing capability and cost feasibility, suggests that plant-made biologics have a bright future.

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