Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN

0976-9595

Review Article

Plant Molecular Farming: An Emerging Opportunity For Biopharmaceuticals

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Received: 08-05-2024; Accepted: 28-05-2024; Published: 29-06-2024

https://doi.org/10.55218/JASR.2024150601

ABSTRACT

Conventional drug production methods are costly. Now, it has been proved that plants are potentially a new source of pharmaceutical proteins, including vaccines, antibodies, blood substitutes, and other therapeutic entities. Unlike mammalian-derived rDNA drugs, plant-derived antibodies, vaccines, and other proteins are particularly advantageous since they are free of mammalian viral vectors and human pathogens. Plants-made therapeutics are cheaper, safer, and can be abundantly produced and easily stored. Recombinant proteins and other metabolites are produced in transgenic plants for industrial or pharmaceutical purposes, which is known as molecular farming. Transgenic plants carry one or more foreign genes transferred through the techniques of transformation. Although initially, these were produced only in a limited number of plant species (e.g., tobacco, petunia, tomato, etc.), later, these could be produced in any plant species, including both dicots and monocots. Transgenic plants resistant to herbicides, insects, viruses, and a host of abiotic stresses have already been produced. These plants have also been produced for improved nutritional quality and are suitable for food processing. The aim of this review paper is to understand plant molecular farming, the advantages and limitations of the process, and biosafety concerns.

Keywords: Transgenic plants, Carbohydrate, Starch, Protein, Lipids, Transformation.

INTRODUCTION

Transgenic plants have been considered a better production system for producing metabolites of pharmaceutical interest. These plants are aimed at producing novel biochemicals like interferon, insulin, immunoglobulin, etc., or useful biopolymers like polyhydroxybutyrate, which normal plants do not produce. These compounds are extracted from transgenic plants and can be used as pharmaceutical or industrial substrates.[1, 2] Many biotechnological companies are developing transgenic plants to be used as bioreactors for the production of biopharmaceutical products.[2, 3] Like edible vaccines, the biopharmaceutical products obtained from transgenic plants are distributed, which can be stored as seeds, tubers, and fruits and either directly used for oral ingestion or for the extraction of the biopharmaceutical. This helps developing countries make immunization programs cheaper and easier since the delivery by direct ingestion of modified plant products eliminates the need for product purification, which is an expensive process in the pharmaceutical industry. These genetically modified plants provide excellent production systems to produce these chemicals cost-effectively on an industrial scale. Other advantages of using transgenic plants as production systems for the production of biopharmaceuticals include (i) comparatively higher yields at a relatively low cost, (ii) reduced health risk due to pathogen contamination, (iii) production in seeds or other storage organs, thus obviating the need for purification; and (iv) little capital investment since the infrastructure for cultivation, harvesting, storage, and processing of transgenic crops would already exist.[3] For the first time in the year 1997, a biopharmaceutical called 'hirudin', (because it was originally isolated from the leech *Hirudo medicinalis*), was produced, which was also commercially produced from a transgenic crop in Canada. In forensic medicine, it has already been used for identification of individuals who could be criminals (murderers or rapists).[3] A variety of crop species have been utilized for molecular farming. However, according to the literature, tobacco and corn responded best to this technique (Table 1). Apart from these two species, alfalfa, barley, canola, rice and sunflower are also used for the experimental purpose.[4, 5]

Transformation Techniques

The uptake of foreign genes by plant cells is called transformation. The techniques used to introduce transgenes into plant cells can be grouped into the following two broad categories:[1] *Agrobacterium*-mediated and.[2] direct gene transfers. The plant cells used for transformation depend on the objectives of the study. These two techniques are fundamentally different in mechanism and are,

Table 1: Some examples of plants used in molecular farming

Category	*Plants used*
Model plants	*Arabidopsis thaliana*
Leafy crops	Lettuce, tobacco, alfalfa, and clover
Cereals	Rice, maize, wheat, barley
Legumes	Pea, soybean, pigeon pea
Vegetables and Fruits	Carrot, Banana, Potato, Tomato,
Oil crops	*Camelina sativa*, Oilseed rape
Simple plants	*Marchantia polymorpha, Lemna* sp., *Physcomitrella patens, Chlamidomonas reinhardti*

Source: 5,6

Table 2: Direct gene transfer in plants

Direct gene-transfer methods	Comments
Particle bombardment	Very successful method. Risk of gene rearrangements and a high copy number. Useful for transient expression assays
Electroporation	Transgenic plants obtained from a range of cereal crops.
Low Efficiency	Requires careful optimization.
DNA uptake into protoplasts	Used for all major cereal crops. Requires optimization with a regenerable cell suspension that may not be available.
Silicon carbide fibres	Regenerable cell suspensions required. Transgenic plants obtained from a number of species.

Source: (8)

in general, applied to different crops. Agrobacterium-mediated transformation is most widely used with dicotyledonous crops.[2] Several different methods for direct gene transfer have been used over the years, of which gene bombardment has been widely adopted by plant biotechnologists.

Direct gene transfer methods have been found to have widespread use in the transformation of cereal crops and have some advantages and disadvantages when compared with *Agrobacterium*-mediated transformation (Table 2).[5] One of the major disadvantages of direct gene transfer methods is that they tend to lead to a higher frequency of gene rearrangement and a higher transgene copy number. This can lead to a high frequency of gene silencing, due to which there are difficulties in using *Agrobacterium* to transform monocotyledonous plants. However, all plant transformation methods can suffer from a problem known as gene silencing, where transgene (and homologous endogenous gene) expression is actually repressed. For studies on gene regulation, etc., (i) the cells should be competent to take up DNA and allow the expression of transgenes. (ii) For the production of transgenic tissues, the cells, in addition, must be meristematic. Finally, (iii) transgenic plants can be produced only when the cells are also capable of regenerating complete plants.[7]

In the case of plants, stable transformations may be either non-integrative or integrative. In non-integrative stable transformation, the transgene is maintained stably in an extrachromosomal state, e.g., in the case of virus vectors. However, this type of transformation is not expressed to the next generation. The integrative stable transformation results when the transgene becomes integrated into the plant genome; these integrations are heritable.[1,7,8]

Production of Metabolites

Production of carbohydrate

Plants produce a variety of commercially valuable carbohydrates. The two most important and abundant carbohydrates are cellulose and starch. Some biotechnological efforts is being made towards improving the yield and quality of these bulk carbohydrates. Some other carbohydrates that could be useful to produce in transgenic plants include oligofructose, cyclodextrins, and trehalose. The carbohydrates are produced and stored in a number of different cellular compartments. Starch and its derivatives are produced in plastids, whereas fructans (storage carbohydrates) in plants are produced and stored in the vacuole. Sugars and alcohols are produced in the cytosol and accumulate throughout the cell, often in response to abiotic stress. For example, starch is stored transiently in the leaves of cereals, and starch reserves accumulate in the amyloplasts of the grain endosperm.[8]

Production of starch

Starch is mainly used as for thickener in the food industry and as a sweetener in the beverage industry; about one-third is also used either in the paper, packaging, and textile industries or just as raw material in the chemical industry.[9] The demand for amylose-free starch may increase since it is easily digestible and can make clear pastes that do not retrograde.[3]

Many higher plants are used for their stored starch. These include (i) seeds of cereals and legumes and (ii) Tubers or roots of potato, yam, and cassava. In potatoes, 75% of the dry weight is starch, making it a model system for improvement in starch quantity and quality. However, corn produces 17 million tons of starch per year, compared to potatoes, which produce 2 million tons per year. Transgenic corn is more difficult to produce, and potato starch is certainly better. The major components of starch are (i), amylose, a linear α (1-4) D-glucan polymer (mol. wt. 104–106), and (ii), amylopectin (mol. wt. 104), which is a branched α (1-4 and 1-6) D-glucan polymer. Besides these two components, starch also contains small amounts of lipids, proteins, and phosphorus, which determine the starch quality. Starch is present in two types of plastids: chloroplasts as 'transitory starch' and amyloplasts as amylose. Amylose makes up 11 to 370% of total reserve starch.[3]

Most of the starch in higher plants is synthesized from sucrose, involving at least 13 enzymes, of which only the following three are considered the key enzymes: (i) ADP-glucose pyrophosphorylase (AGPase), (ii) soluble starch synthase (SSS), and (iii) branching enzyme (BE). Many genes in starch biosynthesis have been isolated and are used in the production of transgenic plants. Genetic engineering has given the following results in potatoes: (i) the antisense approach was successfully pursued for inhibiting the action of the granule-bound starch synthase (GBSS) enzyme. This gives a 70 to 100% inhibition of the activity of this enzyme, resulting in a decrease, complete, or absence of amylose content, thus giving amylose-free starch. (ii) Starch content could be increased by introducing a bacterial gene encoding AGPase (glgC) into potatoes, coupled with a strong

Table 3: Bacteria, which produce CGTases

Organism	*CGTase type*	*Gene cloned*
Klebsiellaoxytoca M5al	α	+
Bacillus macerans	α	+
Bacillus stearothermophilus	α	+
Bacillus circulans	β	+
Bacillus megaterium	β	–
Bacillus ohbensis	β	–
Micrococcus sp.	β	–
Alkalophilic Bacillus 38-2	β	+
Alkalophilic Bacillus 17-1	β	+
Alkalophilic Bacillus 1011	β	+
Alkalophilic Bacillus 1-1	β	+
Bacillus subtilisNo. 313	γ	+
Alkalophilic Bacillus 290-3	γ	+

Source: 3

Table 4: The fatty acid composition of major oil crops

Fatty acid	Composition (%)				
	Peanut oil	*soya oil*	*Palm oil*	*Rapeseed oil (LEAR)*	*Sunflower oil*
16.0	11	42	04	05	10
18.0	03	05	01	01	03
18:1	22	41	60	15	50
18:2	55	10	20	79	30
18:3	08	00	09	00	00
20:1	00	00	02	00	03
Others	01	00	02	00	00

LEAR, low erucic acid rape. Percentages may not add up to 100% due to rounding and non-inclusion of other constituents. Source:

promoter belonging to the patatin gene. This promoter induces high expression in tubers. Starch content was increased by at least 50% .[3] The simple diagram of starch biosynthesis in Fig. 1 is shown. There is a branch in the biosynthetic pathway leading to one or the other form of starch. Thus, at three points, this pathway could be altered in different ways. The production of ADP-glucose could be altered, which would affect the overall level of starch biosynthesis. Thus, increasing the amount of the enzyme ADP-glucose pyrophosphorylase should increase the amount of starch produced. On the other hand, manipulation of the branches in the pathway would affect the ratio of amylose to amylopectin.[7]

The proportion of amylose to amylopectin is normally about 20 to 30% amylose to 70 to 80% amylopectin, and it is this ratio that has the greatest influence on the physicochemical properties of the starch. Many of the uses of starch in food production involve the formation of a gel after heating the starch in water and cooling, so increasing the proportion of amylopectin could be advantageous to amylose molecules tend to aggregate and crystalize on cooling, whereas amylopectin gels are more stable and generally more desirable

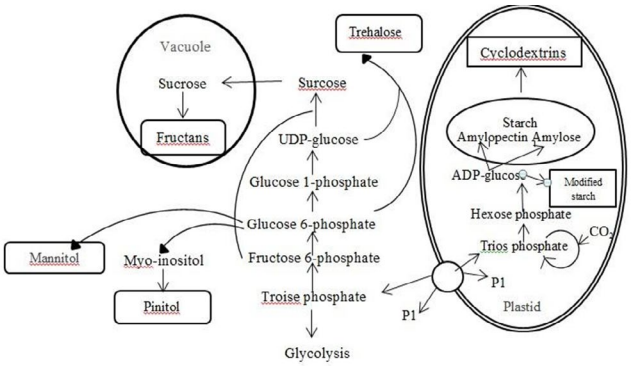


Fig. 1: Metabolic pathways for the biosynthesis of carbohydrate products for molecular farming (Source:7).

for food processing. The waxy phenotype is characterized by a starch with no amylose that gelatinizes easily, giving clear pastes that are ideal for thickening and stabilizing food products. It is now known that the waxy gene encodes the GBSS protein, and waxy starches have been produced in transgenic potatoes by antisense inhibition of GBSS1 and in sweet potatoes by sense suppression of the same gene.[8]

Cyclodextrins from starch

Cyclodextrins are high-value products that could be made from starch. These compounds are typically six, seven, or eight-membered rings comprising glucopyranose subunits attached in (1→4) linkage and are normally produced by the bacterial fermentation of maize starch, particularly for pharmaceutical applications. Structurally, the cyclodextrins form a cone-shaped ring (Fig. 2) with hydrophilic residues on the exterior and a hydrophobic pocket in the center of the ring. The seven-membered ring has the ideal dimensions to form a pocket for small hydrophobic compounds. Thus, in a concentrated suspension, the cyclodextrins will effectively solubilize hydrophobic pharmaceuticals such as steroids. It has been identified as a suitable target for molecular farming .[3,7,8]

A bacterial cyclodextrin glycosyltransferase (CGTase) gene from *Klebsiella pneumoniae* was incorporated into a plastid targeting sequence and kept under the control of the promoter from the patatin gene. However, the transformation of potatoes with this construct resulted in very little conversion (0.001-0.01%) of starch to cyclodextrins (Table 3).[3, 7, 8]

Mannitol from starch

Mannitol is a six-carbon, linear chain(Fig. 2). It is a sorbitol-related diuretic and renal diagnostic tool. Other names of mannitol are osmitrol, mannite, manna sugar, and D-mannitol. The World Health Organization's list of essential medicines includes it as the most efficient and secures medication in the healthcare system.[7]

An increase in the level of mannitol in transgenic tobacco plants has been achieved by the transfer of a gene for mannitol dehydrogenase from E. coli to tobacco.[3] It is used as a sweetener in foods for diabetics because it is barely absorbed by the intestine.[5]

Trehalose

Trehalose is produced in some plants and microorganisms (e.g., yeasts), often in response to osmotic stress (Fig. 2). It is used for

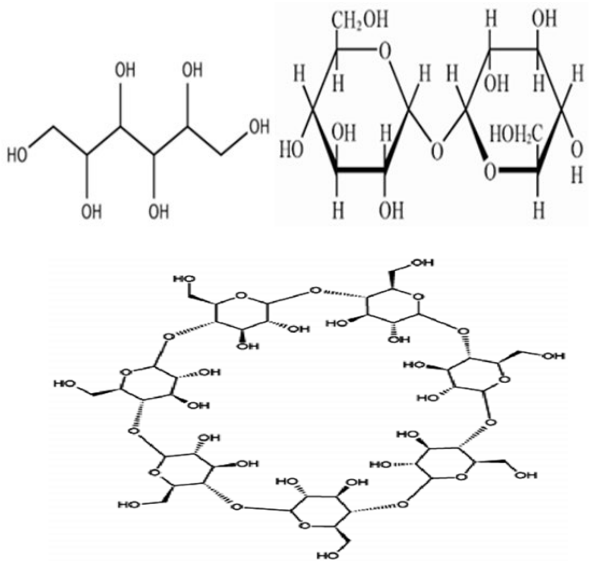


Fig. 2: Structure of mannitol, trehalose and cyclodextrin Source: 8

food processing, dehydration, and flavor retention. Trehalose can be synthesized from yeast and *E. coli*. Genes for its synthesis have been introduced into tobacco, potatoes, and rice.[8]

Production of polyfructans

Polyfructans are soluble polymers of fructose that are synthesized and stored in the vacuole. They have a typical structure of glucose-fructose (G-F-F). The use of fructans as a carbohydrate reserve is widespread throughout the plant kingdom. There are different glycosidic linkages possible between the fructose residues, giving different straight and branched polymers. The inulins are the major storage carbohydrate found in bulbs such as onions and storage roots such as chicory and Jerusalem artichoke and are formed by (1→2β) linkages. Levans are widespread in the leaves and stems of grasses, including major cereal crops such as wheat, and comprise (6→2β) linkages. Graminae-type fructans found, for example, in grasses are a mixed type and have both (1→2β) and (6→2β) linkages.[8]

The first step of the biosynthetic pathway of fructans involves the transfer of fructose from a donor sucrose molecule to an acceptor sucrose molecule to form ketone by the enzyme sucrose fructosyltransferase (SST).

$$G\text{-}F+G\text{-}F \rightarrow G\text{-}F\text{-}F+G$$

Here, G is glucose, and F is fructose. In the next step, the kestose (GFF or GF2) acts as the fructose donor to the growing fructan chain *via* fructan-fructosyltransferase (FFT) activity, and the sucrose molecule is recycled.[8]

$$G\text{-}F\text{-}Fn+G\text{-}F\text{-}F \rightarrow G\text{-}F\text{-}Fn+1+G\text{-}F$$

In certain bacteria, such as Bacillus subtilis, very high molecular mass levans are produced by a single reaction in which sucrose acts directly as the fructose donor to the growing chain (sucrose fructanfructosyltransferase, SFT). The first sugar in the fructan chain is always glucose, as sucrose is the initial acceptor molecule of the chain. A glucose residue is released for each remaining fructose residue attached to the chain, which in plants is transported back from the vacuole into the cytosol.[8]

Many transgenic plants producing polyfructans have been developed. In earlier experiments, the sacB gene of *Bacillus amyloliquifaciens*, which encodes levan sucrose catalyzes a 6→2β linkage, was transformed into maize, tobacco, potato, and sugar beet.[8]

Production of Lipids

Major crops such as oilseed rape (canola), soybeans, and maize produce lipids in large quantities for industrial and food purposes. Molecular farming will, therefore, have a role in the improvement of existing lipid products and the engineering of novel lipid products.[8]

Improvement of plant oils

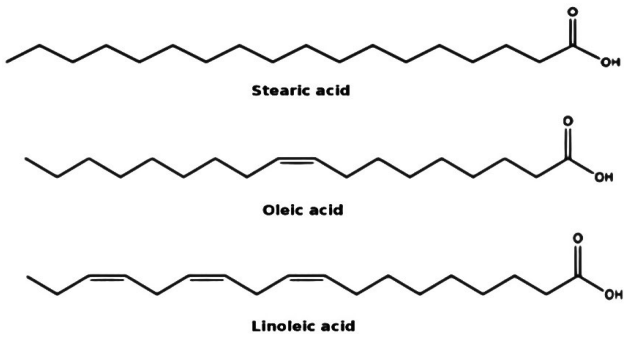
Oils are already extracted from a number of different types of crops. Oilseed rape, or canola, and soybean are major sources of oil that are often unspecified, but sunflower oil, corn oil, and olive oil are often identified because of the improved characteristics of these oils for particular purposes (Table 4). They are used in the industry for the manufacture of soaps and detergents, lubricants, and biofuel. However, non-food applications currently comprise only about 10% of the total vegetable oils produced. A major long-term goal is, therefore, the improvement of plant oils for industrial applications since they must eventually replace non-renewable, petroleum-based products.[8]

The site of fatty acid synthesis is exclusively in the stroma of the plastid, whereas most modifications of the fatty acids occur in the cytoplasm and on the endoplasmic reticulum (ER). Assembly of TAGs takes place in the membrane of ER and is stored in oil bodies. The oil bodies of seeds (and other tissues that undergo extreme desiccation) also contain proteins called oleosins in the lipid monolayer.[8]

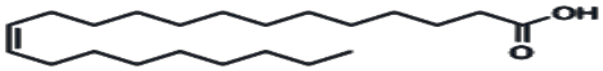
The fatty acid biosynthesis starts in the plastid with the formation of malonyl-coenzyme A (CoA) from acetyl-CoA in ATP-dependent reactions catalyzed by acetyl-CoA carboxylase (ACCase). Three subsequent steps (reduction of the carbonyl group, removal of water to form a double bond and reduction of the double bond) produce an acyl-ACP, which is two carbons longer than the original. A fatty acid synthase multienzyme complex brings the entire sequence of elongation reactions from the initial binding to ACP. In many oil-producing crops, this process stops at the 16-carbon stage, and the palmitoyl-ACP (16:0) is elongated to stearoyl-ACP (18:0) by a specific synthase. After this step, desaturation by a soluble 9C-stearoyl desaturase in the stroma of the plastid converts the stearoyl-ACP to oleoyl-ACP ($18:1\Delta^9$). After the formation of fatty acids in the plastid, the fatty acids are set free from the ACP and exported to the cytoplasm, where they are converted to acyl-CoA esters. TAGs are formed by the stepwise acylation of glycerol 3-phosphate in the ER membrane. Fatty acid modifications further occur after they have been attached to various glycerophosphatides.[8]

Production of shorter-chain fatty acids

The oils produced by the major oil crops of the world consist mainly of oleic, stearic, palmitic, linoleic, and linolenic acids, which are all C16, or C18, in length (Fig. 3). Hydrolysis of the acyl-ACP by the enzyme thioesterase can terminate the synthesis of fatty acids at a particular length. The introduction of this gene into oilseed rape causes fatty acid synthesis to terminate at the 12:0 stage and a high proportion of lauric acid (58% of total fatty acids) to accumulate in the seed oil. Attempts to produce caprylic acid (8:0) in oilseed rape transformed



Source: (under cc)
Fig. 3: Shorter chain fatty acids



Source: (under CC)
Fig. 4: Structure of Erucic acid

with only the acyl-ACP thioesterase gene from Cupheahookeriana were less successful, with only 12% of fatty acids represented by caprylic acid. It is thought that this could be due to the low availability of short acyl-ACP pools, and further work indicated that crossing plants expressing condensing enzymes (3-ketoacyl-ACP synthase, KAS) from Cuphea (with unique specificity for 6.0-caproic and 8.0-acyl-ACP) with plants expressing a Cuphea acyl-ACP thioesterase gene improved the proportion of short fatty acids.[8]

Production of longer chain fatty acids

One of the important targets is to synthesize fatty acids longer than C18 for use as industrial oils (Fig. 4). In oilseed rape and other Brassica species, there is a two-step elongation pathway from oleoyl-CoA (18:1Δ9) to erucoyl-CoA (22:1Δ13), such that erucic acid is one of the constituents of Brassica oils. Erucic acid is unsuitable for human consumption but is valuable as an industrial oleochemical. The highest erucic acid content of HEAR is about 50% of the total fatty acids, which makes the cost of separating out and disposing of the other fatty acids uncompetitive with mineral oil sources. The transformation of oilseed rape with a combination of β-ketoacyl-CoA synthase and 22:1 acyl-CoA:lysophosphatidic acid acyltransferase produced 60% erucic acid.[8]

Table 5: Therapeutic recombinant proteins produced in transgenic plants

Protein	*Application*	*Host Plant*
α –Tricosanthin	HIV therapy	Tobacco
Angiotensin-1-converting	Hypertension	Tobacco, Tomato enzyme
Collagen Human	Tissue repair	Tobacco
Factor XIII (A-domain)	Bleeding	Tobacco
Glucocerebrosidase Gaucher disease		Tobacco
Hrudin Leech	Anticoagulant	Canola
Human α -antitrypsin	Cystic fibrosis, Liver disease, Haemorrhage	Rice
Human α -interferon	Hepatitis C and B	Rice, Turnip
Human aprotinin transplantation surgery	Trypsin inhibitor for	Corn
Human enkephalins opiate activity	Anti-hyper analgesic by	ThaleCress, Canola
Human epidermal factor	Wound repair/Control of cell proliferation	Tobacco growth
Human eythropoitin Anemia		Tobacco
Human granules macrophage colony simulating factor	Neutropenia	Tobacco
Human growth hormone hemoglobin	Dwarfism, Wound healing Blood substitute	Tobacc Human Tobacco
Human hirudinvanant Ethiopain	C Anticoagulant	Mustard Tobacco, Canola,
Human hometimene	Collagen I, Collagen synthesis	Tobacco
Human protein C	Anticoagulant	Tobacco
Human serum albumin	Liver cirrhosis	Potato, tobacco
Interleukin- 2, 4, 10, 12, 18	Antiviral, anticancer	Potato, tobacco
Lactofemin	Antimicrobial	Potato tubers, rice, tobacco
Lysozyme	Antimicrobial	Rice
Pancreatic lipase	Exocrine pancreatic deficiency	Tobacco, maize
Synthetic elastin	Tissue repair	Tobacco

Source: 7

Table 6: Examples of antibodies that have been produced in plants

Application	*Plant*	*Antibody*	*Signal sequence*
S.mutans SA I/II (dental carries)	Tobacco	sIgA (hybrid)	Murine IgG
S.mutans SA I/II (dental carries)	Tobacco	IgG (Guy's 13)	Murine IgG
Surface antigen (colon cancer)	Tobacco	IgG Co17-1A	Murine IgG/KDEL
Herpes simplex virus	Soybean	IgG (anti HSV-2)	Tobacco extension
Single-chain Pv Lymphoma	Tobacco	scFv (38C13)	Rice a-amylase
Carcinoembryonic antigen (cancer)	Cereals	scPVT84.66	Murine IgG/KDEL

Source: 8

Table 7: Examples of vaccines produced in plants

Origin	*Recombinant protein*	*Plant*	*Production Level Human*
Escherichia coli	Heat-labile enterotoxin B	Tobacco	0.001% SLP
Vibrio cholera	CholeraCloxA and	Potato	0.3% TSP CtoxB subunits
Hepatitis B	Envelope surface protein	Tobacco/potato	<0.1% FW
Norwalk virus	Capsid protein	Tobacco/potato	0.23/0.37% TSP
Rabies virus	Rabies virus glycoprotein	Tomato	1% TSP Animal
Foot-and-mouth	Virus epitope VP1	Alfalfa/ Arabidopsis	N/A disease virus
Porcine coronavirus	Viral glycoprotein	Tobacco/maize	0.2% TSP/0.01%
Mink enteritis virus	Viral epitopeVP2	Black-eyed bean	N/A (CAMV)6
Canine parvovirus	Peptide from VP2	Arabidopsis	3% SLP capsid protein

Source: 5 ("FW, fresh weight; N/A, not available, SLP, soluble leaf protein, TSP, total soluble protein. Produced using a CPMV expression system.)

Production of proteins

Numerous recombinant proteins have been produced in transgenic plants. These proteins are often produced and accumulated in plant organs like leaves, fruits, roots, tubers, and seeds (Table 5). Extraction and purification of proteins from biochemically complex tissues become a major barrier to large-scale production of these proteins in transgenic plants. It is a laborious and expensive process. Even *in-vitro* systems, the manufacture of recombinant proteins can be slow, low-yielding, and expensive. In view of this, a plant system based on natural secretion from the roots of intact plants has been suggested and tried. The plant roots have developed a mechanism involving the secretion of different chemicals into the rhizosphere. In 1999, three heterologous genes (genes for green fluorescent protein, or GFR, from jellyfish, Aequorea victoria, placental secreted alkaline phosphatase, or SEAP, and xylanase from bacteria, *Clostridium*, and xylanase from thermocellum) were used for the formation of transgenic plants, demonstrating that root secretion (termed rhizosecretion) was successfully exploited for the production of recombinant proteins.[8]

Recombinant proteins made in plants are divided into three parts: antibodies, vaccines, and other medically related proteins.[7] Proteins are nitrogenous compounds and are the most important and abundant solids in the protoplasm of all living organisms. Proteins are high-molecular-weight polymers consisting of low-molecular-weight monomer units called amino acids, or L-α-aminoacids, linked together to form long chains (polypeptide chains) of varying length. The cell nucleus contains nucleoproteins, which are intimately associated with cell division and heredity. Cytoplasm contains thousands of enzymes, which are also proteinaceous. Proteins also serve as a major component of blood, epithelial tissue, and connective tissue in animals and, when in excess, as a source of energy and fat.[8]

Production of antibodies

Antibodies are produced by the vertebrate immune system. These are used for many applications, including the diagnosis, prevention, and treatment of human and animal diseases.[8] Plants have been used to produce a variety of antibodies, including whole antibodies, antigen-binding fragments, and scFvs (single-chain Fv protein). Despite the now well-characterized differences in the glycosylation pattern seen between antibodies produced in plant and mammalian expression systems, antibodies produced in plants generally seem to exhibit similar properties to antibodies produced in other mammalian systems. scFvs have recently been made in tobacco as a C-terminal fusion to an elastin-like polypeptide that is expressed in seed.[6] This approach was developed to take advantage of high levels of protein expression and the seed's ability to keep proteins functional during extended storage periods at ambient temperature.[3, 8]

The first example of a functional antibody (a mouse immunoglobulin IgG1) being produced in plants was reported in 1989. The antibody can be constructed in a two-step process. In the first stage, two separate transgenic tobacco lines were produced: one contained the gene for the heavy chain, and the other contained the gene for the light chain. The second stage of the process was to cross these plants to generate progeny that expressed both genes. It was found that in the F1 progeny expressing both chains, significant amounts (up to 1.3% of total leaf protein) of assembled antibodies were produced. For efficient assembly of the antibody in plants, these immunoglobulin signal sequences are targeted at the nascent light and heavy chains to the ER.[8]

Antibodies that are produced in plants are thought to be suitable for topical immunotherapy. The antibody does this by recognizing the native streptococcal antigen (SA) I/II cell-surface adhesion molecule, thereby preventing colonization.[5] Transgenic plants have been used for the production of antibodies directed against many diseases (Table 6).[10,11,13]

Production of Plant Derived Vaccines

Plant-based systems have the potential to provide low-cost, easily administered, locally produced edible vaccines that could provide mucosal immunity against the infectious agents that are responsible for the deaths of millions of people, particularly children, annually.

Plant-derived vaccines have been produced against many diseases, such as *Vibrio cholerae*, enterotoxigenic *E. coli*, hepatitis B virus, Norwalk virus, rabies virus, human cytomegalovirus, etc.[11]

One of the first successful attempts in this area used a transient expression system. Recombinant CPMV was used as a vector for a linear epitope (antigenic determinant) from the VP2 capsid protein of the mink enteritis virus. The recombinant CPMV was replicated in black-eyed beans (*Vignaunguiculata*), from which chimeric virus particles (CPMV-VP2), displaying the VP2 epitope, were isolated and utilized for the subcutaneous injection into mink. The vaccine was capable of inducing resistance to clinical infection by the mink enteritis virus. A vaccine for swine fever has been produced in plants using a fusion of the E2 glycoprotein fused to the CP of PVX. Many other vaccines have now been produced in plants, and Table 7 lists some examples.

Production of other proteins of medical importance

Trichosanthin

Trichosanthin is a component of the tuber of the plant Trichosantheskirilowii, which is used in Chinese medicine. Trichosanthin is a ribosome-inactivating protein that inhibits tumor growth and the immune response. It may be helpful in the treatment of HIV/acquired immunodeficiency syndrome (AIDS). Relatively high levels of trichosanthin accumulation (2% of total soluble protein 2 weeks after inoculation) have been achieved in *N. benthamiana* using a viral RNA-based transfection system. Trichosanthin is produced on a large scale by this method. The rapid production also offers the opportunity to rapidly produce mutated forms of trichosanthin for screening in trials aimed at identifying forms with increased efficacy or reduced side effects.[7]

Glucocerebrosidase

Gaucher's disease is an inherited disorder in which glucocerebroside (a component of red blood cells that is normally broken down into glucose and ceramide as old and damaged red blood cells are removed by the body) accumulates in lysosomes due to a deficiency in the enzyme glucocerebrosidase. Gaucher's disease, which results in swelling of the spleen and liver and severe bone damage, can be extremely debilitating and painful. A process to produce glucocerebrosidase in tobacco has recently been patented, which will hopefully result in cheaper glucocerebrosidase being available for patients with Gaucher's disease.[3, 7]

Human serum albumin

Human serum albumin (HSA) has many potential medical applications. HSA has been expressed in tobacco and potatoes under the control of the 355 promoter. Two forms of the HSA protein were expressed, with different signal sequences to ensure secretion of the HSA. One form had the human prepro sequence, while the other had the signal sequence from the tobacco extracellular PR-S protein. Although both forms of HSA were secreted, analysis showed that the form with the human prepro sequence was not properly processed, leading to proHSA being secreted. However, the form with the PR-S signal sequence was correctly processed, and mature HSA indistinguishable from the human protein was produced.[7]

Hirudin (an anticoagulant) for 'thrombosis'

Hirudin is another important drug that was produced from the leech (*Hirudo medicinalis*) and is utilized as an anticoagulant for the treatment of thrombosis. But now it is synthesized by recombinant bacteria and yeast.[3]

Biosafety during industrial production of metabolites

The production of metabolites from genetically modified crops introduces several unique challenges. Plants are generally grown in an open environment and this leads to potential gene flow to weeds or related crops through pollination or seed contamination.[11] Most of the developing countries lack biosafety legislation for genetically modified plants.[14] There is some negative opinion about transgenic plants among the general public, is that genetically modified plants are against nature.[15, 16]

Transgenic plants can generate biohazards. Therefore, several non-infectious illnesses can be associated with this. Microbes or microbial products can enter the body through inhalation. During the extraction of intracellular enzymes, the greatest risk is posed by the handling of large quantities of cell debris. An effective monitoring strategy should be followed for the environmental assessment of emissions at all stages of the manufacturing process.[1, 17]

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity has been framed to cover the handling and use of all living modified organisms that may have adverse effects on the conservation and risks to human health.[1] The National Institute of Health (NIH, USA) has developed guidelines for recombinant DNA research with a view to specifying the practices for constructing and handling i) recombinant DNA molecules and ii)organisms and viruses containing recombinant DNA molecules. These guidelines require approval and clearance from NIH or another Federal agency. All experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into human subjects cannot be initiated without submission of the required information to NIH and other specified agencies.[1]

In India, the Ministry of Environment and Forests (MOEF) in December 1989 framed the rules and procedures for the manufacture, import, use, research and release of GMOs and the products made from such organisms. This was done under the provisions of the Environment Protection Act, 1986 (EPA). The Indian Recombinant DNA safety Guidelines and Regulations have been prepared by (initially in 1990; revised in 1994) and are available on request from the Recombinant DNA Advisory Committee, Department of Biotechnology (DBT), New Delhi.[12]

CONCLUSION

Molecular farming is an emerging industry and is growing very fast. Plants offer a very wide range of options for the production of useful metabolites. The cost-effective production, rapid scalability, the absence of human pathogens, and the capability to fold and assemble complex proteins accurately make the plants suitable for molecular farming. Hence, transgenic plants have been used for the bulk production of biopolymers, vaccines, therapeutic proteins, nutraceuticals, hormones, and industrial enzymes. The need for recombinant proteins and other useful metabolites will certainly increase in the future, and molecular farming will provide very good future prospects to meet the increasing demand.

CONFLICT OF INTEREST

The author declares no conflicts of interest in this work.

SOURCE OF FUNDING

None declared.

REFERENCES

1. Singh B D. Genetics, First Edition Kalyani Publishers Delhi; 2003.
2. Ramessar K, Capell T, Christou P. Molecular Pharming in Cereal Crops. Phytochem Rev 2008a; 7:579–92
3. Nikolov Z, Hammes D. Production of Recombinant Proteins from Transgenic Crops. In: Hood E E, Howard JA. (eds) Plants as Factories for Protein Production; 2002 ; 159-174.
4. Sahu P K, Patel T S, Singh S, Tirkey P and Sharma D. Molecular Farming: A biotechnological Approach in Agriculture for Production of useful Metabolites. International Journal of Research in Biotechnology and Biochemistry. 2014; 4(2): 23-30.
5. Shah S, Yadav VK, Singh AV, Kumar VA. Molecular Farming in Plants: Synthesis of Novel Biomaterials. Journal of Hill Agriculture. January-June 2010; 1(1):6-12.
6. Rastogi S, Dwivedi UN. Plant Transgenic Technology. In: Kumar A, Pareek A, Gupta S M, editors. Biotechnology in Medicine and Agriculture Principles and Practices. I. k. Publishing House Pvt. Ltd. 2013; 576-580.
7. Slater A, Scott W. Nigel and Fowler R M. Plant Biotechnology: The Genetic Manipulation of Plants, Second Edition, Oxford University Press, United Kingdom, 2008.
8. Goyal S, Gupta P. Food, Nutrition and Health, S. Chand and Company Limited, New Delhi, 2012; 74.
9. Fischer R, Hoffmann K, Schillberg S and Emans N. Antibody Production by molecular farming in plants. J Biol Regul Homeost Agents, 2000; 14(2):83-92.
10. Thomas BR, Van D A and Bradford, KJ. Production of Therapeutic Proteins in Plants. Agricultural Biotechnology in California Series, Publication. 2002; 8078.
11. Horn ME, Woodward SL, & Howard JA. Plant Molecular Farming: Systems and Productions. Plant cell reports, 2004; 22: 711-720.
12. Sao R, Sahu P K. Molecular Farming as an Approach for Production of useful Metabolites. Journal of Plant Development Sciences 2019; Vol. 11(12): 697-704.
13. Smyth S, Phillips PW. Labeling to Manage Marketing of GM foods. Trends Biotechnol 2003; 21:389–93.
14. Streiffer R, Hedemann T. The political import of intrinsic objections to Genetically Engineered Food. J Agri Environ Ethics . 2005; 1 8:191–210.
15. Sharma A K, Sharma M K. Plants as Bioreactors: Recent Developments and Emerging Opportunities, Biotechnology Advances 27 , 2009; 811–832.
16. Ahmad K. Molecular Farming: Strategies, Expression systems and Biosafety Considerations. Czech J. Genet. Plant Breed, 2014; 50: 1–10.

HOW TO CITE THIS ARTICLE: Kumari K. Plant Molecular Farming: An Emerging Opportunity For Biopharmaceuticals. *J Adv Sci Res*. 2024;15(6): 1-8 **DOI:** 10.55218/JASR.2024150601