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Transgenic Plants as Expression Factories for Bio Pharmaceuticals

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Commentary

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INTRODUCTION

At present agriculture not only provides food, but is also used for the production of pharmaceuticals or industrial compounds such as pharmaceutical drugs, vaccines along with biodegradable plastic and industrial chemicals. Since last three decades, plant genetic engineering has played a vital role in the production of bio-pharmaceutical products from crops. Due to technological advancement of genetic engineering, biotechnologist are able to engineer plants by using living organism with the help of different transformation techniques like *Agrobacterium* mediated transformation, biolistic gene gun, and so on to produce biopharmaceuticals products for diagnostic purposes as well as nutritional supplements. Scientists are also able to control gene expression, protein targeting, growth and other parameters in order to adjust the structural and functional properties of the product[1]. Progress in this study has led to a shift from basic research towards commercial exploitation as molecular farming has become a method of choice to produce pharmaceutical products. Development of transgenic plants as a source of bio pharmaceuticals production is referred to as Molecular Farming. It involves use of plants and animals for the production of proteins and other valuable metabolites having therapeutic value in medical field or industry[2,3]. In 1986, Batra derived the first pharmaceutically relevant protein made in plants, a human growth hormone, which was expressed in transgenic tobacco demonstrated that functional recombinant antibodies can be expressed in tobacco[4]. Later, showed that foreign proteins with economic value can be produced by using transgenic higher plants[5]. After that, plants have been used intensively for the production of pharmaceutical products. Similarly other studies carried out such as egg proteins with important properties-avidin blood substitutes[6,7]. In the 1999 a first molecularly farmed pharmaceutical proteins, a bovine protease inhibitor, was produced in transgenic plants named Aprotinin as well as the first vaccines[8,9]. It has been shown that transgenic plants are extremely versatile and they can be widely used for the production of wide range of proteins[10]. Although transgenic animals, bacteria and fungi are also used for the production of proteins, plants can be used to gain more economic profit[11]. Preferably, higher plants are used for the production of protein instead of animals due to following reasons: (a) production cost is lesser than transgenic animals; (b) handling is easier than animals, expertise already exist for planting, harvesting and processing of plant material; (c) plants are free from known human pathogen (such as virions), so there is no chance of contamination in the final product; (d) higher plants generally synthesize proteins from eukaryotes with correct folding, glycosylation, and activity; (e) stability is higher than animals because plant cells can store proteins to subcellular endocompartments that reduce degradation and therefore increase stability[11,12]. For long term storage, transgenic plants can also produce organs rich in a recombinant protein[13]. By the propagation of stably transformed plants lines in the field, large amount of biomass and protein can be produced[14]. Due to these reasons, plant molecular farming has become more attractive for modern biotechnologist especially for plastid and chloroplast engineering[11,15,16]. Nowadays, mainly rice, wheat, maize, banana, tomato, tobacco, Arabidopsis and oilseed rape are used for molecular farming. Among them mainly *Nicotiana tabacum* is used as model expression system because it has the ability to produce large quantity of green leaf materials per acre. But for the production of biopharmaceuticals in seeds, the crop such as corn, soybean and canola are preferred because tobacco seeds are extremely small.

One of the upcoming technology is multiple-transgene direct DNA transfer which has major advantage over alternative gene transfer, in this technique all the required components for the expression of complex recombinant macromolecules is transferred into the plant genome[17]. Transferred the four transgene in different plasmids into rice and showed that 20% transgenic plants carried all four genes. Resulting transgenic plants showed that multiple transgenes are inherited in a linked fashion[18]. Another example of direct DNA transfer is introduction of expression cassettes containing promoter, open reading frame, terminator and without vector backbone, in this case stability of transgene and level of expression is higher[19]. Another technology is manufacturing pharmaceutical products in plastids because due to large number of transgene copies in homoplasmic tranformants, which produces large number of recombinant produced the tetanus toxin fragment (TetC) using plastids as vaccine producing platform. TetC was produced at 25% of total soluble cellular protein in tobacco chloroplasts showed that using plastids for vaccine production is a promising approach[20-22]. Later on it was found that there were several cases in which target pharmaceutical molecules in plastid gave little or no expression so it was concluded that in case of TetC expression level it was an issue solved with its codon optimization. High level of rotavirus VP6 protein was expressed in chloroplast of young tobacco leaves by but their expression was reduced adversely as the leaves matured[23].

Before choosing the production system, there are many factors which must be kept in mind while choosing plant species, tissue and target subcellular organelles that will be used as chosen host for production of recombinant proteins. One of the most important factors is the expression level, another one is from the biosafety point of view, the impact of exposure on the environment, food and feed chains. One of the potential risks is the pollen transferability from transgenic to related outcrossing species and other nontransgenic crops which may lead to persistence of genetically engineered material in environment and non-target organisms[24,25]. According to overexpression of aglycosylated CTB causes massive tissue necrosis and poor accumulation unless retained in the endoplasmic reticulum (ER)[26]. They also reported as, gCTB's potential as an oral immunogen and point to a potential role of N-glycosylation in increasing recombinant protein yields in plants. Some examples of various Bio-pharmaceuticals produced in transgenic plants and in pipeline for commercialization are shown in Table 1.

Table 1: Examples of Various Bio-pharmaceutical produced in transgenic plants in the pipeline for commercialization.

Name of crops	Products	Category	Applications
Tobacco	Human protein C (Serum protease)	Anticoagulants	Used in protein.C pathway
Tobacco, Oilseed, Ethiopian Mustard	Human hirudin variant 2		For indirect thrombin Inhibitors
Tobacco	Neutropenia	Recombinant hormones/proteins	In Human granulocyte-macrophage
Tobacco	Human erythropoietin		In Anemia disease
Thale cress, Oilseed	Human enkephalins		Useful in antihyperanalgesic by opiate activity
Tobacco	Human epidermal growth factor		It helps in Wound repair and control of cell proliferation
Rice, turnip	Human interferon-α		Used in Hepatitis C and B
Potato, Tobacco	Human serum albumin		In Liver Cirrhosis
Tobacco	Human haemoglobin		For Blood substitute
Tobacco	Human homotrimeric collagen I		In Collagen
Tobacco, Lettuce	CTB Cholera toxin B Subunit-proinsulin		Diabetes
Rice	Human α-1 antitrypsin	Proteins/Peptide Inhibitors	In cystic fribrosis, liver disease, and haemorrhage
Maize	Human aprotinin		In trypsin inhibitor for transplantation surgery
Tobacco/tomato	Angiotensin-I-converting enzyme		In hypertension
Nicotiana bethamiana	α-trichosanthin from TMV-U1 subgenomic coat protein		For HIV therapies
Tobacco	Glucocerebrosidase	Recombinant enzymes	For Gaucher's disease
Rice	Daffodil phytoene synthase	Nutraceuticals	Used for provitamin A deficiency
Potato	*Amaranthus hypochondriacus* Ama1 seed albumin		In amino acid deficiency
In pipeline for commercialization			
Viral vectors in Tobacco	Various single-chain Fv antibody fragments	Antibody	For non-Hodgkin's lymphoma
Transgenic tobacco	CaroRx		In dental caries

Transgenic maize	Gastric lipase	Therapeutic enzymes	In cystic fibrosis, pancreatitis
Transgenic maize, Transgenic potato	*E. coli* heat labile toxin	Vaccine	For diarrhoea
Transgenic potato	Norwalk virus capsid protein		In norwalk virus infection
Viral vectors in spinach	Rabies glycoprotein		In rabies
Transgenic potato	Hepatitis B virus surface antigen		For Hepatitis B
Transgenic lettuce			
Transgenic Arabidopsis	Human intrinsic factor	Dietary	In Vitamin B_{12} deficiency
Transgenic maize	Lactoferrin		In Gastrointestinal infection
Transgenic rice	Lysozyme, Lactoferrrin, Human serum albumin		For diarrhoea
Transgenic tobacco	Cyanoverin-N	Microbicide	In HIV
Transgenic safflower	Insulin	Hormone	In diabetes

Conclusion and Future Perspectives

Plant molecular farming is being used to fulfil the increasing demand of recombinant proteins at a lower cost and higher quantity which can't be produced in microbial as well as animal cell cultures. The increase in amount and lowering the cost helps in easy availability of the drugs to the patients. Like other recombinant originating system, biopharmaceutical products derived from the transgenic plants must meet the standard and safety by a risk assessment analysis. We can reduced the risk of contamination by using contained production facilities like green houses, development of phenotypic and fluorescent markers which can be used for the visual selection of lines expressing pharmaceuticals[27]. Efficient purification system is required while using non-edible plants as host to ensure the pharmaceuticals products safety. For example in case of tobacco, it is not co-purified with other potentially toxic proteins or antigenic plant metabolites. In biopharmaceuticals, nature of glycosylation is one of major concern that is sometimes different from that found in animals. In mammals, oligosaccharides provide the substrate for extensive elongation and modification processes to give rise to the final diversification of N-glycosylation whereas in plants, modifications of these oligosaccharides are more limited. Many products are already developed from plants and human beings are constantly exposed to plant glycoproteins in food without ill effect. Some carbohydrates moieties are unique to plants and when administered regularly, which may present an antigenic challenge to immune systems which leads to sensitization. Therefore, to get rid from sensitization, researcher should try to develop such a mutant transgenic plants lacking of enzymes involved in glycosylation pathway. Other concern is the development of regulatory frameworks to commercialize the human therapeutics which will help the public to rapidly believe and accept this technology.

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