PLANTS AS BIOFACTORIES FOR THE PRODUCTION OF BIOPHARMACEUTICALS:
A BRIEF REVIEW

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ABSTRACT

Plant genetic engineering is conventionally used for transferring beneficial traits to plants. However in recent years the application of this technology for the production of commercially valuable simple or complex therapeutic proteins and industrial enzymes makes it more important and emerging field of study. The whole plant or plant parts or plant cell culture have been used for the production of biopharmaceuticals like cytokines, blood proteins, milk proteins, hormones, antibodies or antibody fragments, metabolic enzymes, hormones, antigens or vaccine epitopes and many more biological molecules used in animal, specially human health care. Safe, cost effective and considerable high level of recombinant protein production with the ability to properly fold and assemble eukaryotic proteins makes it an alternative competitive system with microbial and mammalian hosts. The increase in recombinant protein production varies with varying host plant species, transformation strategies, location of protein accumulation and the expression of foreign gene which can be optimized. Some of the strategies used till date to optimize the process with their success stories, have been discussed in this review. Although the increases in protein accumulation and their safe downstream processing is vital factor, but the acceptance of this technology also depends on human acceptance which also seems to be promising.

KEYWORDS: Plant Biotechnology, Plant Genetic Engineering, Biopharmaceuticals, Recombinant Protein, Human Health Care

INTRODUCTION

One of the most precious gifts of the nature is plant which has been cultivated by humans for thousands of years not only for foods, but also as useful raw materials and medicines. From the ancient times, plants are being used in medicines. Although the proper documented records about medicinal plants dates back at least 5000 years to the Sumerians [1], the use of plants and plant parts in many folk medicines around the world is long back in history. Till today, one quarter of drugs prescribed is of plant origin [2]. Plant’s use for the traditional medicine is mainly attributive to the medicinal property of secondary metabolic compounds. Among the several thousands of compounds synthesized by plants, some are essential for its own metabolism and universally present in all plants: the primary metabolites, the other group of chemicals do not have essential role in plant’s metabolism, but are helpful in disease resistance, stress tolerance and are generally produced to protect plants against pathogen infection, herbivore attack or UV irradiation: the secondary metabolites. The secondary metabolites do not have any fundamental biochemical role in the normal building and maintaining of plant cells [3].

In the twenty-first century, with the help of advanced technology, plants can be modified at the genetic level: arising in the concept of ‘Metabolic engineering’ (altering the biosynthetic routes of many valuable secondary metabolites) and ‘Molecular farming’ (production of pharmaceutically important and commercially valuable proteins). Genetic engineering of plants was convincingly proved in 1980’s [4,5] and later on progressed in the development of abiotic or biotic stress tolerant transgenic plants, plants with improved nutritional quality and plant products with extended shelf life.
Progress in biotechnological research made it possible to understand the mechanisms of synthesis and production of several compounds and proteins with desirable properties. With the help of biotechnological methods, it is now feasible to manipulate plants own metabolism by altering the enzyme activity, introducing new genes to change the availability of intermediate compound(s) of a biosynthetic pathway, altering the expression of regulatory genes and also by over-expressing key enzyme(s) for the enhanced production of desirable compound(s). At the same time, plants can be also be used for the production of foreign proteins including those that are therapeutic in humans [6]. This opened up the avenue for the utilization of plants as bio-factories for the production of pharmaceutically important and commercially valuable proteins, which is popularly referred to as Molecular farming [7]. From the year 1986 when the first therapeutic protein expressed in tobacco and sunflower callus [8] there was a gradual trend to express foreign proteins having pharmaceutical importance in an increasingly diverse range of crops. Many pharmaceutically important proteins had also been expressed in plants like antibodies [9-18] and vaccines [19]. Progress in the field of genomics, proteomics and metabolomics becomes a boon for the human health care sector which started flourishing with the knowledge in molecular basis of disease development and the development of new therapeutic proteins [19].

**SELECTION OF EXPRESSION SYSTEM**

Expression foreign gene in prokaryotic or eukaryotic host for the production of recombinant proteins of therapeutic importance becomes an interesting field of study. Although the most extensively used expression host had been *E. coli* in prokaryote and Chinese hamster ovary cells, *Pichia pastoris, Saccharomyces cerevisiae* in eukaryotes, there are several disadvantages in using those expression systems. For large scale production of recombinant proteins, microorganisms seem to be the best suitable host. But several disadvantages including the lack of post translational modifications (require for antibodies), difficulty in purification as the host endotoxins are often co-purified and form inclusion bodies (requires in vitro refolding), make it less suitable system for expressing proteins that are therapeutic for human. On the other hand, proteins expressed in mammalian cells are similar in their properties to those of the natural origin [20]. But still, the mammalian expression system is less suitable for expressing the therapeutic proteins due to the inability of high scale production and expensive culture cost [21]. In this context, plants proved to be a good system for expressing foreign proteins soon after the confirmation of the structural authenticity of plant-derived recombinant proteins in 1992 when plants were used for the production of hepatitis B virus (HBV) surface antigen, an experimental vaccine [22] for the first time. The ability of assembling complex functional glycoprotein with several subunits by plant cells [12] made it more important system for future use. Progress in expressing a series of HIV neutralizing antibodies and peptide lectins in plant proves it to be a better alternative system for the production of pharmaceutically important proteins [23].

Plants offer several advantages for the recombinant protein production not only for the lower production cost, high scale production and their ability of correct folding and post translational modifications of the expressed proteins, but also as safe expression systems as the plant cells are free from potential human pathogens or prions, oncogenic DNA sequences and endotoxins. Recombinant proteins expressed in plants can be targeted to various organelles for eg. in chloroplasts where high level of expression is possible. The proteins expressed in edible parts of the plant can be used directly or after minor processing which reduce the downstream processing costs. The concept was further applied to the production of edible vaccine.

Progress towards development of novel expression systems remains successful. *Chlamydomonas reinhardtii*, a microalga is capable of expressing human therapeutic proteins in short period of time and the recombinant protein showed the similar folding pattern. This makes it an attractive system for the expression human therapeutic proteins [24]. One of the disadvantages of plant expression system lies in the post translational modification. Plant-specific protein N-
glycosylation makes the recombinant protein immunogenic. The moss, Physcomitrella patens becomes an alternative expression system to avoid the difficulty. Among the other advantages, the most important one of Physcomitrella expression system compared to other plant systems is the feasibility of targeted gene replacements [25].

CHOICE OF HOST PLANT SPECIES

The choice of plant species or tissue for commercial recombinant protein production varies. Although tobacco and alfalfa seems to be the potential crops due to the high yield of biomass and seeds and short life cycle, the use of other plant species like corn, potato, soybean, rice and wheat has also been noticed. Lettuce is also reported to express the edible vaccine for hepatitis B [26]. Recombinant protein expression in vegetative organs may interfere with plant growth and development and also less advantageous because of presence of chlorophyll and other poly-phenolic compounds which renders difficulty in downstream processing. Low yield of recombinant proteins in leafy crops due to their unstable in aqueous environment and exposure of herbivores to pharmaceutical proteins are two major obstacles. The plant has to grow in controlled area. But there is an added advantage of using leafy part in expressing therapeutic proteins over the tubers, seeds or fruits. When the fruits, tubers and seeds are the expression system, the plants have to go through a flowering cycle to produce fruits and seeds, while proteins expressed in vegetative organs can be harvested before flowering. This eliminates the possibility of the release of pollen and flow of genetic material by pollen transfer [27].

Seed or tubers are more advantageous over leafy plant materials for longer period of storage of the recombinant protein in plants. Seed based expression system using canola, corn or soybeans have been adopted by companies like, Sem BioSys, Genetics, Agracetus, Mogen International and Plantzyme [28]. Proteins of different molecular weight (6000 daltons to 272, 000 daltons) have been expressed in plant seeds [29]. Flax, cotton and oilseed rape may also prove to be the potential system for expressing foreign proteins. In this case the production costs of recombinant protein can be subsidized to a certain degree by secondary revenues from alternative products. Oleosin-fusion technology for targeting the recombinant proteins to oil bodies, developed by SemiBioSys Genetics, can be used to facilitate purification.

Potatoes also proved to be a good expression system and transgenic potatoes have been administered to humans for clinical trials. Among several other antigens expressed in potatoes for human trial, the enterotoxigenic E. coli (ETEC) labile toxin B-subunit (LTB), one potent oral immunogens [30] and norwalk virus capsid protein (NVCP) [31] hold significant success. Recent trial using LTB expressed in processed corn seed produced nearly similar results to that of potato study [32]. Another candidate crop, tomatoes were successfully used for the first time for the production of rabies vaccine [27]. Among the fruit crops, banana is a significant one with multiple advantages. Bananas consumed as staple food, available at low cost in tropical and subtropical countries and also palatable and easily digestible. Bananas are triploid and are propagated vegetatively. Therefore it is an ideal crop for biological containment. According to CJ Arntzen, 1997 [33], one banana can potentially contain ten doses of vaccine.

Plant cell suspension culture which combines the advantages of whole-plant system have become promising alternatives for the production of recombinant proteins [34]. The unique features include product safety, cost-effective manufacturing and easy separation and purification of the recombinant protein [35]. In the controlled system, the plant cells are free from contaminating pathogens, herbicides and pesticides. A wide number of recombinant antibodies have been expressed in BY-2 and NT-1 cell line of tobacco and also in rice cell suspension cultures [36].

TRANSFORMATION OF THE HOST PLANT

To express the protein in host plant, the foreign transgene has to be transferred in plant cell. The choice of a
transformation generally vary according to the variation of plant material used for transformation, the vector and the delivery method, desired type of expression and also on the sub cellular location where the expression is desired. Several physical methods for transformation like particle gun (biolistics), silicon-carbide whiskers, coated nanoparticles, PEG (polyethylene glycol)-mediated transformation, liposomes, electroporation are available of which the biolistics method is more popular. Although the physical methods are very simple, there is chance of DNA recombination before integration to plant gene and multiple copies of foreign gene may be introduced in cells resulting in increased expression or gene silencing and also the tissue specificity is lost in this process [37]. On the other hand the biological methods which are more popular include mainly two methods: agroinfiltration and infection with modified viral vectors.

Transient expression is well suited to verify expression constructs, to avoid harmful long term effect of foreign protein in plant cell, to avoid transcriptional gene silencing and to produce small amounts of products for functional analysis before proceeding to transgenic plants. While stable transformation is required to generate transgenic plants and can be done after several optimization of either physical (biolistics method) or biological methods. Transient expression is also possible by transformation of plant cells by physical or biological methods like biolistic method and agroinfiltration respectively. The most popular method of transient expression is by transforming the host plant with Agrobacterium containing transgenes using a vacuum chamber, the agroinfiltration method. Agrobacterium based transformation strategies use the advantages of the natural genetic engineering property of Agrobacterium itself which can transfer the T-DNA harboring the recombinant DNA cloned into the region between the right and left border sequences in plant cell for integration [38]. While the viral vector can systemically infect most cells in a plant and the RNA viral vectors can replicate in cytoplasm without entering into the nucleus or integrating into the host chromosome and this results a high quantities of heterologous protein production in very short time [39]. Though a large number and variety of viral vectors are available [40], Tobacco mosaic virus (TMV), Cow pea mosaic virus (CPMV) and Tomato bushy stunt virus (TBSV) based vectors are more popular. Newly developed a defective RNA (dRNA)-based TMV vector (dRT-V) eliminates the limitation of plant viral vectorslike TMV for the co-expression of multiple genes in a single cell [41].

Transformation of the plant gene in plastid has reported to be advantageous as the amount of product is high in this case. Particle bombardment and PEG mediated transformation of plant protoplast are good and suitable for plastid transformation [42]. In this case, the targeted DNA is integrated into the plastid genome through homologous recombination. Several fold increase in the expression level of recombinant proteins has been reported when they are expressed in plastids [43]. This may be due to the increase in copy number of the foreign gene within the cell. A mature leaf cell contain as many as 100 plastids and each plastid contains 10-100 genomes resulting in the abundance of transgene in each cell to be around 10,000 copies [44]. The expression level of human somatotropin increases 300 fold when it transformed to tobacco chloroplast compared to nuclear transformed plants [45].

OPTIMIZATION OF TRANSGENE EXPRESSION IN HOST PLANT

In order to get notable amount of product, targeted or tissue specific expression and accumulation different strategies are taken to optimize the transgene expression level in host plant.

Optimization by Codon Usage

In plants including other eukaryotes, synonymous codons of the genetic code are not used with equal frequency; rather some codons are preferred over others [46]. Codon usage generally refers to the frequency of use of alternate and equivalent codons for a particular amino acid. The use of synonymous codons for a particular amino acid differs between taxonomic groups, primarily in the use of G + C in the degenerate third base [47]. Bacterial genes contain AT rich
sequences and the GC content of plant genes is generally higher. The choice of codons influences the rate of translation and mRNA degradation and is correlated to the levels of available tRNAs for that amino acid and the ribosome may pause encountering a rare codon, because it may take longer time for a rare iso-accepting t-RNA to enter the A-site of the ribosome [47]. Several software programs are available for optimization of codon usage, like Gene Designer (https://www.dna20.com/; [48]), and OPTIMIZER (http://genomes.urv.cat/OPTIMIZER/; [49]).

Complete codon engineering by replacing codons with more favourable codons throughout the whole gene may generate partially or fully modified synthetic gene which can enhance the expression levels of the transgene [50]. This strategy were applied increase the expression levels of transgenes like, the winter flounder antifreeze protein (AFP) in corn [51], porcine alpha lactalbumin gene in the kernels of transgenic maize and the B- subunit of heat labile enterotoxin of Entertoxigenic E. coli (LT-B) [52,53]. Optimization of plant mRNA processing by changing the AAT codon to GTG surrounding the translation initiation site of LT-B gene resulted in 5-40 fold higher amount of LT-B expression level when transformed in potato [54]. It is also reported that specific sequences within mRNA itself containing destabilizing motifs and cryptic splice sites affect its stability and the translation performance within the plant cells [55,56]. The use of 5’ and 3’ untranslated leader sequence has also positive effect on transgene expression. The 3’ sequences of soybean vegetative storage protein gene and potato pin II gene are reported to enhance the expression level of hepatitis B surface antigen (HBsAg) in potato tubers [57]. The 5’ untranslated leader sequence from alfalfa mosaic virus when used with RSV-F gene, the transient expression in apple leaf protoplasts increased by 5.5 fold. Site directed mutagenesis method is also applied sometimes to partially modify the coding sequence of the transgene to increase the expression level. Crystal protein genes of Bacillus thuringiensis were poorly expressed in plants, but partial modification of the gene sequence (3% nucleotide difference), cryIA (b) gene showed 10-fold higher level of expression, although it is quite low than the fully modified (21% nucleotide difference) cryIA(b) which had a 100-fold higher level of expression compared to the control one [58].

Optimization of Promoters and Terminators

The selection of a suitable promoter is one of the most important factors for over expressing a protein by increasing the transcription of the foreign gene by strong promoters. Promoter sequences may originate from plants or their pathogens as in case of native promoters or may be synthetic in nature. They may be constitutively expressed or inducible in nature. The most commonly used constitutive promoter is cauliflower mosaic virus 35S (CaMV 35S) promoter [59]. Constitutive promoters of plant origin include maize ubiquitin-1 promoter (ubi-1) and rice actin-1 promoter (act-1). Constitutive promoters of viral origin renders very high levels of expression, but some deleterious effects such as gene silencing via co-suppression have also been reported [60]. With the aid of constitutive promoters, widespread expression of foreign gene in plant cell is achieved and this may lead to accumulation of recombinant proteins in the cells leading to cellular toxicity.

This problem can be eliminated with the use of inducible or tissue specific promoters. Wound inducible defense gene derived promoters are well reported to be used for over expression of recombinant proteins in plants [61,62]. The light-inducible promoters like rubisco-small subunit dicot promoter for overexpressing E1 cellulase enzyme in tobacco [63] and chalcone synthase promoter from parsley [64] is well reported inducible promoters which triggers the expression of the protein in response to light. Expression of the foreign gene can be targeted to particular tissues of lower metabolic activity like seed embryo or endosperm by the use of tissue specific promoters. The maize globulin-1 promoter for targeting the protein expression to the embryo of monocots [65] is one of the popular tissue specific expression promoters. Other tissue specific promoters like sps1 promoter for leaf specific expression [66], patatin promoter for tuber-specific expression [67] are well characterized. Several other seed specific or embryo specific promoters [28] are also reported.
Several other promoters for various applications are also reported [37].

Further factors affecting the expression of foreign gene in host plant relates to the type of polyadenylation signal or transcription terminator to be used. Most common transcription terminator/poly(A) signals derived from the CaMV 35S RNA gene or the nopaline synthase (nos) gene from A. tumefaciens are successfully used in a wide variety of species including dicot and monocot plants [68]. The 3' noncoding regions of the Me1 gene has led to several fold enhancement in gene expression from different promoter sequences tested [69] and proved to be a promising one. The other promising candidate of plant-derived transcription terminator is wheat histone H3 gene [70].

**Protein Targeting**

Protein targeting is an important factor that has to be optimized for better output. After translation, proteins are folded and then translocated to intercellular locations in presence of molecular chaperones which protect the native from degradation. Protein targeting is essential for its stability, modification and in general for the survival of the plant. Proteins secreted in cytosol are often degraded by proteases and till date it is not possible to establish protease free plant line. On the other hand glycosylation and disulfide bridge formation to recombinant protein is only possible in the endoplasmic reticulum (ER) and golgi apparatus of plant cells [71-73] which makes it mandatory for the glycoproteins to be transported to ER or golgi apparatus after translation. Targeting proteins to intracellular organelles (ER, chloroplast, and vacuole) [74] or secretory pathway (like ER or golgi apparatus) will facilitate proper folding of proteins which is essential for their correct functioning and increased expression level [75,76]. It is reported that by incorporating C-terminal ER retention signal, 10-100 fold increases in target protein yield is possible as compared to targeting them to secretary pathway [77]. Enhanced accumulation of LT-B in tobacco and potato were observed by directing the recombinant protein to microsomes [78]. Targeting the recombinant protein for single chain variable fragments (ScFvs) towards ER enhances its antigen binding activity and the recombinant protein were reported to be stored for more than 3 weeks without loss of activity [79].

**Post Translational Modifications**

The proper biological activity, stability and folding of the recombinant protein depends on its modification after translation. Many proteins require complex post translational modification steps to assemble into the active multimeric form. Glycosylation is one of the important post translation modifications which have been shown to play a critical role for various physiological activities of glycoproteins [80]. The N-linked glycans which are generally found in plants are mostly of Man3GlcAc2 structure with or without β 1,2 xylose and or α1,3 fucose residues [81,82], but in the mammalian system the Man3GlcAc2 (M3) core structure is further extended with the addition of penultimate galactose and terminal sialic acid residues [83]. Although they are rarely found in plants, the complex type N-glycans is formed by the addition of additional α1,4 fucose and β 1,3 galactose residues giving rise to mammalian Lewis A like structures [84]. The presence of xylose and fucose residues in plant derived recombinant protein makes raises the problem of immunogenicity in mammals as they are generally absent in animal glycoprotein. One possible solution by transferring the human β 1,4-galactosyltransferase to tobacco results in the formation of in glycans with galactose residues and the absence of the dominant xylosidated and fucosylated type sugar chains confirms that the transformed cells are can produce glycoproteins that are no more immunogenic for mammals [85].

Phosphorylation is another post translational modification which is important for the recombinant enzymes that are only active after being phosphorylated. Animals mainly use tyrosine phosphorylation, while in plant both the serine-threonine phosphorylation and tyrosine kinase pathway is active [86]. Hence, proteins that require tyrosine phosphorylation for their function can safely be produced in plants and it extends the range of recombinant proteins made in plants to
include industrial enzymes, too [87].

**DOWNSTREAM PROCESSING OF RECOMBINANT PROTEIN**

Designing effective and commercially viable methods for the purification of the recombinant proteins is a most important part for commercializing plant based transgenic products. Although for edible vaccines which does not require purification, the plant or plant parts where the proteins are expressed, can directly be used for consumption while for others several strategies like affinity tag based purification, subcellular targeting, oleosin fusion protein production, have been developed.

In affinity tag based purification, the recombinant protein is produced as a fusion protein containing a small protein or peptide which has affinity to a specific ligand. Actually the vector sequence itself contains the coding sequence of the small peptide or protein that has to be tagged to the recombinant protein. The tagged protein or peptide sequence is eliminated from the transgenic protein by the use of external protease after the purification of the recombinant protein by affinity chromatography. The protease used for cleave is costly increasing the downstream processing cost and also the purification steps. Sometimes the affinity tag may alter the folding or processing of the recombinant proteins. Some common affinity tag and ligand pairs which are in use include histidine residues-metal ions (nickel or cobalt), maltose binding protein-amylose and *Staphylococcus A* protein –IgG. Human glucocerebrosidase -flag epitope fusion protein has been reported to be purified using affinity tag based method from tobacco [61].

The export of proteins out of cells or targeting them to various compartments within the cell can simplify the purification process, although this may sometimes interfere with the post-translational modifications of the recombinant protein. When the recombinant protein is produced by cell suspension culture and root culture, the purification of extracellular secreted protein from the culture medium is more easy and cost effective. Secretion of hepatitis B surface antigen by the tobacco cell suspension culture (suspension culture of NT-I cells) into cell culture medium is reported to be possible by the addition of ER retention signal. Some other examples include the targeting of human serum albumin (HSA) to the apoplastic space by fusing with tobacco pathogenesis related protein, PR-S signal sequence [88] and of xylanase which was fused with potato proteinase inhibitor II protein signal peptide to facilitate the secretion of the protein into apoplastic space [89]. Secreation of the recombinant protein through guttation fluid is also possible and also reported for tobacco plant by the incorporation of ER retention signal at ‘C’ terminus end [90]. Tobacco produces very less amount of guttation fluid and is not so suitable for this method. But tomato and some grasses which produce larger quantities of guttation fluid can preferentially be used for this method and may open up an alternative method for collecting recombinant proteins avoiding costly and time taking downstream processing steps. Targeting the recombinant protein to the oil bodies enables simple and rapid purification of the recombinant proteins as oil bodies from soluble contaminants by centrifugation. The central domain motif of the oleosin bears the signal sequences for their localization to oil bodies [91] and foreign proteins can be fused to either N or C terminal end of the sequence. Canadian company Sem BioSys uses this technology for the plant based protein production and purification [28].

Purification of the recombinant protein in a single step has also been reported with the help of chaperones which fold the protein into cuboidal crystals that can be purified simply by centrifugation [92]. Another novel approach for one step purification depends upon the hydrophobic interaction between the recombinant protein and the protein polymer based on the five repeated amino acid sequences, GVGVP. The GVGVP based polymers are encoded by synthetic genes and exists as an extended molecule at low temperature, but raising the temperature near the threshold level, the polymer hydrophobically folds into β-spirals and further aggregates due to hydrophobic association. Purification of recombinant
insulin in the form of insulin-polymer complex is reported to be done in one step.

SOME EXAMPLES OF THERAPEUTIC PROTEINS EXPRESSED IN PLANTS

Genetic engineering techniques extend to the level that many biopharmaceuticals like human serum albumin, α-interferon, enkephalins, granulocyte-macrophage colony stimulating factor, glucocerebrosidase, monoclonal antibodies and vaccines can be expressed and produced in plants. Since the early 1980’s plant genetic engineering focused basically on the improvement of the agronomic traits, which gradually shifted towards the production of recombinant pharmaceutically important proteins. The expressions of human erythropoietin in tobacco cells [93] and further patenting the production of lysosomal enzyme glucocerebrosidase in transgenic tobacco by Cramer and his colleagues in 1999 in USA hold promise for exploiting transgenic plants for the production of commercially viable enzymes. The production of hirudin, an important anti-coagulant in transgenic oil seed rape by the Canadian company Sem BioSys [94] is worth citing in this context. Lactoferrin, an important iron binding glycoprotein found in human milk, was expressed in potato and tomato plants [95] and also in rice [96]. Human milk protein β casein has also been reported to be expressed in potato [97]. Human α lactalbumin when expressed in tobacco plants along with galactosyltransferase, was found to be fully active in the synthesis of lactose [98]. Expression of bioactive human fibroblast growth factor 8b in tobacco plant [99] is also a promising example. Anemia due to iron deficiency is a common disease and attempts were taken to overcome the problem by transforming three genes (Ferritin, metallothionin like gene) in rice to increase iron content [100]. Rice plants engineered with carotenoid biosynthetic pathway genes for the production of β-carotene in the endosperm [101] is also a good attempt to overcome the vitamin A deficiency in human.

Vaccination is the safest way for disease prevention and more economical, too. But several limitations arise, like difficulty in culturing pathogen and preparing vaccine for contagious disease like Hepatitis B, Cervical cancer, appearance of disease in immuno-suppressed individuals after the use of certain live attenuated vaccines and presence of reactogenic components in whole inactivated vaccines (eg. Bordetella pertussis. The concept of producing vaccine in edible plant parts, popularly called edible vaccines [78,102] serve as an attractive alternative to develop cost effective and safe vaccines at low farming cost [22,103]. There are several reports citing the example of expressing full sized recombinant antibodies and single chain antibody fragments (ScFvs) in transgenic tobacco plants [9,104,76] and secretory IgA in potato tubers [105]. The produced recombinant antibody shows no loss of affinity. Human anti-herpes simplex virus (HSV) antibody expressed in soybean and the recombinant protein was fully efficient in preventing vaginal HSV-2 transmission in a mouse model [17]. Expression of antibody against carcinoembryonic antigen in rice and wheat [106] highlights on the possibility of antibody based cancer therapy. Recent success in expressing chimeric murine monoclonal antibody (mAb) 62-71-3 which is an anti-rabies antibody in Nicotiana benthamiana is promising. The recombinant antibody was shown to be glycosylated and had good affinity to rabies virus [107].

CONCLUSIONS

With the progress in plant biotechnology and new innovations in the agriculture, food and pharmaceutical industry, the area of research in developing plants as a vehicle for the industrial and pharmaceutical products is flourishing. The demand for new biopharmaceuticals for treating different infectious diseases as well as the existing one is increasing. Attention have been paid to evaluate different host plant species for their performance as alternative production system, optimization of transformation strategy, foreign gene expression, downstream processing steps for the availability of safe recombinant biopharmaceuticals in a cost-effective manner. When all the optimized parameters will be fully integrated, the final outcome will surely be the best. But unlike simple unicellular systems, plants are more complex system and
integrating all these parameters needs more time and effort. There are several regulatory issues and risk factors associated with the use of recombinant molecules that are the major public concern put a question mark on the use of recombinant products. The purity, safety and reliability of the plant derived biopharmaceuticals have to be addressed. Close cooperation between learned professional from medical, industrial field and plant biotechnologist to address the issues will be beneficial. There is no question on the beneficial effect of the recombinant technology on common people. Recombinant plant and plant products which are already commercially available indicates the positive trend among common people for the acceptance of the recombinant products. The technology advances and the possibility of safe large-scale production of biopharmaceuticals in cost effective manner using plants or plant cell culture as bioreactor appears very promising.

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