REVIEW ARTICLE

Transgenic plants for the production of edible vaccines and antibodies for immunotherapy

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Vaccines and antibodies play a key role in healthcare. However, the cost of production and maintaining a chain for vaccine distribution has so far hampered realizing their full potential. Expression of antigens as vaccines, and of antibodies against antigens of pathogens in transgenic plants is a convenient and inexpensive source for these immunotherapeutic molecules. Various antigens and antibodies have already been expressed successfully in plants and have been shown to retain their native functional forms. Edible plant vaccine against diarrhoea, expressed in potato, and antibody against dental caries, expressed in tobacco, is already in preclinical human trials. Attempts are being made to express many proteins of immunotherapeutic use at high levels in plants and to use them as bio-reactors of the modern era.

MOST of the drugs used by man, until very recently, were being derived from plants, which subsequently led to pharmaceutical companies starting chemical synthesis of the medicinal compounds. Recent progress in the area of transgenic plants has, however, once again attracted attention of the scientists, and plants are being looked upon as potential bio-reactors or bio-factories for the production of immunotherapeutic molecules. Transgenic material, in the form of seed or fruit, can be easily stored and transported from one place to another without fear of its degradation or damage. Furthermore, a large amount of bio-mass can be easily produced by cultivation in fields with relatively few inputs. In addition, transgenic plants capable of producing several different products can be created at any given time by crossing plants producing different products.

It was therefore not surprising when in 1989 Hiatt and co-workers[1], attempted to produce antibodies in plants which could serve the purpose of passive immunization. Though the first report on production of edible vaccine appeared in 1990 in the form of a patent application[2], the concept of edible vaccine got impetus after Arntzen and co-workers[3] expressed hepatitis B surface antigen in tobacco in 1992 to produce immunologically active ingredient via genetic engineering of plants. This generated a good deal of excitement among biotechnologists, particularly in light of the potential of edible vaccines and antibodies for immunotherapy for countries like India. In this paper we highlight the facts on this state-of-the-art technology and its potential for therapy.

Transgenic plants for immunotherapy

Since 1984, when transformation of tobacco – the first plant to be transformed with a foreign gene – was reported[4], great effort has gone into developing efficient methods for genetic transformation of plants, and optimizing expression of foreign genes in plants. The techniques used to introduce foreign genes into plants have been extended to major crops, including vegetables, as well as into ornamental, medicinal, fruit, tree and pasture plants[5]. Various foreign proteins including serum albumin, human α-interferon, human erythropoetin, and murine IgG and IgA immunoglobulins have been successfully expressed in plants[6]. In recent years, several attempts have been made to produce various antigens and antibodies in plants[2,7]. Antigens or antibodies expressed in plants can be administered orally as any edible part of the plant, or by parenteral route (such as intramuscular or intravenous injection) after isolation and purification from the plant tissue. The edible part of the plant to be used as a vaccine is fed raw to experimental animals or humans to prevent possible denaturation during cooking, and avoid cumbersome purification protocols.

While *Agrobacterium*-mediated transformation still remains the method of choice for dicots, a general method, the biolistics method, of transformation of plants, including monocots, has come into existence[5,8]. Various strategies for expression of foreign genes in high amounts in plants include use of strong and organ-specific plant promoters, targeting of the protein into endoplasmic reticulum (ER) by incorporating ER-targeting and ER-retention signals, creation of optimized translation start site context as well as alteration of codons to suit the expression of prokaryotic genes in a plant[9,10]. Though promoters of genes, like maize ubiqutin and rice actin, have been reported to direct

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high level of expression in monocots, the 35S promoter of cauliflower mosaic virus remains the promoter of choice for dicots[11]. Targeting of the protein to appropriate cellular compartment may be helpful in stabilizing the protein. Retention of heat labile *E. coli* enterotoxin in ER of potato by using ER-retention signal has been reported to elevate the expression levels of the recombinant protein[12]. Though signals for membrane targeting, protein folding, oligomerization and *N*-glycosylation are highly conserved in animals and plants[13], while expressing bacterial proteins targeted to ER, it is important to consider the sequence of a signal peptide as the signal peptide for targeting to periplasmic space in bacterium may not be equally efficient in plants. Substitution of signal peptide of bacterial origin with a plant-specific ER-targeting sequence was observed to dramatically increase the glycosylation and secretion efficiency of chitinase[14]. For production of edible vaccines or antibodies, it is desirable to select a plant whose products are consumed raw to avoid degradation during cooking. Thus, plants like tomato, banana and cucumbers are generally the plants of choice. While expression of a gene that is stably integrated into the genome allows maintenance of the material in the form of seeds, some virus-based vectors can also be used to express the gene transiently to develop the products in a short period (Figure 1). This may have the additional advantage of allowing expression of the product at very high level; not always attainable in transgenic systems.

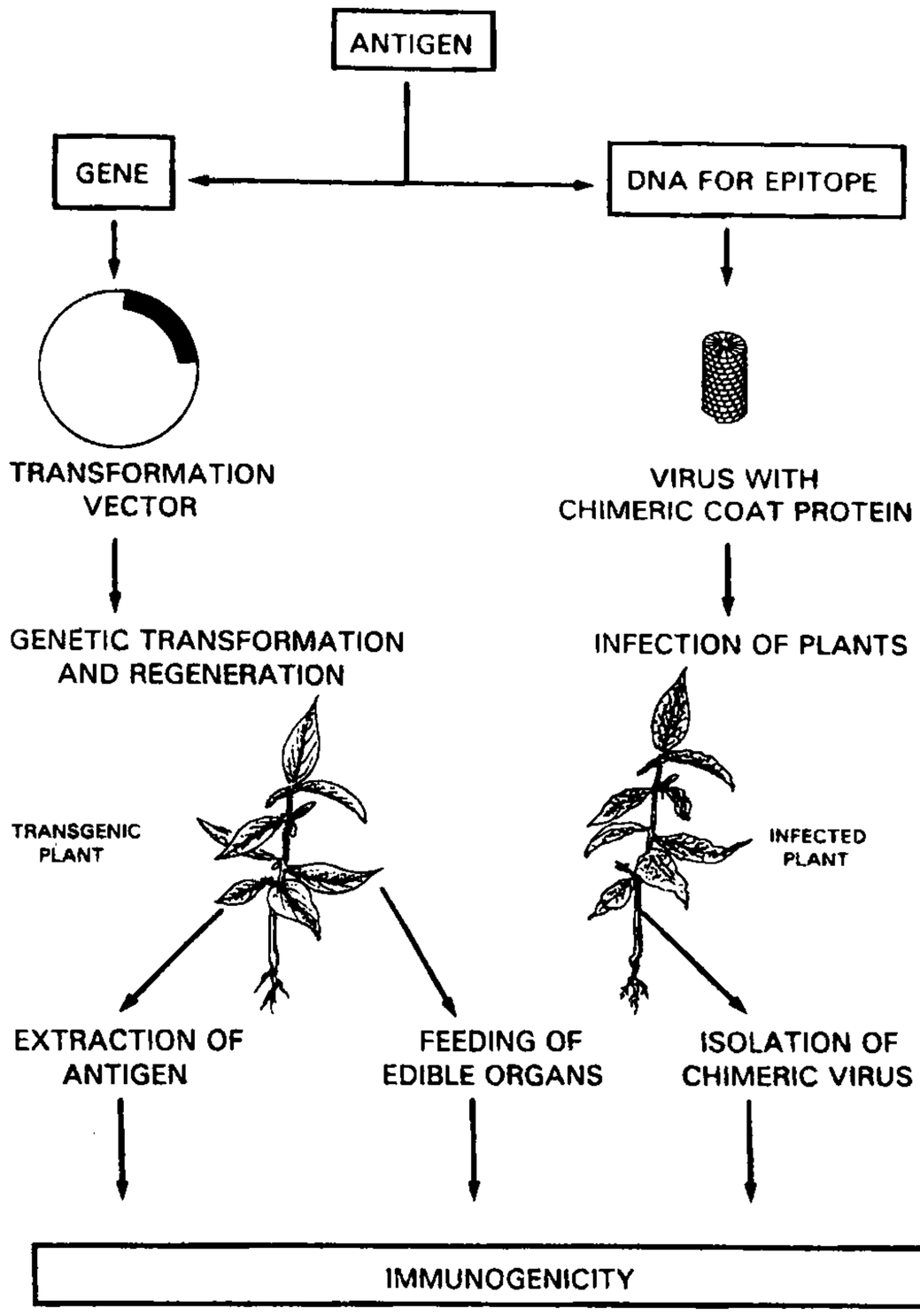

Figure 1. Strategies for expression of antigens in plants[2].

Vaccines

While plant system may have the capability of producing any vaccine in large amounts and in a less expensive manner, purification of the product may require the use of existing or even more cumbersome procedures. Attention therefore has been paid to mainly those antigens that stimulate mucosal immune system to produce secretory IgA (S-IgA) at mucosal surfaces, such as gut and respiratory epithelia. In general, a mucosal response is achieved more effectively by oral instead of parenteral delivery of the antigen. Thus, an antigen produced in the edible part of a plant can serve as a vaccine against several infectious agents which invade epithelial membranes. These include bacteria and viruses transmitted via contaminated food or water, and resulting in diseases like diarrhoea and whooping cough.

Table 1. Antigens produced in transgenic plants

Protein	Plant	Refs
Hepatitis B surface antigen	Tobacco	3, 15
Rabies virus glycoprotein	Tomato	22
Norwalk virus capsid protein	Tobacco	16
E. coli heat-labile enterotoxin B subunit	Potato	12, 17
Cholera toxin B subunit	Potato, tobacco	18–20
Mouse glutamate decarboxylase	Potato	21
VP1 protein of foot-and-mouth disease virus	*Arabidopsis*	23
Insulin	Potato	24
Glycoprotein of swine-transmissible gastroenteritis coronavirus	*Arabidopsis*	25

The first report of the production of edible vaccine (a surface protein from *Streptococcus*) in tobacco, at 0.02% of total leaf protein level, appeared in 1990 in the form of a patent application published under the International Patent Cooperation Treaty[2]. Subsequently, a number of attempts were made to express various antigens in plants[15–25] (Table 1). Since acute watery diarrhoea is caused by enteroxigenic *Escherichia coli* and *Vibrio cholerae* that colonize the small intestine and produce one or more enterotoxin, an attempt was made towards the production of edible vaccine by expressing heat-labile enterotoxin (LT-B) in toabacco and potato[12].

The enterotoxin (LT) from *E. coli* is a multimeric protein, quite similar to cholera toxin (CT) structurally, functionally and antigenically. LT has one A subunit

(27 kDa) and a pentamer of B subunits (11.6 kDa). Binding of the non-toxic LT-B pentamer to GM_1 gangliosides, present on epithelial cell surfaces, allows entry of the toxin LT-A subunits into the cells. LT-B and CT-B are both potent oral immunogens. An oral vaccine composed of the cholera toxin-B subunit (CT-B) with killed *V. cholerae* cells has been reported to give significant level of protection against cholera[26]. But the cost of production of CT-B by conventional methods is too high to allow distribution of this vaccine. The recombinant LT-B (rLT-B) produced in tobacco and potato showed partial pentamerization after the engineering of subunit gene in a way that allowed retention of the protein in microsomal vesicles. On testing immunogenicity of rLT-B by feeding potato tubers to mice, both humoral and mucosal immune responses were reported to be stimulated. This vaccine has gone through pre-clinical trials in humans. The antigenic protein retained its immunogenecity after purification from the transgenic potato expressing it[27]. Fourteen healthy individuals, who ate 50–100 g raw potatoes, were screened for gut-derived antibody secreting cells (ASC), which were detectable 7–10 days after immunization. Presence of both anti-LT IgA-secreting cells and anti-LT IgG-secreting cells was detected in the peripheral blood.

Cholera toxin, which is very similar to *E. coli* LT, has also been expressed in plants. Hein *et al.*[18] generated tobacco plants expressing CT-A or CT-B subunits of the toxin. CT-A produced in plant was not cleaved into A1 and A2 subunits, which happens in epithelial cells. Plants expressing CT-B showed the presence of a protein that migrated to the same position in denaturing gel as the CT-B derived from *V. cholerae*, and was recognized by mouse anti-CT-B antibody. Cholera toxin-B subunit, when expressed in potato, was processed in a natural way: the pentameric form (the naturally occurring form) being the abundant form. Antigenically it was found to be similar to the bacterial protein. Even after boiling transgenic potato tubers till they became soft, approximately 50% of the CT-B was present in the pentameric GM_1 ganglioside-binding form[19,20].

Similarly, a rabies virus coat glycoprotein gene has been expressed in tomato plants[22]. The protein that was expressed had molecular mass of 62 kDa compared to 66 kDa observed from virus grown in BHK cells. Since the orally administered protein elicited protective immunity in animals, it was expected that continued efforts would lead to development of an edible oral vaccine against rabies which could be used as a preventive strategy. While the results with antigenic properties of the components produced in plants are encouraging, their value as a vaccine can be improved by providing other adjuvants which either enhance the immunogenic potential or reduce the degradation of the active ingredient by the gut microflora.

The Hepatitis B surface antigen (HBsAG) has been reported to accumulate to 0.01% of soluble protein level in transgenic tobacco[3]. The antigens, delivered in a macromolecular form, are known to survive the gut atmosphere and perform better. Interestingly, the recombinant HBsAG was recovered in virus-like particles of 22 nm diameter (similar to yeast-derived HBsAG-based vaccine) which is known to be a prerequisite for better immunogenicity. A crude extract from plants was used for parenteral immunization in mice. The immune response included all IgG subclasses as well as IgM against hepatitis B[15]. Carrillo *et al.*[23] expressed structural protein, VP1, of foot-and-mouth disease virus in *Arabidopsis*. The mouse that was immunized intraperitoneally with a leaf extract elicited immune response to synthetic peptides carrying various epitopes of VP1, or to complete VP1. Furthermore, all the mice immunized with the leaf extract were protected against challenge with virulent foot-and-mouth disease virus.

One of the alternative strategies of producing a plant-based vaccine is to infect the plants with recombinant viruses carrying the desired antigen that is fused to viral coat protein. The infected plants have been reported to produce the desired fusion protein in large amounts in a short time. The technique involved either placing the gene downstream a subgenomic promoter, or fusing the gene with capsid protein that coats the virus (Table 2, Figure 1). The latter strategy is perhaps the strategy of choice since fusion proteins in particulate form are highly immunogenic. It should, however, be kept in view that recombinant viruses need to be highly purified for parenteral administration or partially purified for oral administration. Modelska *et al.*[29] have shown that immunization of mice intraperitoneally or orally by gastric incubation or by feeding of plants infected with the recombinant alfalfa mosaic virus (AIMV) carrying rabies peptide CPDrg 24 mounted local as well as systemic immune response. Oral administration could stimulate both serum IgG as well as IgA synthesis. After immunization, 40% of the mice were protected against the challenge with a lethal dose of the virus.

Table 2. Transient production of antigens in plants after infection with plant viruses expressing a recombinant gene

Protein	Plant	Carrier	Refs
Influenza antigen	Tobacco	TMV	28
Murine zona pellucida antigen	Tobacco	TMV	28
Rabies antigen	Spinach	AIMV	29
HIV-1 antigen	Tobacco	AIMV	30
Mink enteritis virus antigen	Black eyed bean	CPMV	31
Colon cancer antigen	Tobacco	TMV	32

AIMV, alfalfa mosaic virus; TMV, tobacco mosaic virus; CPMV, cowpea mosaic virus.

Likewise, a 13-amino-acid epitope of zona pellucida, ZP3, protein and another epitope from malarial sporozoites have been expressed as fusion proteins with TMV capsid protein with the idea of developing anti-fertility and anti-malarial vaccines[28]. The antigenicity of the products has been found to be positive. The same is true for epitopes derived from human immunodeficiency virus which were expressed as alfalfa mosaic virus coat protein fusion products[30]. Recently, scientists at Axis Genetics, Cambridge, have shown that injecting mink with extracts of plants infected with a cowpea mosaic virus, that expresses a mink enteritis antigen gene, protects the animal against subsequent virus challenge[31]. While much remains to be done, indications are that plant-based vaccines can compete with vaccines produced by other approaches, particularly keeping in view the low cost and ease of production/distribution.

Modulation of immune response to acquire immune tolerance

One of the utilities of producing antigens in plants in large amount is in treatment of autoimmune diseases like diabetes mellitus which involve production of antibodies against glutamic acid decarboxylase (GAD) and insulin, leading to destruction of insulin-producing pancreatic cells[33,34]. The antigens targeted for autoimmune response can be fed to the animals to induce immune tolerance. However, since the use of antigens for inducing oral tolerance requires production in large amounts of the human antigens that are generally difficult to produce by conventional means, attempts have been made to produce such antigens in plants. Insulin[24] and GAD[21] have been produced in potato and tobacco, respectively. To direct the delivery of plant-synthesized insulin to the gut-associated lymphoid tissue, insulin was linked to cholera-toxin B subunit. Non-obese diabetic mice which were fed with the transformed potato tuber tissue containing microgram level of the recombinant insulin delayed the progression of clinical diabetes. Similarly, GAD-producing tobacco plants, given as a dietary supplement, inhibited the development of diabetes in the non-obese diabetic mouse.

Expression and assembly of antibodies in plants

Transgenic plants are also being looked upon as a source for producing large-scale antibodies which can serve the purpose of passive immunization by direct application, in addition to providing a tool for drug targeting or interactive inactivation of undesirable molecules[7,35]. Gene technology has provided great impetus to the utility of antibodies, since antibody genes can be altered to order. Thus not only genes coding for both the

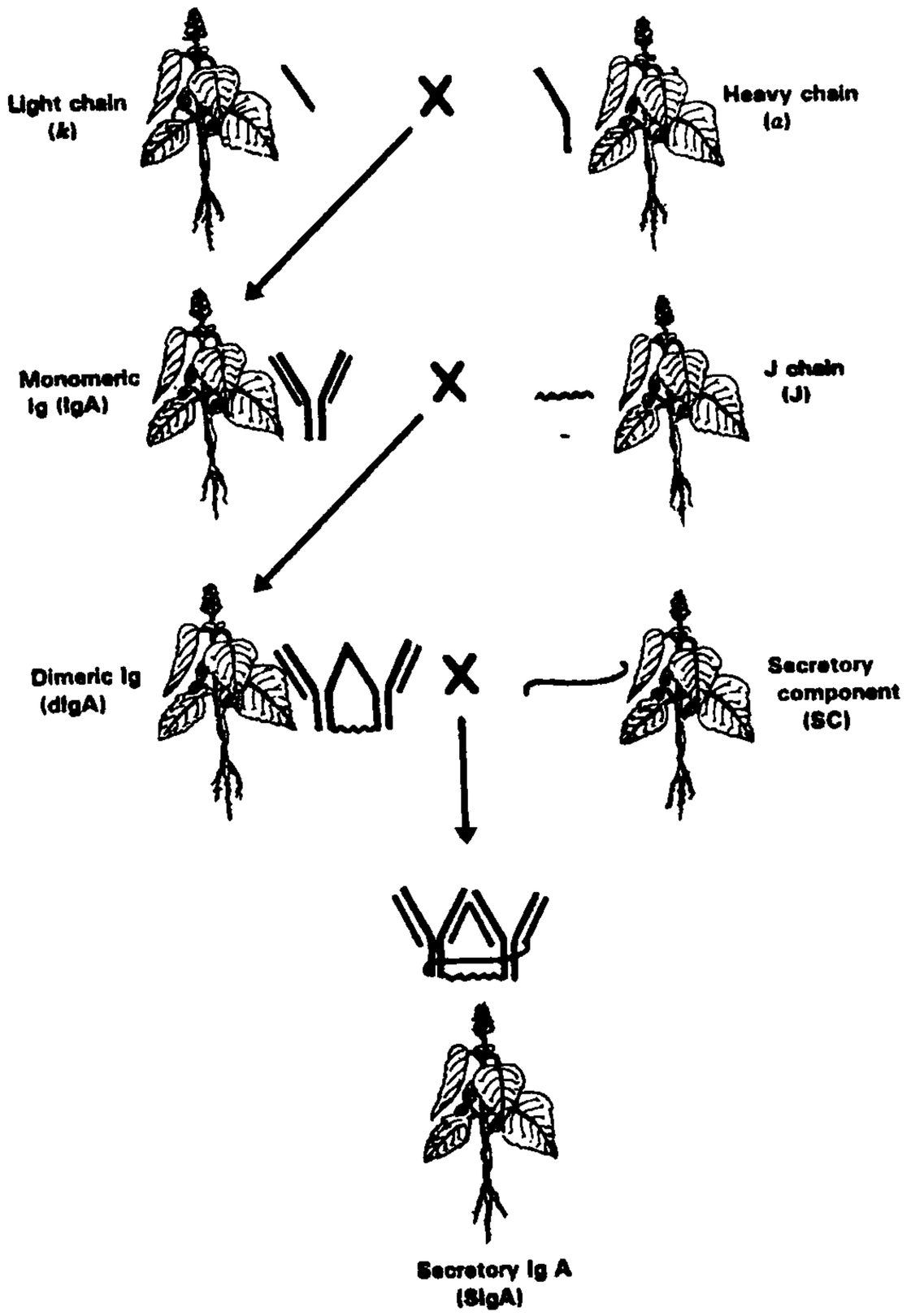

Figure 2. Strategy for production of secretory antibody in plants[7].

light and heavy chains have been expressed, but modified genes capable of expressing only Fab fragments (assembled light chains and shortened heavy chains) or scFV (single peptide chains where variable domains of heavy and light chains are covalently linked by a short flexible peptide) have also been expressed in bacteria and mammalian cells[36–48] (Figure 2, Table 3). Murine antibodies have been humanized by changing the constant and framework domains. In addition, recent technology involving PCR and phage display allow cloning and screening of antibodies with suitable avidity easily.

Transgenic plants not only provide the means to express antibodies but also enable the glycosylation and entry into secretory pathway which allow assembly of complete antibodies and Fab fragments. Variable fragments (Fv) can be produced in cytosol, directed to different compartments and fused with proteins such as protein A and phosphatase to improve the detection and purification of single chain Fv (scFv). In plants, antibody production (1–5% of total plant protein) has been

Table 3. Antibodies and antibody fragments produced in transgenic plants

Antibody	Antigen	Plant	Refs
IgG (*k*)	Transition stage analog	Tobacco	1
IgM (*λ*)	NP(4-hydroxy-3-nitrophenyl)acetyl hapten	Tobacco	36
Single domain (dAb)	Substance P	Tobacco	37
Single chain Fv	Phytochrome	Tobacco	38
Single chain Fv	Artichoke mottled crinkle virus coat protein	Tobacco	39
Fab; IgG (*k*)	Human creatin kinase	*Arabidopsis*	40
IgG (*k*)	Fungal cutinase	Tobacco	41
IgG (*k*) and SIgG/A hybrid	*S. mutans* adhesin	Tobacco	42, 43
Single chain Fv	Abscisic acid	Tobacco	44
Single chain Fv	Nematode antigen	Tobacco	45
Single chain Fv	*β* glucuronidase *β*-1,4 endoglucanase	Tobacco	46
Single chain antibody fragment	Atrazine, Paraquat	Tobacco	47
IgG	Glycoprotein B of herpes simplex virus	Soybean	48

achieved by cross-pollination of individually transformed plants expressing light or heavy chains[2]. Other approaches involve double transformation, or transformation by constructs having genes for both light and heavy chains on the same vector. Despite the fact that production of antibodies in plants takes longer, the low cost of production and capability of increasing production simply by increased propagation make plant antibodies an attractive proposition.

Aiming at therapeutic treatment, Ma and co-workers[43] have succeeded in producing multimeric secretory IgA (SIgA) molecules in plants which represent the predominant form of immunoglobulin in mucosal secretions. SIgA not only contains heavy and light chains but it is also dimerized by a J chain, and protected from proteolysis by a fourth polypeptide, the SC. Production of such antibodies in mammalian cells is very complex because of the requirement of B cell as well as gut epithelial cells for the formation of the SIgA. Thus, four transgenic tobacco plants were produced by genetic engineering which produced a murine monoclonal antibody light *k* chain, the hybrid IgA-G antibody heavy chain, murine J chain and rabbit secretory component. A series of sexual crosses was carried out to allow expression of all the four proteins simultaneously. The progeny produced a functional secretory immunoglobulin very efficiently. This demonstrated the potential of plants in assembly of antibodies, and the flexibility of system (Figure 2). Recently, a humanized monoclonal antibody against glycoprotein B of herpes simplex virus 2 (HSV-2) has been expressed in soybean. This antibody was found to possess the same efficacy for prevention of vaginal HSV-2 infection in mice and similar stability in human semen as the antibody expressed in human cell culture[48].

Topical application of antibodies has already been shown to control infection by way of passive immunization. A hybrid monoclonal antibody (IgA/G), having constant regions of IgG and IgA fused, has been used successfully against human dental caries caused by the bacterium *Streptococcus mutans*[42]. Ma *et al.*[49] compared the secretory antibody generated in transgenic tobacco (SIgA/G) and the original mouse IgG. Though both had similar binding affinity to surface adhesion protein of *S. mutans*, SIgA/G survived for 3 days in the oral cavity, whereas IgG could survive for just one day. The plant antibody provided protection against the colonization of the *S. mutans* for at least four months. These results show that this strategy could be useful for many other mucosal infections in humans and animals.

Prospects

Although the first human clinical trials for plant-based vaccine[27] and antibody[42] have been performed recently, many challenges including maximization of expression levels, stabilization during post-harvest storage, remain to be met. Edible vaccines can be improved for their oral immunogenecity by the use of appropriate adjuvant which could be used either as a fusion to the candidate gene or as an independent gene. Concern about immune tolerance and allergy to plant-based vaccines has been expressed and needs to be addressed suitably. In addition, we need to select the best targets for vaccine or antibody production in plants, particularly from the viewpoint of edible nature of plant parts. Antigens produced by diarrhoea and whooping cough causing organisms are promising candidates. It is also desirable that the concept of edible vaccine should first be tested in animals. For this purpose, edible vaccine against rabies and anthrax diseases may be considered as suitable to target dogs and cattle.

1. Hiatt, A., Cafferkey, R. and Bowdish, K., *Nature*, 1989, **342**, 76–78.
2. Mason, H. S. and Arntzen, C. J., *Trends Biotechnol.*, 1995, **13**, 388–392.
3. Mason, H. S., Lam, D. M. and Arntzen, C. J., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 11745–11749.
4. De Block, M., Herrera-Estrella, L., van Montagu, M., Schell, J. and Zambryski, P., *EMBO J.*, 1984, **3**, 1681–1689.
5. Birch, R. G., *Annu. Rev. Plant Physiol. Mol. Biol.*, 1997, **48**, 297–326.
6. Cramer, C. L., Weissenborn, D. L., Oishi, K. K., Grabau, E. A., Bennett, S., Ponce, E., Grabowski, G. A. and Radin, D. N., *Ann. NY Acad. Sci.*, 1996, **792**, 62–71.
7. Ma., J. K. and Hein, M. B., *Trends Biotechnol.*, 1995, **13**, 522–527.
8. Christou, P., *Plant Mol. Biol.*, 1997, **35**, 197–203.
9. McElroy, D. and Brettell, R. I. S., *Trends Biotechnol.*, 1994, **12**, 62–68.

10. Tyagi, A. K., Bajaj, S., Mohanty, A., Chaudhury, A. and Maheshwari, S. C., *Crit. Rev. Biotechnol.*, 1999, **19**, 41–79.
11. Schledzewski, K. and Mendel, R. R., *Transgenic Res.*, 1994, **3**, 249–255.
12. Haq, T. A., Mason, H. S., Clements, J. D. and Artnzen, C. J., *Science*, 1995, **268**, 714–715.
13. Chrispeels, M. J. and Tague, B. W., *Int. Rev. Cytol.*, 1991, **125**, 1–45.
14. Lund, P. and Dunsmuir, P., *Plant Mol. Biol.*, 1992, **18**, 47–53.
15. Thanavala, Y., Yang, Y.-F., Lyons, P., Mason, H. S. and Arntzen, C., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 3358–3361.
16. Mason, H. S., Ball, J. M., Shi, J. J., Jiang, X., Estes, M. K. and Arntzen, C. J., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 5335–5340.
17. Mason, H. S., Haq, T. A., Clements, J. D. and Arntzen, C. J., *Vaccine*, 1998, **16**, 1336–1343.
18. Hein, M. B., Yeo, T.-C., Wang, F. and Sturtevant, A., *Ann. NY Acad. Sci.*, 1996, **792**, 51–56.
19. Arakawa, T., Chong, D. K. X., Merritt, J. L. and Langridge, W. H. R., *Transgenic Res.*, 1997, **6**, 403–413.
20. Arakawa, T., Chong, D. K. X. and Langridge, W. H. R., *Nature Biotechnol.*, 1998, **16**, 292–297.
21. Ma, S.-W., Zhao, D. L., Yin, Z. Q., Mukherjee, R., Singh, B., Quin, H. Y., Stiller, C. R. and Jevnikar, A. M., *Nature Med.*, 1997, **3**, 793–796.
22. McGarvey, P. B., Hammond, J., Dienelt, M. M., Hooper, D. C., Fu, Z. F., Dietzschold, B., Koprowski, H. and Michaels, F. H., *BioTechnology*, 1995, **13**, 1484–1487.
23. Carrillo, C., Wigdorovitz, A., Oliveros, J. C., Zamorano, P. I., Sadir, A. M., Gomez, N., Salinas, J., Escribano, J. M. and Borca, M. V., *J. Virol.*, 1998, **72**, 1688–1690.
24. Arakawa, T., Yu, J., Chong, D. K. X., Hough, J., Engen, P. C. and Langridge, W. H. R., *Nature Biotechnol.*, 1998, **16**, 934–938.
25. Gomez, N., Carrillo, G. N., Salinas, J., Parra, F., Borca, M. V. and Escribano, J. M., *Virology*, 1998, **249**, 352–358.
26. Svennerholm, A.-M., Jertborn, M., Gothefors, L., Karim, A. M. M. M., Sack, D. A. and Holmgren, J., *J. Infect. Dis.*, 1984, **149**, 884–893.
27. Tacket, C. O., Mason, H. S., Losonsky, G., Clements, J. D., Levine, M. M. and Arntzen, C. J., *Nature Med.*, 1998, **4**, 607–609.
28. Beachy, R. N., Fitchen, J. H. and Hein, M. B., *Ann. NY Acad. Sci.*, 1996, **792**, 43–49.
29. Modelska, A., Dietzschold, B., Sleysh, N., Fu, Z. F., Steplewski, K., Hooper, D. C., Koprowski, H. and Yusibov, V., *Proc. Natl. Acad. Sci. USA*, 1997, **95**, 2481–2485.
30. Yusibov, V., Modelska, A., Steplewski, K., Agadjanyan, M., Weiner, D., Hooper, D. C. and Koprowski, H., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 5784–5788.
31. Dalsgaard, K., Uttenthal, A., Jones, T. D., Xu, F., Merryweather, A., Hamilton, W. D. O., Langeveld, J. P. M., Boshuizen, R. S., Kamstrup, S., Lomonossoff, G. P., Porta, C., Vela, C., Casal, J. I., Meloen, R. H. and Rodger, P. B., *Nature Biotechnol.*, 1997, **15**, 248–252.
32. Verch, T., Yusibov, V. and Koprowski, H., *J. Immunol. Methods*, 1998, **220**, 69–75.
33. DeAizpura, H. J., Wilson, Y. M. and Harrison, L. C., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 9841–9845.
34. Tisch, R. and McDevitt, H., *Cell*, 1996, **85**, 291–297.
35. Hiatt, A. and Mostov, K., in *Transgenic Plants: Fundamentals and Applications* (ed. Hiatt, A.), Marcel Dekker, Inc, New York, 1993, pp. 221–237.
36. During, K., Hippe, S., Kreuzaler, F. and Schell, J., *Plant Mol. Biol.*, 1990, **15**, 281–293.
37. Benvenuto, E., Ordas, R. J., Tavazza, R., Ancora, G., Biocca, S., Cattaneo, A. and Galeffi, P., *Plant Mol. Biol.*, 1991, **17**, 865–874.
38. Owen, M., Gandecha, A., Cockburn, B. and Whitelam, G., *Biotechnology*, 1992, **10**, 790–794.
39. Tavladoraki, P., Benvenuto, E., Trinca, S., De Martinis, D., Cattaneo, A. and Galeffi, P., *Nature*, 1993, **366**, 469–472.
40. De Neve, M., De Loose, M., Jacobs, A., Van Houdt, H., Kaluza, B., Weidle, U. and Depicker, A., *Transgenic Res.*, 1993, **2**, 227–237.
41. Van Engelen, F. A., Schouten, A., Molthoff., J. W., Roosien, J., Salinas, J., Dirkse, W. G., Schots, A., Bakkar, J., Gommers, F. J., Jongsma, M. A., Bosch, D. and Stiekema, W. J., *Plant Mol. Biol.*, 1994, **26**, 1701–1710.
42. Ma, J. K.-C., Lehner, T., Stabilla, P., Fux, C. I. and Hiatt, A., *Eur. J. Immunol.*, 1994, **24**, 131–138.
43. Ma, J. K.-C., Hiatt, A., Hein, M. B., Vine, N., Wang, F., Stabila, P., Van Dolleweerd, C., Mostov, K. and Lehner, T., *Science*, 1995, **268**, 716–719.
44. Artsaenko, O., Peisker, M., Zurniedan, U., Fielder, U., Weiler, E. W., Muntz, K. and Conrad, U., *Plant J.*, 1995, **8**, 745–750.
45. Baum, T. J., Hiatt, A., Parrott, W. A., Pratt, L. H. and Hussey, R. S., *Mol. Plant Microbe Interact.*, 1996, **9**, 382–387.
46. Schouten, A., Roosien, J., de Boer, J. M., Wilmink, A., Rosso, M. N., Bosch, D., Stieckema, W. J., Gommers, F. J., Bakker, J. and Schots, A., *FEBS Lett.*, 1997, **415**, 235–241.
47. Longstaff, M., Newell, C. A., Boonstra, B., Strachan, G., Learmonth, D., Harris, W. J., Porter, A. J. and Hamilton, W. D., *Biochem. Biophys. Acta*, 1998, **1381**, 147–160.
48. Zeitlin, L., Olmsted, S. S., Moench, T. R., Co, M. S., Martinell, B. J., Paradkar, V. M., Russell, D. R., Queen, C., Cone, R. A. and Whaley, K. J., *Nature Biotechnol.*, 1998, **16**, 1361–1364.
49. Ma, J. K.-C., Hikmat, B. Y., Wycoff, K., Vine, N. D., Chargelegue, D., Yu, L., Hein, M. B. and Lehner, T., *Nature Med.*, 1998, **4**, 601–606.

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