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REVIEW

A banana or a syringe: journey to edible vaccines

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Abstract Constant emergence of diseases, along with the expanding size of world population creates demands for newer vaccines which can meet the challenges that conventional vaccines have not been able to overcome. The application of transgenic plants in the production of pharmaceuticals has led to the new approach of plant-based, orally-delivered vaccines. In recent years a number of recombinant vaccine antigens have been expressed in different plant tissues. The review highlights the generation of edible vaccines, their mode of action and their clinical application in various human diseases. Though the road ahead seems promising, there are several constraints which restrict the success and public acceptability of these vaccines. These include problems of choice of plants, storage, delivery, dosage, safety, public perception, quality control and licensing.

Keywords Vaccination · Genetically modified plants · Edible vaccines · Immune response · Clinical trials

Introduction

Search for an easier and affordable means of immunization has led researchers to the idea of using fruit and vegetable plants as factories for synthesizing vaccines known as "edible vaccines". Invented by Charles J. Arntzen (Biodesign institute, Arizona State University) these subunit vaccines are made up of antigens that can be grown in genetically modified plants and delivered through the edible parts of the plant (Arntzen 1997). They do not contain the genes responsible for pathogenesis, making them safe as they can generate an immune response in the body without causing disease. Edible vaccines are likely to overcome the hurdles posed by traditional vaccines, as they can be delivered without needles, do not require refrigeration and can be made, less expensively, right in the area in which they will be delivered.

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Transgenic plants and edible vaccines

The concept of modifying food crops to achieve better health is nothing new. For centuries, food crops have been altered through selective breeding (Goodman et al. 1987). A selected gene from a related or a completely different species can be transferred to another to achieve better characteristics like improved nutritional quality, insect resistance, herbicide resistance, disease resistance or salt tolerance. Ever since the transformation of the tobacco plant (Horsch et al. 1984), efficient methods for genetic transformation and optimizing expression of foreign genes in plants have been explored.

Many pharmacologically relevant proteins have been produced in a diverse range of crops e.g. human growth hormone in transgenic tobacco (Barta et al. 1986), antibody expressed in tobacco (Hiatt et al. 1992), milk proteins (Chong et al. 1997), human serum albumin in tobacco (Daniell 2003), many industrial enzymes (Hood et al. 2003), and new protein polymers with both medical and industrial uses (Ruggiero et al. 2000). The use of transgenic plants for the large-scale production of heterologous

proteins is gaining worldwide recognition, and could be a take-off point for the cost-effective production of vaccine proteins also (Mishra et al. 2008).

Plants for vaccine production

Plants used for oral vaccine production should produce edible parts that can be consumed uncooked since vaccine antigens are heat sensitive. Moreover it should be rich in protein because vaccine protein will only be a small percentage (0.01–2.0%) of the total protein content of a plant. It should grow widely, be capable of being transformed by genetic engineering and should not produce toxic products (Prakash 1996). Different plants like tobacco, banana, potato, tomato, lettuce, rice and papaya have been used for the production of edible vaccines (Table 1) for preventing a number of human diseases like diarrhea, cholera, anthrax, measles etc. (Mishra et al. 2008).

Generation of transgenic plants is species dependent and takes 3–9 months. Foreign DNA integrates randomly into the plant genome, resulting in a different gene expression level for each independent line, so that 50–100 plants are transformed together at a time. Transformed cells and whole plants expressing the desired product are selected using the antibiotic resistance genes. The plant expressing the highest levels of antigen and least number of adverse effects is selected. Each antigen so expressed in plants is tested for its proper assembly and verified by animal studies, Western blot; and quantified by enzyme-linked immunosorbent assay (Haq et al. 1995).

Creating edible vaccines

The concept of edible vaccines got impetus after Arntzen and co-workers expressed hepatitis B surface antigen in tobacco (Mason et al. 1992). These vaccines are prepared by introducing the desired gene into plants and inducing these genetically modified plants to manufacture the encoded protein. Among the different epitopes, protective ones can be selected for vaccine development. Some methods for the preparation of edible vaccines are as follows.

Using Ti plasmid

Agrobacterim tumefaciens known as the 'natural genetic engineer' is a widely used vector for carrying out transformation in plants. This soil bacterium possesses a circular Ti (tumor inducing) plasmid (200 Kb) that contains genes for synthesis of cytokinin, auxin and phytohormones. The bacterium transfers T-DNA of its plasmid into the plant genome upon infection, and forms a mass of cells known as crown gall at the wound site. The plasmid is disarmed by

Table 1 Characteristics of various plant crops used for the production of edible vaccines

Plant	Storage	Consumption	Production	Advantages
Tobacco (*Nicotiana tabacum*)	Seeds can be stored for long time; lowered cost of preservation	Leaves and seeds can be consumed	Ease of production and number of harvests are large	Antibodies from seeds can be purified easily
Potato (*Solanum tuberosum*)	Can be stored for long periods without refrigeration	Needs cooking which can denature the antigen	Easy and cost effective propagation	Easily transformed and can be propagated easily from its 'eyes'
Banana (*Musa* spp.)	Spoils rapidly after ripening	Does not need cooking	Grown widely in developing countries	Proteins not destroyed even if cooked
Tomato (*Solanum lycopersicum*)	Spoils readily; can be prevented by freeze-drying	Can be pulverized into heat stable tablets	Grow quickly and can be cultivated broadly	High content of vitamin A may help to boost the immune response
Rice (*Oryza sativa*)	Easy storage/transportation	Consumed after cooking as the expressed protein is heat stable	Grows slowly and requires specialized conditions	High expression of proteins/antigens, heat stable and low allergenic potential
Lettuce (*Latuca sativa*)	Spoils readily	Consumed raw	Grows fast	High yield
Papaya (*Carica papaya*)	Limited shelf life	Consumed raw	Easy and cost effective propagation	High antigenic expression

deleting the genes for auxin and cytokinin synthesis so that there is no tumor (gall) formation upon transformation. T-DNA carrying the transgene is integrated into the plant genome at random sites (Gustavo et al. 1998). Another soil bacterium, *Agrobacterium rhizogenes* carrying the Ri (root inducing) plasmid (Gustavo et al. 1998), can also be used for transformation of the phenotype of plant cells.

Using chimeric viruses

Plant viruses including cowpea mosaic virus (CPMV), alfalfa mosaic virus (ALMV), tobacco mosaic virus (TMV) and cauliflower mosaic virus (CaMV) can be used to express fragments of antigenic epitopes on their surface with the help of overcoat and epicoat technology (Walmsley and Arntzen 2000). CPMV has been genetically modified to express multiple epitopes like HIV-1 and human rhinovirus 14 (Porta et al. 1994); foot and mouth disease virus (Usha et al. 1993); canine parvovirus and fungal epitopes from *Plasmodium falciparum* on its surface. These viruses are used as vectors for transformation of plants for vaccine production.

Other methods like biolistics method, electroporation and lipofection can also be adopted for the gene transfer.

Generation of immune response

Edible vaccines are taken orally, so they mimic the process of natural infection and stimulate both mucosal and systemic immune responses in the body (Koo et al. 1999), which is a big advantage as compared to traditional vaccines.

As plant parts are fed directly, the antigen is protected from attack by the enzymes, gastric and intestinal secretions due to bio-encapsulation. The plant cell wall breaks in the intestine to release the antigens. The M cells of the Peyer patches and gut-associated lymphoid tissue (GALT) take up the antigens first (Lal et al. 2007). M cells pass the antigen to macrophages and other antigen-presenting cells for display to the helper T cells. Th cells stimulate B cells to make and release antibodies (IgG, IgE, IgA and memory responses) that neutralize the antigen in a way similar to conventional vaccines.

Advantages of edible vaccines

Preparation of either injectible or oral vaccine (like polio vaccine), on an industrial scale is an expensive process and requires specially-built manufacturing facilities, cold chain storages, medical personnel and sterile injections. One of the key goals of the edible vaccine pioneers is to reduce immunization expenses.

Edible vaccines are safer, as they possess antigenic subunits only and are devoid of the pathogenic epitopes. They can induce both systemic and mucosal immunity. Their scale-up requires planting more acreage of value-added plants only (Korban et al. 2002) and processing in the form of juice, powder or sauce, is less complicated and cost effective.

Vaccines synthesized in mammalian cells can be contaminated with pathogenic organisms but plant based vaccines are not, as plant viruses do not infect humans (Lal et al. 2007). Feasibility in oral administration as compared to injections and enhanced compliance with children are other advantages of edible vaccines. Moreover, needles used in conventional vaccination also cause environmental contamination and carry the risk of spreading second-hand diseases (Yu 2008).

Edible vaccines can be integrated with other vaccine approaches or multiple antigens may be delivered for improved efficacy. The "multi-component" ability is possible by crossing two plant lines harboring different antigens. These "second-generation" vaccines allow several antigens to approach M cells simultaneously, e.g. a trivalent vaccine against cholera, ETEC (enterotoxigenic *E. coli*) and rotavirus, elicits a significant immune response to all three antigens (Yu and Langridge 2001).

Clinical applications

A large number of clinical trials have been carried out to validate the potential of edible vaccines for the prevention of various human diseases (Table 2).

Other therapeutic potentials

In addition to being used as vaccines, transgenic plants have additional therapeutic applications. Transgenic soybeans produce a tumor-reactive monoclonal antibody called BR-96 which can be used as a drug carrier to treat breast, colon, ovarian and lung cancers (Moffat 1995). An engineered contraceptive developed in tobacco mosaic virus contains Zona pellucida ZB3 protein, which covers the unfertilized eggs preventing fertilization (Prakash 1996). A vaccine against Alzheimer's disease using a beta amyloid gene inserted into the tomato genome could induce a strong immune response in mice (Youm et al. 2008).

The green vaccines

Plant chloroplasts are now being used for the production of green vaccine (Verma and Daniell 2007). Chloroplast expression has advantages of hyperexpression of protein, efficient oral delivery and transgene containment via

Table 2 Clinical studies carried out for evaluating different edible vaccines for various human diseases

Organism/Disease	Antigen expressed	Plant sp.	Outcome
ETEC Enterotoxigenic *Eshcherichia coli* (Diarrhea)	LT-B	Potato	First successful human trial done in 1997 generating systemic and mucosal immune responses. LT-B has also been found to be immunogenic in pre-clinical trials (Yu 2008)
Norwalk virus (Gastroenteritis)	Viral capsid protein	Potato tubers	Systemic and mucosal response observed, with no safety concerns. Freeze-dried tomato powder containing NV capsid has been found to be immunogenic in pre-clinical trials (Yu 2008)
Measles	Measles virus heamaglutinin (MV-H) antigen	Tobacco	The vaccine does not cause atypical measles as seen with the current vaccine (Polack et al. 1999)
			Prime boost strategy induced 20 times higher protective titers (Webster et al. 2002)
Hepatitis-B	Viral major surface antigen (HBs Ag)	Potato tubers; lettuce leaves	Lettuce leaves produced systemic immune response.
			Potato-based vaccine enhanced the protective immunity when used as a booster (Yu 2008)
Rabies	Antigenic peptides of glycoprotein and nucleoprotein fused to alfalfa mosaic virus coat protein	Spinach leaves	Protective immune response observed without major safety concerns (Modelska et al. 1998)
Yersinia pestis (Plague)	F1-V antigen	Tomato, dried and pulverized into tablets	Trials in mice successfully produced protection (Alvarez et al. 2006)
Vibrio cholerae (Cholera)	Cholera toxin B (CT-B)	Tobacco	Daniell et al. (2001) achieved accumulation of CT-B level of 4.1% of total soluble protein in tobacco plants.
HIV	Envelope protein gp-120, in Tobacco mosaic virus	Plastids of Tobacco and tomato	Neutralizing antibodies produced in mice (Yusibov et al. 1997).
	Tat protein	Spinach	Mice fed with spinach followed by DNA vaccination resulted in higher antibody titre (Karasev et al. 2005)
	p24 and Nef		Fusion of p-24 and Nef triggered antigen accumulation and lead to protective immunity (Zhou et al. 2008)
Bacillus anthracis (Anthrax)	*B. anthracis* protective antigen (pag A)	Tobacco chloroplast	Antibody response against the protective antigen seen in the sera of animals (Aziz et al. 2005)
			Vaccine devoid of edema factor, lethal factor and other toxic side effects
		Tomato and spinach also under trial	
Taenia crassiceps (cysticerci) and *Taenia solium* (cysticercosis)	Three synthetic-peptides of anti-cysticerosois vaccine (KETc1, KETc12 and KETc7)	Seeds of papaya (*Carica papaya* L.)	Proposed vaccine found to be immunogenic and reduction in parasite load was observed (Sciutto et al. 2002, Hernández et al. 2007)

maternal inheritance. To date, 23 vaccine antigens against 16 different bacterial, viral and protozoan antigens have been expressed in chloroplasts (Davoodi-Semiromi et al. 2009) which include, cholera (Daniell et al. 2001), anthrax (Koya et al. 2005), plague (Arlen et al. 2008), HIV antigens p24 (Zhou et al. 2008), Rotavirus (Birch-Machin et al. 2004), amoebiasis (Chebolu and Daniell 2007) and malaria (Davoodi-Semiromi et al. 2010).

Obstacles on the way

The concept of edible vaccines looks attractive, but implementation can be a little difficult. There are many issues that need to be addressed for developing a plant-based vaccine i.e. antigen selection, efficacy in systems, choice of plants, delivery, dosage, safety, public perception and quality control and licensing (Lal et al. 2007). Antigen selection involves safety concerns of whether or not selected antigens are compatible enough with the selected plant type to be expressed. Dosage is determined by a patient's weight, age, the fruit or plant size and the ripeness of the fruit or plant (Yu 2008). One tomato or banana is never the same size as another, so significant differences in protein content might occur. This could lead to the risk of underdosing leading to lesser production of antibodies or overdosing leading to tolerance. Consistency of dosage from fruit to fruit, plant to plant, and generation to generation is thus a matter of concern (Tripurani et al. 2003).

Shelf life of the plant crops is very crucial. Since these fruits are being used as vectors for the vaccines in question, they have to be properly stored to avoid infection or disease through spoilage (Richter and Kipp 1999). Another concern could be of transgene escape and identification of "vaccine" fruit versus a normal fruit to avoid the misadministration of the vaccine (Tripurani et al. 2003).

Methods employed for increasing the antigenic protein content in transgenic plants by stunted growth of plants and reduction of fruit formation may introduce excess mRNA which may cause gene silencing in the plant genome (Lal et al. 2007). Moreover, there could be an allergic reaction or other side effects like cytokine-induced sickness, central nervous system toxicity or autoimmune diseases on consumption of plant-based vaccines.

Regulatory concerns

The public perception as well as ecological and environmental risks of edible vaccines are the considerations for their acceptance (Lal et al. 2007).

It is still not clear if edible vaccines would be regulated under food, drugs or agricultural products, and what vaccine component would be licensed, the antigen or the genetically modified fruit or the seeds that contain the transgene. They would be subjected to a very close scrutiny by the regulatory bodies to make sure that they never enter the food supply, which means that they would need to be grown in containment because cross-pollination may result in the modified crops unknowingly making their way into our normal food supply (Lal et al. 2007). Moreover, results must show that the edible vaccines are as effective and safe as the injectable vaccines, to gain FDA approval.

Future prospects

Edible vaccines may not be available as of now, but researchers from fields as diverse as agronomy and biotechnology make it possible to believe that the image of the child being vaccinated as she eats a banana (Arntzen 1997) is not far fetched. Future research and work on edible vaccines would determine if these vaccines will be able to meet the WHO standards of quality i.e. purity, potency, safety, and efficiency (Sala et al. 2003).

The massive increase in global area utilized in cultivating transgenic crops suggests the acceptance of transgenic crops in most developing countries (Lal et al. 2007). If these vaccines become a reality, vaccination for most diseases would be possible worldwide.

Conclusion

The economic and technical payback offered by edible vaccines makes them ideal substitutes for conventional vaccines. Vaccine crops, though still not in commercial production would soon become a reality if significant challenges can be overcome. The scientific community hopes that with today's technology, the techniques of production will likely be conquered to make plant derived vaccines more efficient and dependable. Above all, social acceptance of this technology would govern the commercial achievability of this vaccine.

References

Alvarez ML, Pinyerd HL, Crisantes JD, Rigano MM, Pinkhasov J, Walmsley AM, Mason HS, Cardineau GA (2006) Plant-made subunit vaccine against pneumonic and bubonic plague is orally immunogenic in mice. Vaccine 24:2477–2490

Arlen PA, Singleton M, Adamovicz JJ, Ding Y, Davoodi-Semiromi A, Daniell H (2008) Effective plague vaccination via oral delivery of plant cells expressing F1-V antigens in chloroplasts. Infect Immun 76:3640–3650

Arntzen CJ (1997) Edible vaccines. Public Health Rep 112:190–197

Aziz MA, Sikriwal D, Jarugula S, Kumar PA, Bhatnagar R (2005) Transformation of an edible crop with the pagA gene of *Bacillus anthracis*. FASEB J 19:1501–1503. doi:10.1096/fj.04-3215fje

Barta A, Sommergruber K, Thompson D, Hartmuth K, Matzke MA, Matzke AJM (1986) The expression of a nopaline synthase human growth hormones chimeric gene in transformed tobacco and sunflower callus tissue. Plant Mol Biol 6:347–357

Birch-Machin I, Newell CA, Hibberd JM, Gray JC (2004) Accumulation of rotavirus VP6 protein in chloroplasts of transplastomic tobacco is limited by protein stability. Plant Biotechnol J 2: 261–270

Chebolu S, Daniell H (2007) Stable expression of Gal/GalNAc lectin of *Entamoeba histolytica* in transgenic chloroplasts and immunogenicity in mice towards vaccine development for amoebiasis. Plant Biotechnol J 5:230–239

Chong DK, Roberts W, Arakawa T, Illes K, Bagi G, Slattery CW, Langridge WH (1997) Expression of the human milk protein beta-casein in transgenic potato plants. Transgenic Res 6: 289–296

Daniell H (2003) A chloroplast transgenic approach to hyperexpress and purify human serum albumin, a protein highly susceptible to proteolytic degradation. Plant Biotechnol 1:77–79

Daniell H, Lee SB, Panchal T, Weibe PO (2001) Expression of native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts. J Mol Biol 311:1001–1009

Davoodi-Semiromi A, Samson N, Daniell H (2009) The green vaccine: A global strategy to combat infectious and autoimmune diseases. Hum Vacc 5:488–493

Davoodi-Semiromi A, Schreiber M, Nalapalli S, Verma D, Singh ND, Banks RK, Chakrabarti D, Daniell H (2010) Chloroplast-derived vaccine antigens confer dual immunity against cholera and malaria by oral or injectible delivery. Plant Biotechnol J 8: 223–242

Goodman RM, Hauptli H, Crossway A, Knauf VC (1987) Gne transfer in crop improvement. Science 236:48–54

Gustavo A, González-Cabrera RJ, Vázquez-Padrón R, Camilo AP (1998) *Agrobacterium tumefaciens*: a natural tool for plant transformation. Electron J Biotechnol [online]. doi:10.4067/S0717-34581998000300002

Haq TA, Mason HS, Clements JD, Arntzen CJ (1995) Oral immunization with a recombinant bacterial antigen produced in transgenic plants. Science 268:714–716

Hernández M, Cabrera-Ponce JL, Fragoso G, López-Casillas F, Guevara-García A, Rosas G et al (2007) A new highly effective anticysticercosis vaccine expressed in transgenic papaya. Vaccine 25:4252–4260

Hiatt A, Cafferkey R, Bowdish K (1992) Production of antibodies in transgenic plants. Proc Natl Acad Sci USA 89:11745–11749

Hood EE, Bailey MR, Beifuss K, Megallanes M, Horn ME, Callaway E, Drees C, Delaney DE, Clough R, Howard JA (2003) Criteria for high-level expression of a fungal laccase gene in transgenic maize. Plant Biotechnol J 1:129–140

Horsch RB, Fraley RT, Rogers SG, Sanders PR, Lloyd A, Hoffmann N (1984) Inheritance of functional foreign genes in plants. Science 223:496–498

Karasev AV, Foulke S, Wellens C, Rich A, Shon KJ, Zwierzynski I, Home D, Koprowski H, Reitz M (2005) Plant based HIV-1 vaccine candidate: Tat protein produced in spinach. Vaccine 23:1875–1880

Koo M, Bendahmane M, Lettieri GA, Paoletti AD, Lane TE, Fitchen JH, Buchmeier MJ, Beachy RN (1999) Protective immunity against murinehepatitis virus (MHV) induced by intranasal or subcutaneousadministration of hybrids of tobacco mosaic virus that carries anMHV epitope. Proc Natl Acad Sci USA 96:7774–7779

Korban SS, Krasnyanski SF, Buetow DE (2002) Foods as production and delivery vehicles for human vaccines. J Am Coll Nutr 21:212–217

Koya V, Moayeri M, Leppla SH, Daniell H (2005) Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge. Infect Immun 73:8266–8274

Lal P, Ramachandran VG, Goyal R, Sharma R (2007) Edible vaccines: Current status and future. Indian J Med Microbiol 25:93–102

Mason HS, Lam DMK, Arntzen CJ (1992) Expression of hepatitis B surface antigen in transgenic plants. Proc Natl Acad Sci USA 89:11745–11749

Mishra N, Gupta PN, Khatri K, Goyal AK, Vyas SP (2008) Edible vaccines a new approach to oral immunozation. Indian J Biotechnol 7:283–294

Modelska A, Dietzschold B, Sleysh N, Fu ZF, Steplewski K, Hooper DC, Koprowski H, Yusibov V (1998) Immunization against rabies with plant-derived antigen. Proc Natl Acad Sci USA 95:2481–2485

Moffat AS (1995) Exploring transgenic plants as a new vaccine source. Science 268:658–660

Polack FP, Auwaerter PG, Lee SH, Nousari HC, Valsamakis A, Leiferman KM (1999) Production of atypical measles in rhesus macaques: evidence for disease mediated by immune complex formation and eosinophils in the presence of fusion-inhibiting antibody. Nat Med 5:629–634

Porta C, Spall VE, Loveland J, Johnson JE, Barker PJ, Lomonossoff GP (1994) Development of cowpea mosaic virus as a high-yielding system for the presentation of foreign peptides. Virology 202:949–955

Prakash CS (1996) Edible vaccines and antibody producing plants. Biotechnol Dev Monit 27:10–13

Richter L, Kipp PB (1999) Topics in microbiology and immunology. In: Hammond J, McGarvey P, Yusibov V (eds) Plant biotechnology: new products and applications. Springer, Heidelberg, pp 159–176

Ruggiero F, Exposito JY, Bournat P, Gruber V, Perret S, Comte J, Olagnier B, Garrone R, Theisen M (2000) Triple helix assembly and processing of human collagen produced in transgenic tobacco plants. FEBS Lett 469:132–136

Sala F, Manuela Rigano M, Barbante A, Basso B, Walmsley AM, Castiglione S (2003) Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. Vaccine 21:803–808

Sciutto E, Fragoso G, Manoutcharian K, Gevorkian G, Rosas-Salgad G, Hern á ndez-Gonzalez M, Herrera-Estrella L, Cabrera-Ponce JL et al (2002) New approaches to improve peptide vaccine against porcine *Taenia solium* Cysticercosis. Arch Med Res 33:371–378

Tripurani SK, Reddy NS, Sambasivarao KRS (2003) Green revolution vaccines, edible vaccines. Afr J Biotechnol 2:679–683

Usha R, Rohll JB, Spall VE, Shanks M, Maule AJ, Johnson JE, Lomonossoff GP (1993) Expression of an animal virus antigenic site on the surface of a plant virus particle. Virology 197: 366–374

Verma D, Daniell H (2007) Chloroplast vector systems for biotechnology applications. Plant Physiol 145:1129–1143

Walmsley AM, Arntzen CJ (2000) Plants for delivery of edible vaccines. Curr Opin Biotechnol 11:126–129

Webster DE, Thomas MC, Strugnell RA, Dry IB, Wesselingh SL (2002) Appetising solutions: an edible vaccine for measles. Med J Aust 176:434–437

Youm JW, Jeon JH, Kim H, Kim YH, Ko K, Joung H, Kim HS (2008) Transgenic tomatoes expressing human beta-amyloid for use as a

vaccine against Alzheimer's disease. Biotechnol Lett 30:1839–1845

Yu N (2008) Edible vaccines MMG 445. Basic Biotechnol eJournal 4:104–109

Yu J, Langridge W (2001) A plant based multicomponent vaccine protects mice from enteric diseases. Nat Biotechnol 19:548–552

Yusibov V, Modelska A, Steplewski K et al (1997) Antigens produced in plants by infection with chimeric plant viruses immunize against rabies virus and HIV-1. Proc Natl Acad Sci USA 94:5784–5788

Zhou F, Badillo-Corona JA, Karcher D, Gonzalez-Rabade N, Piepenburg K, Borchers A-MI, Maloney AP, Kavanagh TA, Gray JC, Bock R (2008) High-level expression of HIV antigens from the tobacco and tomato plastid genomes. Plant Biotechnol J. doi:10.1111/j.1467-7652.2008.00356.x