Supplementary Material

Plant-made vaccine antigens and biopharmaceuticals

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Table S1. Vaccine antigens expressed via the plant nuclear genome

Antigen	Expression system	Expression level	Functional evaluation	Refs
Human vaccines Stable expression				
Bacterial antigens				
Enterotoxigenic *E. coli* Heat-labile toxin B subunit (LTB)	Tobacco leaf	0.001% TSP	Immunogenic and protective in mice after oral administration	[S1]
	Potato tuber	0.19% TSP	Immonogenic and protective by oral delivery to mice	[S2]
	Potato tuber	7.3 to 17.2 μg g^{-1} FW	Immunogenic in mice and human after oral administration	[S3]
	Maize seeds	0.01-0.05% TSP	Immunogenic and protective in mice after oral administration	[S4]
	Maize seeds	0.8% TSP	Immunogenic response in mice after oral administration	[S5]
	Potato tuber	17μg g^{-1} FW	Immunogenic in mice following oral administration	[S6]
	Carrots	0.3% TSP	Immunogenic and protective against CT challenge	[S7]
	Corn	Not Reported	Immunogenic in human following oral administration	[S8]
	Soybean	1.8-2.4% TSP	Immunogenic and partial protection against LT challenge in mice	[S9]
	Lettuce	1.0-2.0% TSP	Strong affinity to GM1-ganglioside	[S10]
Enterotoxigenic *E. coli* fimbrial subunit FanC	Soybean	0.4-0.5% TSP	Immunogenic by intraperitoneal delivery in mice.	[S11]
Cholera toxin B subunit (CTB)	Potato tuber	0.3% TSP	Immunogenic and protective immunity in mice against bacterial enterotoxin following oral administration	[S12,S13]
	Tomato, leaves Fruits	0.02% TSP 0.03-0.04% TSP	Strong affinity to GM1-ganglioside	[S14]
	Tomato	0.034-0.081% TSP	Immunogenic by oral delivery to mice	[S15]
	Rice	2.1% TSP	Immunogenic and protective against CT challenge to mice following oral administration	[S16]
	Carrot	0.48% TSP	Strong affinity for GM1-ganglioside	[S17]
	Rice	1.5-2.1% TSP	Strong affinity for GM1-ganglioside	[S18]
	Lettuce	0.16-0.24% TSP	Strong affinity for GM1-ganglioside	[S19]
Accessory colonization factor subunit A (ACFA) or CTB-acfA	Tomato leaves Fruits	0.0003-0.06 and 0.006- 0.02% TSP or 0.006-0.25 and 0.06-0.08% TSP	Not reported	[S20]
Toxin co-regulated pilus subunit A (TCPA) P4 or P6 epitope fused with CTB	Tomato	0.007-0.17 or 0.019-0.096% TSP	Strong affinity for GM1-ganglioside	[S21]
Bacillus anthracis protective antigen (PA)	Tobacco leaf	Not Reported	Biological activity demonstrated by cytolytic assay on macrophage like cell lines with lethal factor	[S22]
Cholera toxin B subunit fused to rotavirus and enterotoxigenic *E. coli* heat labile toxin B subunit	Potato tuber	Not Reported	Immunogenic and protective (passive immunity) by oral delivery to mice	[S23]
Antigenic lipoprotein (Ag473)	Rice leaves Seeds	0.18-0.6 or 0.05-0.75% TSP	Immunogenic in mice following intraperitoneal administration.	[S24]
Diphtheria–tetanus–pertussis (DTP)	Tobacco Carrot cell suspension	0.2-0.3 mg 0.5 kg^{-1} biomass	Induced strong antigen-specific antibody response in injected mice	[S25]
Tuberculosis antigen ESAT6 fused to LTB	*Arabidopsis*	11-24.5 μg g^{-1} FW	Strong affinity for GM1-ganglioside	[S26]

Viral antigens				
Hepatitis B virus (HBV) surface antigen	Tobacco leaf	0.0002-0.0066 % TSP	Immunogenic by intraperitoneal delivery to mice	[S27,S28]
	Potato	8.5 µg g^{-1} FW	Immunogenic response in humans following oral administration	[S29]
	Potato	8.35 and 1.1 µg g^{-1} FW	Immunogenic to mice following oral administration	[S30, S31]
	Lupin callus Lettuce	11-150 and 1-5.5 ng g^{-1} FW	Immunogenic to mice by oral delivery	[S32]
	Potato	Not reported	Not reported	[S33]
	Banana	19.92-38 ng g^{-1} FW	Not reported	[S34]
	Cherry tomatillo, Leaf Stem Fruit	90-300 ng, 5-15 ng and 6-7 ng g^{1} FW	Plant-derived rHBsAg had same immunogenicity as commercial vaccine in stimulating production of HBsAg specific antibodies in animal	[S35]
	Tomato, Fruit	0.02% TSP	Not reported	[S36]
	Tomato	73.2-255.6 ng g^{-1} DW	Not reported	[S37]
Hepatitis B virus (HBV) surface S and preS2 antigens	Potato	0.03-0.09 and 0.003-0.012% TSP	Immunogenic in mice	[S38,S39]
Hepatitis B virus surface antigen fused with preS1 epitop	Rice	15.8-31.5 ng g^{-1} DW	Immunogenic by intraperitoneal delivery to mice	[S40]
Norwalk virus capsid protein (NVCP)	Tobacco leave, potato tubers	0.23% TSP	Immunogenic by oral delivery to mice	[S41]
	Potato tubers	215-751 µg $150g^{-1}$ FW	Immunogenic by oral delivery to human	[S42]
	Tomato Fruit and Potato	8 and 0.4 % TSP	Elicit systemic and mucosal antibody responses in mice following oral administration	[S43]
	Tomato	20 µg g^{-1} FW	Immunogenic in mice	[S44]
Rotavirus fused with cholera toxin B and enterotoxigenic *E. coli* heat labile toxin B subunit	Potato tubers	0.01-0.1% TSP	Immunogenic by oral delivery to mice. Reduced symptoms following rotavirus challenge in pups	[S23,S45]
Rotavirus capsid protein (VP6)	Potato leaves Potato tubers	0.006% TSP 0.002% TSP	Immunogenic in mice following intraperitoneal injection	[S46]
Human group A rotavirus (VP6) protein	Alfalfa	0.06-0.28% TSP	Immunogenic in mice and offspring developed less severe diarrhea after challenge with simian rotavirus SA-11, indicating that antibodies generated in the dams provided passive heterotypic protection to the pups	[S47]
Rotavirus (VP7)	Potato tubers	0.3-0.4 % TSP	Immunogenic in mice following oral delivery. Neutralization activity against rotavirus	[S48,S49]
Measles Haemgglutinin protein	Tobacco leaf	Not Reported	Immunogenic by intraperitoneal or oral delivery to mice	[S50,S51]
	Carrot	Not reported	Immunization of mice with plant extracts induced high titres of IgG1 and IgG2a antibodies that cross-reacted strongly with the measles virus and neutralized the virus *in vitro*	[S52]
SARS-CoV S protein (S1)	Tomato and tobacco leaf	0.1% TSP	Immunogenic to mice following oral administration	[S53]
Respiratory syncytial virus (RSV)	Tomato fruits	1-32.5 µg g^{-1} FW	Immunogenic to mice following oral administration	[S54]
Human cytomegalovirus glycoprotein B	Tobacco seeds	0.007-0.014% TSP	Not reported	[S55]
Human cytomegalovirus glycoprotein B (gB)	Rice	Not reported	Contained several neutralizing epitopes and stable over 27 months	[S56]
Smallpox recombinant vaccine virus B5 antigenic domain (pB5)	Tobacco and Collard leaf	Not Reported	Antibody response in mice immunized parenterally and protects against lethal dose of vaccinia virus	[S57]
Human papillomavirus-like particles (HPV VLPs) L1 capsid protein	Potato	23 ng g^{-1} FW	Immune response in mice following parenteral administration	[S58]
HPV-11 L1major capsid protein	*Arabidopsis* and tobacco	3-12 µg g^{-1} or 0.2-2.2 µg g^{-1} FW	Weak immune response in rabbits and no neutralization activity	[S59]
HPV major capsid protein L1	Tobacco and potato	0.5 to 0.2% TSP	Plant-derived L1 was immunogenic in mice	[S60]
Human immunodeficiency virus (HIV-1) and hepatitis B virus (HBV) chimeric gene	Tomato	0.3 ng mg^{-1}DW	Oral administration of plant-derived HIV/HBV vaccine antigen stimulates both serum and secretory HIV- and HBV-specific antibodies in mice	[S61]
HIV-1 subtype C p24 antigen	*Arabidopsis*	0.5 mg g^{-1} in stem and 0.2mg g^{-1} in leaf FW	Immunogenic in mice	[S62]
HIV type 1 mucosal vaccine (CTB-MPR) membrane proximal (ectodomain) region of gp41	Tobacco leaf	0.01-0.2% TSP	Affinity for GM1-ganglioside and immunogenic in mice after mucosal prime-systemic boost immunization	[S63]

Japanese encephalitis virus (JEV) envelope protein (E)	Rice	1.1-1.9 μg mg^{-1} TSP	JEV-specific neutralizing antibody detected in mice following intraperitoneal or oral administration	[S64]
Canine parvovirus (CPV) 2L21	*Arabidopsis*	0.15-3.3% TSP	Immunogenic in mice following intraperitoneal or oral administration	[S65]
Small pox vaccine candidate (B5) Viral spike protein (SARS antigen)	Collard Cauliflower	Not reported	Not reported	[S66]
Surface glycoprotein (G protein) of rabies virus	Tobacco	0.001-0.38% TSP	Plant-derived G protein induced complete protective immunity in mice against intracerebral lethal challenge with live rabies virus	[S67]
Tumor-associated colorectal cancer antigen EpCAM	Tobacco	10 mg kg^{-1} FW	Induced humoral immune response in immunized mice. Sera from immunized mice efficiently inhibited the growth of SW948 colorectal carcinoma cells	[S68]
Protozoan antigens				
Plasmodium yoelii merozoite surface protein (PyMSP4/5)	Tobacco	0.02-0.04% TSP	Induce antigen-specific antibodies in mice following parenteral delivery	[S69]
Human vaccines (Viral/Transient expression)				
Bacterial antigens				
Tuberculosis antigen ESAT6	Tobacco leaf (Potato virus X)	0.5-1% TSP	Not reported	[S70]
Yersinia pestis F1 and LcrV antigens	Tobacco leaf tissue	380 and 120 μg g^{-1} FW	Immunogenic and protective in monkeys against *Y. pestis* following subcutaneous injection	[S71]
	Tobacco	17 μg g^{-1} FW	Immunogenic in mice and protection in Cynomolgus Macaques against aerosolized *Y. pestis*	[S72]
Yersinia pestis F1-V and F1 antigens	Tobacco leaf (TMV)	1 and 2mg g^{-1}	Immunogenic and protection in vaccinated guinea pigs against *Y. pestis* aerosol challenge	[S73]
Staphylococcus aureus D2 peptide of fibronectin-binding protein (FnBP)	Tobacco leaf (CPMV) or (Potato virusX)	1.2 mg g^{-1} or 0.2mg g^{-1} FW	Immunogenic to mice and rats following subcutaneous injection. Mice and rat sera completely inhibit the binding of fibronectin	[S74]
Pseudomonas aeruginosa outer membrane protein F	Cowpea leaf (CPMV)	1-1.2 mg g^{-1} FW	Immunogenic and protective in mice following subcutaneous injection	[S75]
	Tobacco leaf (Tobacco mosaic virus)	Not Reported	Immunogenic and protective in mice following intramuscular or subcutaneous injection	[S76]
Bacillus anthracis protective antigen (PA)	Tobacco	Not reported	Immunogenic and were able to neutralized the effects of LeTx *in vitro*	[S77]
Bacillus anthracis protective antigen (PA-D4s)	Tobacco (Alfalfa mosaic virus)	0.3 mg g^{-1} FW	Immunogenic in mice	[S78]
Viral antigens				
HIV type 1 peptide of gp41 protein	Cowpea leaf (Cowpea mosaic virus)	1.2-1.5 mg g^{-1} FW	Immunogenic in rabbit	[S79]
HIV type 1 V3 loop of gp120 protein	Tobacco leaf (Alfalfa mosaic virus)	Not Reported	Immunogenic in mice following intraperitoneal administration	[S80]
HIV type 1 peptide of V3 loop of gp120 protein	Tobacco leaf (Tomato bushy stunt virus)	0.9 mg g^{-1} FW	Immunogenic in subcutaneously injected mice	[S81]
HIV type 1 peptide of transmembrane protein gp41	Cowpea leaf (Cowpea mosaic virus)	Not Reported	Immunogenic in mice following subcutaneous, nasal or oral delivery	[S82,S83]
HIV type 1 glycoprotein (gp) 41	Tobacco leaf (Potatovirus X)	Not Reported	Immunogenic in mice following intraperitoneal or nasal delivery and inhibit syncytium formation	[S84]
HIV entry inhibitors red algal protein griffithsin (GRFT)	Tobacco (TMV)	1 g kg^{-1} FW	Active against HIV at picomolar concentrations, directly virucidal via binding to HIV envelope glycoproteins and capable of blocking cell-to-cell HIV transmission	[S85]
Human rhinovirus type 14 peptide of VP1 protein (HRV 14)	Cowpea leaf (Cowpea mosaic virus)	1.2-1.5 mg g^{-1} FW	Immunogenic in rabbits following intramuscularly or subcutaneous injection	[S79]
Respiratory syncytial virus (RSV) peptide of G protein	Tobacco leaf (Alfalfa mosaic virus)	0.8mg g^{-1} FW	Immunogenic and protective in mice against viral following intraperitoneal injection	[S86]
Rabies virus glycoprotein and nucleoprotein (NF1-g24 & Av/A4-g24)	Tobacco and spinach leaf (Alfalfa mosaic virus)	0.4 mg g^{-1} and 60 μg g^{-1} FW	Immunogenic and protection in intraperitoneally injected mice when challenged intramuscularly with canine street rabies virus 3374L. Immunogenic in humans following oral administration	[S87]

Smallpox recombinant vaccine virus B5 antigenic domain (pB5)	Tobacco leaf transient	Not Reported	Antibody response in mice immunized perenterally and protection against lethal dose of vaccinia virus	[S57]
	Tobacco leaf transient	Not Reported	Immunogenic in mice following intramuscularly or intranasally administration	[S88]
Human papillomavirus (HPV) type 16 E7 protein	Tobacco leaf (Potato virus X, PVX)	3-4 µg g^{-1} FW	Immunogenic in mice following subcutaneous injection and protects from tumor development after challenge with the E7-expressing C3 tumoral cell line	[S89]
Rotavirus major inner capsid protein (VP6)	Tobacco (PVX)	50 µg g^{-1} FW	Not reported.	[S90]
Human Papilloma Virus (HPV) E7 encoprotein or E7 fused to β--1,3-1,4-glucanase (LicKM)	Tobacco	400 mg kg^{-1} FW	Immunogenic and protective in mice challenged with E7-expressing tumor cells following subcutaneous injection.	[S91]
Non-tumourigenic E7 protein of HPV16	Tobacco		Inhibition of tumor growth and increased survival was observed in C57BL/6 mice	[S92]
Encoding domain III of the dengue 2 envelope protein (D2EIII)	Tobacco (Tobacco mosaic virus)	0.28% TSP	Retains antigenicity and immunogenicity as well as inducing neutralizing antibodies in vaccinated animals.	[S93]
Pathogenic avian influenza virus (H5N1 subtype)	Tobacco	60 mg kg^{-1} FW	Immunogenic in mice and ferret and also protects ferrets against challenge infection with virus	[S94]
Pathogenic avian influenza virus (HPAI)	Tobacco	400 mg kg^{-1} FW	Immunogenic in mice	[S95]
H5N1 avian influenza virus hemagglutinin (HA)	Tobacco	1 mg kg^{-1} FW	Induced immune response in mice but no neutralizing activities in a virus micro-neutralization or hemagglutination inhibition assay	[S96]
Hepatitis B virus (HBV) surface antigen	Tomato	64.4-489 ng g^{-1} DW	Not reported	[S37]

Table S2. Bio-pharmaceutical proteins expressed via the plant nuclear genome

Pharmaceutical protein	Expression system	Expression level	Functional evaluation	Refs
Human interleukin-2 (IL-2) and (IL-**4)**	Tobacco suspension cells	0.10 and 0.18 µg ml^{-1}	Both secreted lymphokines have biological activity as determined by cell proliferation of murine CTLL-2 / CTLL-2 cell line	[S97]
Human α1-antitrypsin (AAT)	Rice suspension cells	4.6-5.7 mg g^{-1} dry cells	Not reported	[S98]
Human growth hormone (hGH)	Tobacco seeds	0.07-0.16% TSP	Radioreceptor binding assay showed same receptor-binding properties as the native mature hormone	[S99]
Human serum albumin (HSA)	Potato	0.032-0.2% TSP	Mature HSA sequence was efficiently and reliably processed in potato	[S100]
Human lysozyme (Hlys)	Rice calli or suspension cells	0.25-2.5 or 3-4.2% TSP	Significant bactericidal activity in *Micrococcus lysodeikticus* and *E. coli* cells when exposed to recombinant human lysozyme. Both native and recombinant human lysozyme displayed the same thermostability and resistance to degradation by low pH	[S101]
Recombinant human granulocyte-macrophage colony stimulating factor (hGM-CSF)	Rice suspension cells	4-73 mg g^{-1} cells DW	Recombinant hGM-CSF supported growth of hGM-CSF-dependent TF-1 cells showing biologically active	[S102]
Human interleukin-18 (hIL-18)	Tobacco	0.004-0.05% TSP	Plant-produced hIL-18 stimulated IFN-γ production on J6-1 cells showing biological activity	[S103]
Interferon-gamma (IFN-γ)	Rice suspension cells	Upto 700 ng g^{-1} cell	Conferred an effective antiviral activity as was evident by the inhibition of dengue virus infection in human A549 cells	[S104]
Human epidermal growth factor (hEGF)	Tobacco	0.00001-0.11% TSP	Biologically active in cumulus cells expansion assays	[S105]
Human insulin-like growth factor-1 (hIGF-1)	Tobacco /rice	0.02 and 0.03% TSP	Recombinant plants-derived rthIGF-1 is effective in stimulating the *in vitro* growth and proliferation of human SHSY5Y neuroblastoma cells	[S106]
Human insulin-like growth factor binding protein-3 (hIGFBP-3)	Tobacco seeds	800 µg DW^{-1}	Not reported	[S107]
Human insulin-like growth factor 1(hIGF-1)	Rice seeds	6.8% TSP	Biologically active *in vitro* in MCF-7 cell culture cell proliferation assays and reduced blood glucose of diabetic mice following oral delivery	[S108]
Human insulin (CTB-insulin)	Potato	0.1% TSP	Strong affinity for GM1-ganglioside and reduction in pancreatic islet inflammation and also delayed in the progression of clinical diabetes in NOD mice	[S109]

Recombinant human insulin	*Arabidopsis* seeds	0.13% TSP	Biologically active *in vivo* and *in vitro* as demonstrated by insulin tolerance test in mice and phosphorylation assay performed in a mammalian cell culture system	[S110]
B chain of human Insulin (CTB-InsB3)	Tobacco	0.08-0.11%TSP	Strong affinity for GM1-ganglioside	[S111]
Human GAD65 (hGAD65) and murine IL-4	Tobacco	0.04, 0.1% TSP	Combine feeding of IL-4 plus GAD in NOD mice delayed the onset of diabetes	[S112]
Human lactoferrin (hLF)	Rice	0.5% TSP	Not reported	[S113]
Human acid *β*-glucosidase	Tobacco seeds	Not reported	Plant-derived GCase is uptaken by fibroblasts of a Gaucher Type-II patient and lacks potentially immunogenic glycans	[S114]
Human Glucocerebrosidase Enzyme (GCD)	Carrot cells	Not reported	Recombinant GCD in transgenic plant cells was biologically active. Phase I clinical trial have shown no clinical or laboratory evidence of any significant innate or humoral immune reactions and phase III clinical trial was approved and is currently ongoing	[S115,S116]
Thrombomodulin (Solulin)	Tobacco	Upto 115 µg g^{-1} FW	Not reported	[S117]
Human cytokine granulocyte macrophage colony stimulating factor (GM-CSF)	Sugarcane	Undetectable-0.02% TSP	Human bone marrow cells (TF-1), which require GM-CSF for cell division, proliferated when growth media was supplemented with transgenic sugarcane extracts and had identical activity levels	[S118]
Mouse interleukin-12 (IL-12)	Tomato leaf and fruit	2.7-7.3 and 1-3.4µg g^{-1} FW	Biologically active *in vitro*. The plant-produced mIL-12 induced the secretion of IFNγ by T cells	[S119]
B chain of human Insulin fused to CTB	Tobacco	0.08-0.11% TSP	GM1–ELISA showed that the plant-derived fusion protein retained GM1–ganglioside receptor binding specificity	[S111]
Human basic fibroblast growth factor (bFGF)	Soybean	2.3% TSP	Mitogenic assay demonstrated that bFGF stimulated Balb/c 3T3 cells to proliferate in a dose-dependent manner indicting similar biological activity as native bFGF	[S120]
Human granulocyte-colony stimulating factor (hG-CSF)	Rice suspension cells	0.7% TSP	Plant-derived hG-CSF supports proliferation of the AML-193 cells similar to commercial *E. coli*-derived hG-CSF	[S121]
Bone morphogenetic protein 2 (BMP2)	Tobacco	0.02% TSP	Application of hBMP2 to mouse C2C12 cell line significantly increased cell ALP activity but lower than commercial rhBMP2	[S122]
Macrophage colony-stimulating factor (M-CSF)1	Tobacco	0.02-1.92% TSP	Plant-derived M-CSFsR inhibits colony formation of J6-1 cells	[S123]
Human epidermal growth factor (hEGF)	Tobacco	0.09-0.3% TSP	Plant-produced hEGF significantly stimulated Vero E6 cell expansion and proliferation similar to commercial hEGF products	[S124]
Human granulocyte-macrophage colony stimulating factor (hGM-CSF)	Rice	1.2-1.3% TSP	Rice seed-derived hGMCSF induces proliferation of TF-1 cells similar to *E. coli*-derived hGM-CSF	[S125]
Human growth hormone (hGH)	Rice suspension cells	57 mg l^{-1}	The biological activity of shGH accumulated in the transgenic rice cell suspension culture was similar to that of the *E. coli*-derived recombinant hGH as shown by proliferation of Nb2 node lymphoma cells	[S126]
Human β-amyloid (Ab)	Tomato	80 and 58 ng ml^{-1}	Immunogenic in mice	[S127]
Human *a*-1-antitrypsin	Tomato	0.44-1.55% TSP	Biologically active, showing high specific activity and efficient inhibition of elastase activity	[S128]
Type II collagen (CII)	Rice	1µg $seed^{-1}$	Feeding DBA/1 mice with transgenic rice seeds for 2 weeks showed tendencies of lowering and delaying serum specific-IgG2a response against subsequent and repeated intraperitoneal-injection of type II collagen	[S129]
Human epidermal growth factor (hEGF)	Tobacco (Transient)	0.015% TSP	Biologically active in cumulus cells expansion assays	[S105]
Human IA-2 (IA-2ic), a diabetes-associated autoantigen	Tobacco (Transient)	0.5% TSP	Plant-derived IA-2ic protein is specifically recognized by human IA-2ic autoantibodies	[S130]
Human fibroblast growth factor 8 isoform b(FGF8b)	Tobacco (Transient)	90-150 µg g^{-1} FW	Plant-expressed FGF8b effectively increased the rate of cell proliferation of NIH3T3 as bacterially expressed mouse FGF8b	[S131]
Human growth hormone (hGH)	Tobacco (TMV)	~60 mg Kg^{-1} FW	Biologically active in hypophysectomized female Sprague Dawley rats	[S132]

Table S3. Vaccine antigens and biopharmaceuticals expressed via the chloroplast genome

Vaccines antigens	Expression system	Expression level	Functional evaluation	Refs
Bacterial antigens				
Cholera toxin B (*Ctx*B)	Tobacco Lettuce	4.1, 8 and 12.3% TSP 4.8 and 9.4% TSP	GM1 ganglioside-binding assay. Long-term protection (50% mouse life span) against CT challenge in both oral (100%) and subcutaneously (89%) immunized mice; protection correlated with CTB-specific IgA and IgG1 titers in oral and IgG1 in subcutaneously immunized mice; increasing numbers of IL10$^+$ T-cell but not Foxp3$^+$ regulatory T-cells, suppression of IFN-γ and absence of IL-17 were observed in protected mice	[S133-S135]
E. coli enterotoxin B (*LTB)*	Tobacco	2.3-2.5% TSP	GM1 ganglioside-binding assay	[S136]
Tetanus toxin (*Tet*C)	Tobacco	18-27 and 7-10% TSP	Mice developed systemic immune response and survived the tetanus toxin challenge	[S137,S138]
Mutant of *E. coli* toxin (*LTK*63)	Tobacco	3.6-3.7% TSP	GM1 ganglioside-binding assay	[S139]
Anthrax protective antigen (*Pag*)	Tobacco	4.5-14.2% TSP	Macrophage lysis assay, systemic immune response, toxin neutralization assay, mice survived (100%) challenge with lethal doses of toxin	[S140,S141]
Lyme disease –OspA (OspA, OspA-T)	Tobacco	1 and 10% TSP	Systemic immune response in mice. Protected mice against *Borrelia burgdorferi*	[S142]
Plague F1-V (*CaF*1-*LcrV*)	Tobacco	14.8% TSP	Immunogenic in mice (IgG1 titers). Oral delivery offered greater protection (88%) and immunity than subcutaneous (33%) injection when challenged with 50-fold lethal dose of aerosolized *Y. pestis*	[S143]
E. coli enterotoxin B (*LTB*)	Tobacco	2.3% TSP	GM1 ganglioside-binding assay; oral immunization partially protected mice from CT challenge	[S144]
Multi-epitope DPT fusion protein	Tobacco	0.8% TSP	Immunogenic in orally immunized mice with freeze-dried chloroplast-derived DPT	[S145]
Viral antigens				
Canine parvovirus (*CTB-2L21,GFP-2L21*)	Tobacco	31.1 and 22.6% TSP	Rabbit sera neutralized CPV in an *in vitro* assay	[S146,S147]
Hepatitis E virus (*HEV E2*)	Tobacco Leaves Seeds	0.63-1.09 ng and 0.015-0.018 ng μg $^{-1}$ TSP	Immune response in mice	[S148]
Swine fever virus (*CSFV E2*)	Tobacco	1-2% TSP	Immune response in mice	[S149]
Human Papillomavirus (*L1*)	Tobacco	20-26% TSP	Induced systemic immune response in mice after intraperitoneal injection, and neutralizing antibodies were detected	[S150]
Human Papillomavirus (L1)	Tobacco	0.1-1.5% TSP	Not reported	[S151]
Foot-and-mouth (*CTB-VP1*)	*Chlamydomonas*	3-4% TSP	Not reported	[S152]
Rotavirus (*VP*6)	Tobacco		Not reported	[S153]
P*trc*-VP6	Seedlings	Undetectable		
P*rrn*-VP6	Seedlings	3% TSP		
P*psbA*-VP6	Seedlings	0.6% TSP		
Hepatitis C (*NS*3)	Tobacco	2% TSP	Not reported	[S154]
Partial spike (S) protein of SARS-CoV	Tobacco	0.2% TSP	Not reported	[S155]
Epstein-Barr virus (*VCA*)	Tobacco	0.002-0.004% TSP	Not reported	[S156]
Swine fever virus (*CSFV E2*)	*Chlamydomonas*	1.5-2% TSP	Not reported	[S157]
HIV (*p24*)	Tobacco	2.5% TSP	Not reported	[S158]
HIV (*P24-Nef*)	Tobacco & Tomato leaf Green Fruit Ripe Fruit	40% TSP 2.5% TSP Not detected	Not reported	[S159]
Protozoan antigens				
Amoebiasis (*Lec*A)	Tobacco	7% TSP	Systemic immune response in mice	[S160]
Malaria (*CTB-ama1* & *CTB-msp1*)	Tobacco Lettuce Tobacco Lettuce	12.3% TSP 9.4% TSP 8% TSP 4.8% TSP	Sera of immunized mice completely inhibited proliferation of the malarial parasite and cross-reacted with the native parasite proteins/parasites in immunoblots and immuno-fluorescence studies, at the ring, trophozoite or schizont parasite stages	[S134,S135]

Autoantigens				
Diabetes – Type 1 (*CTB-pins*)	Tobacco and Lettuce	~16% TSP 2.05-2.5% TSP	CTB-prins treated mice showed significant decrease in inflammation (insulitis) in non-obese diabetic mice; insulin-producing β -cells in the pancreatic islets of CTB-Pins-treated mice were highly protected, increase in insulin production with lower blood or urine glucose levels; Increased expression of immunosuppressive cytokines (IL4, IL 10)	[S161]
Diabetes – Type 1 (*hGAD*65)	*Chlamydomonas*	0.25-0.3% TSP	Immunoreactivity to diabetic sera	[S162]
Biopharmaceutical proteins				
Human somatotropin (*hST*)	Tobacco	0.2-7.0% TSP	Growth response of Nb2 cell line in the presence of somatotropin. Rat lymphoma cell line Nb2 proliferated in proportion to the amount of somatotropin in the culture medium, until saturation is reached	[S163]
Interferon gamma (*uidA-IFN-*γ)	Tobacco	6.0% TSP	Protection of human lung carcinoma cells against infection by encephalomyocarditis virus	[S164]
Interferon alpha 2b (*IFN*α*2b*)	Tobacco LAMD Petit havana	8.0-21.0% TSP 2.0-14.0% TSP	Immunogenic in mice. Transgenic IFN-a2b protected baby hamster kidney cells against cytopathic viral replication in vesicular stomatitis virus cytopathic effect assay, HeLa cells from HIV-1 entry and mice from a highly metastatic tumor line. Also, it increased the expression of major histocompatibility complex class I on splenocytes and the total number of natural killer cells	[S165]
Insulin-like growth factor (*IGF*-1n, *IGF*-1s)	Tobacco	32.4% TSP 32.7% TSP	Growth response in cultured HU-3 cells	[S166]
Human epidermal growth factor (*hEGF*)	Tobacco	Not reported (below detection levels)	Not reported	[S167]
Human alpha1-antitrypsin (*A1AT*)	Tobacco	2% TSP	Fully active and binds to porcine pancreatic elastase	[S168]
Antimicrobial peptide (*MSI-99*)	Tobacco	Not reported	Antifungal or antibacterial activities in vitro and in vivo in *Pseudomonas aeruginosa*	[S169]
Antimicrobial peptide (2 lysin-type protein)	Tobacco	~ 30% TSP	Bacteriolytic activity and kills *Streptococcus pneumoniae*, the causative agent of pneumonia	[S170]
Human serum albumin (*hsa*)	Tobacco	0.02-11.1% TSP	Not reported	[S171]
Human cardiotrophin-1 (*hCT*-1)	Tobacco	5% TSP	Biologically active on human hepatocarcinoma cell line, HepG2 assay	[S172]
Monoclonal antibody (*Guy's* 13)	Tobacco	Not Reported	Not reported	[S173]
Lysozyme antibody fragment (*AbL*)	Tobacco	Not detected	Not reported	[S174]

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