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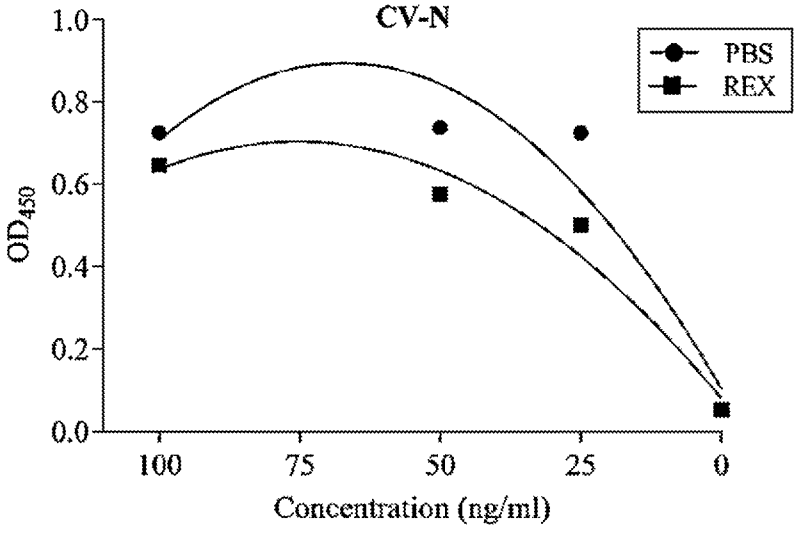


FIG. 1A

(57) Abstract: Microbicidal compositions comprising an endosperm or endosperm extract, and an anti-HIV lectin, an anti-HIV antibody or antigen-binding antibody fragment, or combination thereof, wherein the composition optionally comprises a pharmaceutically acceptable carrier are disclosed. Transgenic plants, methods for preventing an HIV infection in a human, and nucleic acids are also disclosed.

WO 2019/108656 A1

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MICROBICIDAL COMPOSITION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of copending U.S. Provisional Patent Application No. 62/591,569, filed November 28, 2017, which is incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under project number ZIA BC011472 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: one 58,095 Byte ASCII (Text) file named "740347_ST25.txt," dated November 28, 2018.

BACKGROUND OF THE INVENTION

[0004] The rate of infection caused by Human immunodeficiency virus ("HIV") is declining, but the HIV-1 positive population rose to more than 36 million in 2015 and there were 2.1 million new cases in 2013 (see GBD 2015 HIV Collaborators et al., *Estimates of global, regional, and national incidence, prevalence, and mortality of HIV*, 1980–2015: the Global Burden of Disease Study 2015, *Lancet HIV* 3, e361–87 (2016), and UNAIDS). The availability and effectiveness of antiretroviral therapy is increasing, reducing the number of deaths caused by acquired immunodeficiency syndrome ("AIDS") over the last few years. However, the virus continues to spread because there are no effective vaccines, and pre-exposure prophylaxis still remains largely reliant on barrier methods, or the oral administration of tenofovir/emtricitabine (see Ramessar et al., Can microbicides turn the tide

against HIV?, *Curr. Pharm. Des.*, (16): 468–85 (2010); Shattock, R.J. & Rosenberg, Z. Microbicides: topical prevention against HIV, *Cold Spring Harb. Perspect. Med* 2, a007385v (2012); and Pialoux et al., Pre-exposure prophylaxis: a useful tool to prevent human immunodeficiency virus infection?, *Clin. Microbiol. Infect.*, (22): 757–67 (2016)).

[0005] HIV-1 entry into susceptible cells begins when viral surface glycoprotein gp120 engages CD4 on the surface of lymphocytes, followed by binding to co-receptor CCR5 or CXCR4, and then the transmembrane subunit gp41 mediates membrane fusion (Haase, Early events in sexual transmission of HIV and SIV and opportunities for interventions, *Annu. Rev. Med.*, (62): 127–39 (2011)). Molecules that bind to gp120/gp41 in the proper manner could therefore act as HIV entry inhibitors and may be suitable as topical microbicides representing a subset of pre-exposure prophylaxis strategies (McGowan, Microbicides: a new frontier in HIV prevention, *Biologicals,* (34): 241–55 (2006)). Many different entry inhibitors have been tested *in vitro*, in animal studies and in some cases in human clinical trials, but the two most prevalent types of candidate are broadly-neutralizing monoclonal antibodies and lectins, which are carbohydrate-binding proteins. A large panel of antibodies has been tested against HIV *in vitro*, and some such as 4E10, 2F5, 2G12, VRC01, 10-1074 and 3BNC117 have also been tested in human clinical trials (see, for example, McCoy, L.E. & Weiss, R.A., Neutralizing antibodies to HIV-1 induced by immunization, *J. Exp. Med.*, (210): 209–23 (2013); Mascola, J.R. & Haynes, B.F., HIV-1 neutralizing antibodies: understanding nature's pathways, *Immunol Rev.*, (254): 225-44 (2013); Caskey et al., Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117, *Nature,* (522): 487–491 (2015); and Bar et al., Effect of HIV antibody VRC01 on viral rebound after treatment interruption, *New Engl. J. Med.*, (375): 2037–2050 (2016)).

[0006] The lectins griffithsin ("GRFT"), cyanovirin-N ("CV-N"), and scytovirin ("SVN") are HIV fusion inhibitors with low nanomolar to picomolar IC_{50} values against all HIV-1 clades *in vitro* and in animal models, but these have yet to be tested in humans (Mori et al., Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga Griffithsia sp., *J. Biol. Chem.*, (280): 9345–53 (2005); Lagenaur et al., Prevention of vaginal SHIV transmission in macaques by a live recombinant Lactobacillus, *Mucosal Immunol.*, (4): 648–57 (2011); Balzarini, Targeting the glycans of gp120: a novel approach

aimed at the Achilles heel of HIV, *Lancet. Infect. Dis.,* (5): 726–31 (2005); O'Keefe, et al., Broad-spectrum *in vitro* activity and *in vivo* efficacy of the antiviral protein griffithsin against emerging viruses of the family Coronaviridae, *J. Virol.,* (84): 2511–21 (2010); and Emau, et al., Griffithsin, a potent HIV entry inhibitor, is an excellent candidate for anti-HIV microbicide, *J. Med. Primatol.,* (36): 244–53 (2007)).

[0007] The antibodies 2G12, 2F5 and 4E10 have been produced in Chinese hamster ovary ("CHO") cells for prophylactic and therapeutic use, whereas griffithsin and cyanovirin-N were initially expressed as recombinant proteins in *Escherichia coli* and other microbes (Hofmann-Lehmann, et al., Postnatal passive immunization of neonatal macaques with a triple combination of human monoclonal antibodies against oral simian-human immunodeficiency virus challenge, *J. Virol.,* (75): 7470–80 (2001); Stiegler et al., Antiviral activity of the neutralizing antibodies 2F5 and 2G12 in asymptomatic HIV-1-infected humans: a phase I evaluation, *AIDS*, (16): 2019–25 (2002); Giomarelli, et al., Recombinant production of anti-HIV protein, griffithsin, by auto-induction in a fermentor culture, *Protein Expr. Purif.,* (47): 194–202 (2006); Gao, X. *et al.*, Soluble cytoplasmic expression, rapid purification, and characterization of cyanovirin-N as a His-SUMO fusion, *Appl. Microbiol. Biotechnol.*, (85), 1051–60 (2010); Colleluori, et al., Expression, purification, and characterization of recombinant cyanovirin-N for vaginal anti-HIV microbicide development, *Protein Expr. Purif.,* (39): 229–36 (2005); Boyd, et al., Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development, *Antimicrob. Agents Chemother.,* (41): 1521–30 (1997); Giomarelli, et al., The microbicide cyanovirin-N expressed on the surface of commensal bacterium Streptococcus gordonii captures HIV-1, *AIDS,* (16): 1351–6 (2002); Liu et al., Engineered vaginal lactobacillus strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N. *Antimicrob. Agents Chemother.,* (50): 3250–9 (2006); and Mori et al., Functional homologs of cyanovirin-N amenable to mass production in prokaryotic and eukaryotic hosts, *Protein Expr. Purif.*, (26): 42–9 (2002)).

[0008] The production of microbicidal components is expensive because fermenter-based platforms are required and the downstream processing facilities must be compliant with good

manufacturing practice ("GMP") to ensure the removal of viruses or endotoxins (Buyel et al., Extraction and downstream processing of plant-derived recombinant proteins, *Biotechnol Adv.*, (33): 902–13 (2015)). Furthermore, an effective microbicide would ideally contain multiple components targeting different epitopes to (i) prevent the emergence of "escape mutant" HIV strains resistant to individual components, (ii) broaden the coverage of diverse virus strains, and (iii) promote synergies to reduce the absolute concentrations of each component required for efficacy (Garg et al., The future of HIV microbicides: challenges and opportunities, *Antivir. Chem. Chemother.*, (19): 143–50 (2009)). The capacity, scalability and cost issues affecting fermenters are exacerbated when two or three separate products with individual manufacturing processes are required for each microbicide.

[0009] Accordingly, there is a need for new ways to produce and deliver effective microbicides, especially microbicides that are HIV neutralizing.

BRIEF SUMMARY OF THE INVENTION

[0010] An embodiment of the invention provides microbicidal compositions comprising an endosperm or endosperm extract, and an anti-HIV lectin, an anti-HIV antibody or antigen-binding antibody fragment, or combination thereof, wherein the composition optionally comprises a pharmaceutically acceptable carrier.

[0011] Another embodiment of the invention provides transgenic plants that express two or more of cyanovirin-N or a fragment or mutant thereof, griffithsin or a fragment or mutant thereof, scytovirin or a fragment or mutant thereof, other anti-HIV lectin, or an anti-HIV antibody or antigen-binding antibody fragment.

[0012] Yet another embodiment of the invention provides a seed, endosperm, or endosperm extract from a transgenic plant of the present invention.

[0013] Additional embodiments of the invention provide methods for preventing an HIV infection in a human comprising administering the forgoing composition, plant, seed, or endosperm, or extract thereof as provided herein, to a human.

[0014] Further embodiments of the invention provide nucleic acids encoding two or more of cyanovirin-N or a fragment or mutant thereof, griffithsin or a fragment or mutant thereof,

scytovirin or a fragment or mutant thereof, other anti-HIV lectin, or an anti-HIV antibody or antigen-binding antibody fragment.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0015] Figure 1A is a graph showing the results of a gp120-binding ELISA experiment for cyanovirin-N ("CV-N") reconstituted in phosphate-buffered saline ("PBS") (circles) and crude rice seed extracts ("REX") (squares) as detected with a CV-N-specific antibody. The concentrations are listed on the x-axis and the OD_{450} (optical density at 450 nm) are listed on the y-axis.

[0016] Figure 1B is a graph showing the results of a gp120-binding ELISA experiment for griffithsin ("GRFT") reconstituted in PBS (circles) and crude rice seed extracts ("REX") (squares) as detected with a GRFT-specific antibody. The concentrations are listed on the x-axis, and the OD_{450} are listed on the y-axis.

[0017] Figure 1C is a graph showing the results of a gp120-binding ELISA experiment for antibody 2G12 reconstituted in PBS (circles) and crude rice seed extracts ("REX") (squares) as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis, and the OD_{450} are listed on the y-axis.

[0018] Figure 1D is a graph showing the results of a competition ELISA experiment. Specifically, Figure 1D shows antibody 2G12 reconstituted with GRFT in PBS (circles) and crude rice seed extracts ("REX") (squares) as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis, and the OD_{450} are listed on the y-axis.

[0019] Figure 1E is a graph showing the results of a competition ELISA experiment for antibody 2G12 with CV-N reconstituted in PBS (circles) and crude rice seed extracts (squares) as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis, and the OD_{450} are listed on the y-axis.

[0020] Figure 1F is a graph showing the results of a competition ELISA experimentfor antibody 2G12 with CV-N and GRFT reconstituted in PBS (circles) and crude rice seed extracts (squares) as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis, and the OD_{450} are listed on the y-axis.

[0021] Figure 2A is a bar graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}CV-N alone or in combination with gp120 ("OS" refers to rice (*Oryza sativa*)). The controls are listed along the x-axis as C+ (^{EC}CV-N was used as a positive control, starting concentration was 250 ng/mL ["EC" refers to *Escherichia coli*"]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers 1, 6, and 11 along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0022] Figure 2B is a bar graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}GRFT alone or in combination with gp120 ("OS" refers to rice (*Oryza sativa*)). The controls are listed along the x-axis as C+ (^{EC}GRFT was used as a positive control, starting concentration was 250 ng/mL ["EC" refers to *Escherichia coli*"]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers 1, 8, and 11 along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0023] Figure 2C is a bar graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}2G12 alone or in combination with gp120 ("OS" refers to rice (*Oryza sativa*)). The controls are listed along the x-axis as C+ (^{EC}2G12 was used as a positive control, starting concentration was 250 ng/mL ["EC" refers to *Escherichia coli*"]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers 6, 8, and 11 along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0024] Figure 3A shows IC_{50} values for plant extracts containing GRFT alone (left), GRFT and 2G12 (middle left), or GRFT and CV-N (middle right) and GRFT and 2G12 and CV-N (right) tested against NL4.3 pseudotyped virus to evaluate GRFT-mediated neutralization potency. The data reflects the mean GRFT IC_{50} (half maximal inhibitory concentration) from 2 to 3 different extracts for each composition (biological replicates) tested in duplicate (technical replicates). The asterisk denotes significant differences between triple extracts and single/double extracts (determined using Mann–Whitney test).

[0025] Figure 3B is a graph showing the GRFT dose-response curve against NL4.3 pseudotyped virus. One plant extract containing only GRFT was serially diluted in order to evaluate its neutralization potency alone (open circles) or in the presence of 0.2 μg/mL 2G12 (solid triangles pointing up), CV-N (solid triangles pointing down) or both (solid squares), from individual extracts. The concentration is along the x-axis and the % neutralization is along the y-axis.

[0026] Figures 4A is a graph comparing the neutralization IC_{50} values for GRFT in single or triple extracts for the laboratory-adapted NL4.3 using a TZM-bl cell HIV-neutralization assay. The lines indicate the GRFT-2 transgenic line (open circles), transgenic line 11 from Table 2 (solid triangles pointing up), transgenic line 10 from Table 2 (solid triangles pointing down), and transgenic line 9 from Table 2 (solid squares). The concentration of GRFT (ng/mL) is along the x-axis and the % neutralization is along the y-axis.

[0027] Figure 4B is a graph comparing the neutralization IC_{50} values for GRFT in single or triple extracts for the primary isolate SVP16 using a HIV-1 pseudotyped viruses cell in a HIV-neutralization assay. The lines indicate the GRFT-2 transgenic line (open circles), transgenic line 11 from Table 2 (solid triangles pointing up), transgenic line 10 from Table 2 (solid triangles pointing down), and transgenic line 9 from Table 2 (solid squares). The concentration of GRFT (ng/mL) is along the x-axis and the % neutralization is along the y-axis.

[0028] Figure 5A is a graph showing the results of a competition ELISA experiment for antibody 2G12 at different concentrations in PBS (circles) crude rice endosperm extracts ("REX") (squares), crude rice endosperm extract without protein (triangles), protein extract (diamonds), and water extract (hexagons), as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0029] Figure 5B is a graph showing the results of a competition ELISA experiment for antibody 2G12 at different concentrations with CV-N in PBS (circles), crude rice endosperm extracts ("REX") (squares), crude rice endosperm extract without protein (triangles), protein extract (diamonds), and water extract (hexagons), as detected with an immunoglobulin-

specific antibody. The concentrations are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0030] Figure 5C is a graph showing the results of a competition ELISA experiment for antibody 2G12 at different concentrations with GRFT in PBS (circles), crude rice endosperm extracts ("REX") (squares), crude rice endosperm extract without protein (triangles), protein extract (diamonds), and water extract (hexagons), as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0031] Figure 5D is a graph showing the results of a competition ELISA experiment for antibody 2G12 at different concentrations with CV-N and GRFT in PBS (circles), crude rice endosperm extracts ("REX") (squares), crude rice endosperm extract without protein (triangles), protein extract (diamonds), and water extract (hexagons), as detected with an immunoglobulin-specific antibody. The concentrations are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0032] Figure 6A is a graph showing the results of a competition ELISA experiment for GRFT in PBS with increasing CV-N concentration probed with antibodies against GRFT or CV-N. The concentrations of GRFT are represented by the circles in greyscale. The concentrations of CV-N are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0033] Figure 6B is a graph showing the results of a competition ELISA experiment for GRFT in crude seed extract ("REX") with increasing CV-N concentration probed with antibodies against GRFT or CV-N. The concentrations of GRFT are represented by the circles in greyscale. The concentrations of CV-N are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0034] Figure 6C is a graph showing the results of a competition ELISA experiment for CV-N in PBS with increasing GRFT concentration probed with antibodies against GRFT or CV-N. The concentrations of CV-N are represented by the circles in greyscale. The concentrations of GRFT are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0035] Figure 6D is a graph showing the results of a competition ELISA experiment for CV-N in crude seed extract ("REX") with increasing GRFT concentration probed with antibodies against GRFT or CV-N. The concentrations of CV-N are represented by the circles in greyscale. The concentrations of GRFT are listed on the x-axis and the OD_{450} are listed on the y-axis.

[0036] Figure 6E is a graph showing the results of a competition ELISA experiment for 2G12 with CV-N or GRFT in PBS probed with antibodies against 2G12. The concentrations of 2G12 are represented by the circles in greyscale. The concentrations of CV-N (lanes 2-4) and GRFT (lanes 5-7) are listed on the x-axis along with the no lectin control (lane 1). The OD_{450} are listed on the y-axis.

[0037] Figure 6F is a graph showing the results of a competition ELISA experiment for 2G12 with CV-N or GRFT in crude seed extract ("REX") probed with antibodies against 2G12. The concentrations of 2G12 are represented by the circles in greyscale. The concentrations of CV-N (lanes 2-4) and GRFT (lanes 5-7) are listed on the x-axis along with the no lectin control (lane 1). The OD_{450} are listed on the y-axis.

[0038] Figure 6G is a graph showing the results of a competition ELISA experiment for 2G12 with CV-N and GRFT in PBS probed with antibodies against 2G12. The concentrations of 2G12 are represented by the circles in greyscale. The concentrations of CV-N and GRFT (lanes 2-4), and GRFT and CV-N (lanes 5-7) are listed on the x-axis along with the no lectin control (lane 1). The OD_{450} are listed on the y-axis.

[0039] Figure 6H is a graph showing the results of a competition ELISA experiment for 2G12 with CV-N and GRFT in crude seed extract ("REX") probed with antibodies against 2G12. The concentrations of 2G12 are represented by the circles in greyscale. The concentrations of CV-N and GRFT (lanes 2-4) and GRFT and CV-N (lanes 5-7) are listed on the x-axis along with the no lectin control (lane 1). The OD_{450} are listed on the y-axis.

[0040] Figure 7A is a graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}CV-N, ^{OS}GRFT and ^{OS}2G12 alone or in combination with gp120. The binding activity of 2G12 is shown in Figure 7A. The controls are listed along the x-axis as C+ (^{OS}2G12 was used as a positive control, starting

concentration was 250 ng/mL ["OS" refers to rice (*Oryza sativa*)]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0041] Figure 7B is a graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}CV-N, ^{OS}GRFT and ^{OS}2G12 alone or in combination with gp120 ("OS" refers to rice (*Oryza sativa*)). The binding activity of CV-N is shown in Figure 7B. The controls are listed along the x-axis as C+ (^{EC}CV-N was used as a positive control, starting concentration was 250 ng/mL [EC refers *Escherichia coli*]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0042] Figure 7C is a graph showing the binding activity of crude extracts from representative transgenic lines containing ^{OS}CV-N, ^{OS}GRFT and ^{OS}2G12 alone or in combination with gp120 ("OS" refers to rice (*Oryza sativa*)). The binding activity of GRFT is shown in Figure 7C. The controls are listed along the x-axis as C+ (^{EC}GRFT was used as a positive control, starting concentration was 250 ng/mL[EC refers *Escherichia coli*]) and "WT" refers to the negative control which was wild-type endosperm extract. The numbers along x-axis refer to the transgenic lines in Table 2. The OD_{450} are listed on the y-axis.

[0043] Figure 8A illustrates a transformation construct, pRP5-SVN, for stable expression of scytovirin in rice endosperm. The expression cassette contains endosperm specific rice prolamin promoter, thioredoxin coding region, His6 tag, enterokinase site (EK), full-length scytovirin coding region and nos terminator (t-nos).

[0044] Figure 8B illustrates a transformation construct, pRP5-SD1 for stable expression of the first structural domain of scytovirin in rice endosperm. The expression cassette contains endosperm specific rice prolamin promoter, thioredoxin coding region, His6 tag, enterokinase site (EK), coding region of scytovirin's first structural domain and nos terminator (t-nos).

[0045] Figures 9A-9B illustrate that SVN (Figure 9A) and SD1 (Figures 9B) are expressed in rice callus. A sandwich ELISA was used to check the presence of the lectins in rice callus. The plates were coated with HIV gp120, and the lectins were detected with a primary rabbit

anti-SVN polyclonal antiserum and a secondary horseradish peroxidase (HRP)-conjugated antirabbit IgG antibody. The results are the average absorbance at 450 nm (± standard deviation) from triplicate wells. Wild-type endosperm extract ("Wt"), 500 ng/mL of purified SVN/SD1 from *E. coli* ("C+"), and PBS were used for background corrections/controls. The rice callus names are listed on along the y-axis.

[0046] Figures 10A-10B illustrate that SVN (Figure 10A) and SD1 (Figure 10B) lectins accumulate in mature rice seeds. A sandwich ELISA was used to quantify the accumulation of the lectins in rice endosperm. The plates were coated with HIV gp120, and the lectins were detected with a primary rabbit anti-SVN polyclonal antiserum and a secondary horseradish peroxidase (HRP)-conjugated antirabbit IgG antibody. The results are the average absorbance at 450 nm (± standard deviation) from triplicate wells. Four serial dilutions per sample are shown (neat, 1/2 ["Dil 1/2"], 1/4 ["Dil 1/4"] and 1/8 ["Dil 1/8"]). Wild-type endosperm extract ("Wt"), 500 ng/mL of purified SVN/SD1 from *E. coli* ("C+"), and PBS were used for background corrections/controls. The seed cultivar names are listed on along the y-axis.

DETAILED DESCRIPTION OF THE INVENTION

[0047] An embodiment of the invention provides a microbicidal composition comprising (a) an endosperm or endosperm extract, and (b) an anti-HIV lectin, an anti-HIV antibody or antigen-binding antibody fragment, or combination thereof, wherein the composition optionally comprises a pharmaceutically acceptable carrier.

Anti-HIV Lectins

[0048] Anti-HIV lectins are small proteins that have been shown to bind to carbohydrates on HIV viral envelopes *in vitro* and/or *in vivo*. Any anti-HIV lectin known in the art can be used. Anti-HIV lectins can be derived, for example, from plants, algae, cyanobacteria, actinomycetes, and worms. Suitable anti-HIV plant lectins include *Artocarpus heterophyllus* (jacalin) lectin, concanavalin A, *Galanthus nivalis* (snowdrop) agglutinin-related lectins, *Musa acuminata* (banana) lectin, *Myrianthus holstii* lectin, *Narcissus pseudonarcissus* lectin, and *Urtica diocia* agglutinin. Suitable anti-HIV algal lectins include *Boodlea coacta* lectin, griffithsin, and *Oscillatoria agardhii* agglutinin. A preferred anti-HIV algal lectin is

griffithsin. Suitable anti-HIV cyanobacterial lectins include cyanovirin-N, scytovirin, *Microcystis viridis* lectin, and microvirin. Preferred anti-HIV cyanobacterial lectins include cyanovirin-N and scytovirin. A suitable anti-HIV actinomycete lectin is actinohivin. Suitable anti-HIV worm lectins include *Chaetopterus variopedatus polychaete* marine worm lectin, *Serpula vermicularis* sea worm lectin, and C-type lectin Mermaid from nematode (*Laxus oneistus*).

[0049] In one embodiment, the anti-HIV lectin is scytovirin, griffithsin, cyanovirin-N, or a fragment or mutant thereof that retains the ability to bind to carbohydrates on HIV viral envelopes. The scytovirin, griffithsin, cyanovirin-N, or a fragment or mutant thereof can be any known in the art, for example, be any disclosed in US20110183894A1, US20120053116A1, US20080311125A1, US20050084496A1, US20090297516A1, WO2016130628A1, US20160108097A1, US20100221242A1, US20110189105A1, US20090092557A1, US20110263485A1, US20100240578A1, WO2007005766A1, US20080015151A1, US20040258706A1, US20050090643A1, US20020110557A1, US20020127675A1, US20020151476A1, US20030103997A1, US20030166552A1, US20040204365A1, US20040220107A1, WO2003072594A2, WO2002077189A2, WO2000053213A2, WO2000011036A1, WO1996034107A2, the entire disclosures of which are incorporated by reference. It is to be understood, that the lectin (e.g., scytovirin, griffithsin, cyanovirin-N, or a fragment or mutant thereof) can be present in the composition in any form, including as a fusion protein, multimer, or conjugate.

[0050] Scytovirin is a lectin which has been shown to have anti-viral properties. It has a 95-amino-acid sequence (SEQ ID NO: 11) and was isolated from *Scytonema varium*. In one embodiment, the anti-HIV lectin is scytovirin. In some embodiments, the scytovirin, or a fragment or mutant thereof, has about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identity to SEQ ID NO: 11. Sytovirin contains a structural domain, named SD1, which includes residues 1-48 of scytovirin (SEQ ID NO: 34).

[0051] In another embodiment, the anti-HIV lectin is derived from scytovirin. In an embodiment, the anti-HIV lectin comrpises SD1(1-48)Cys7Gly (SEQ ID NO: 12), SD2(49-95)Cys55Gly (SEQ ID NO: 13), SVN(1-40)Cys7Gly (SEQ ID NO: 14), SVN(3-45)Cys7Gly

(SEQ ID NO: 15), SVN(6-45)Cys7Gly (SEQ ID NO: 16), and SVN(11-45) (SEQ ID NO: 17). In another embodiment, the anti-HIV lectin comprises one or more of SD1(1-48)Cys7Gly (SEQ ID NO: 12) or SD2(49-95)Cys55Gly (SEQ ID NO: 13), and SVN(1-40)Cys7Gly (SEQ ID NO: 14). In an embodiment, the anti-HIV lectin comrpises SD1 (SEQ ID NO: 34).

[0052] Griffithsin is a lectin which has been shown to be a potent HIV inhibitor. It has a 121-amino-acid sequence (SEQ ID NO: 9) and was isolated from the red algae *Griffithsia*. In one embodiment, the anti-HIV lectin is griffithsin. In some embodiments, the griffithsin, or a fragment or mutant thereof, has about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identity to SEQ ID NO: 9.

[0053] Cyanovirin-N is a lectin which has anti-viral properties. It has a 101-amino acid sequence (SEQ ID NO: 18) and was isolated from the cyanobacterium *Nostoc ellipsosporum*. In one embodiment, the anti-HIV lectin is cyanovirin-N. In some embodiments, the cyanovirin-N, or a fragment or mutant thereof, has about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identity to SEQ ID NO: 18.

[0054] Included in the scope of an embodiment of the invention are mutants of the lectins described herein. The term "mutants thereof" as used herein refers to a lectin having substantial or significant sequence identity or similarity to a parent lectin, which mutant retains the biological activity (binding to an HIV envelope carbohydrate) of the lectin of which it is a mutant. Mutants encompass, for example, mutants of the lectin described herein (the parent lectin) that retain the ability to treat or prevent HIV to a similar extent, the same extent, or to a higher extent, as the parent lectin. In reference to the parent lectin, the mutant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent lectin.

[0055] A mutant can, for example, comprise the amino acid sequence of the parent lectin with at least one conservative amino acid substitution. Alternatively or additionally, the

functional variants can comprise the amino acid sequence of the parent lectin with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the mutant. The non-conservative amino acid substitution may enhance the biological activity of the mutant, such that the biological activity of the mutant is increased as compared to the parent lectin.

[0056] Amino acid substitutions of the lectins are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (e.g. Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (e.g., Asn, Gln, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (e.g., Ile, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (e.g., His, Phe, Trp, and Tyr), etc.

[0057] The lectin can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, e.g., other amino acids, do not materially change the biological activity of the protein, functional portion, or functional variant.

[0058] The lectins of embodiments of the invention (including fragments and mutants thereof) can be of any length, i.e., can comprise any number of amino acids, provided that the lectins (or fragments and mutants thereof) retain their biological activity, e.g., prevent HIV infection in a human. For example, the lectin can be from about 50 to about 5,000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, or more amino acids in length.

[0059] The lectins of embodiments of the invention (including fragments and mutants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

Anti-HIV Antibody

[0060] The anti-HIV antibody can be any antibody shown to neutralize HIV *in vitro* and/or *in vivo*. The anti-HIV antibody or antigen-binding fragment thereof can be an anti-HIV envelope antibody (e.g., anti-gp120 or anti-gp41) or antigen-binding fragment thereof. Anti-HIV antibodies are known in the art and include, for example, 4E10, 2F5, 2G12, VRC01, 10-1074, or 3BNC117 (see McCoy, L.E. & Weiss, R.A., Neutralizing antibodies to HIV-1 induced by immunization, *J. Exp. Med.,* (210): 209–23 (2013); Mascola, J.R. & Haynes, B.F., HIV-1 neutralizing antibodies: understanding nature's pathways, *Immunol Rev.,* (254): 225-44 (2013); Caskey, M *et al.,* Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117, *Nature,* (522): 487–491 (2015); and Bar K.J. *et al.,* Effect of HIV antibody VRC01 on viral rebound after treatment interruption, *New Engl. J. Med.,* (375): 2037–2050 (2016)). Thus, in one embodiment, the anti-HIV envelope antibody or antigen-binding fragment thereof comprises at least the CDRs or variable regions of 4E10, 2F5, 2G12, VRC01, 10-1074, or 3BNC117.

[0061] In a particular embodiment, the anti-HIV antibody is the 2G12 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable

regions of the 2G12 antibody. In some embodiments, the anti-HIV antibody comprises the heavy chain variable region (SEQ ID NO: 1) and the light chain variable region (SEQ ID NO: 2) of 2G12.

[0062] In another embodiment, the anti-HIV antibody is the 4E10 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable regions of the 4E10 antibody. In some embodiments, the anti-HIV antibody comprises the heavy chain variable region (SEQ ID NO: 5 and the light chain variable region (SEQ ID NO: 6) of 4E10.

[0063] In one embodiment, the anti-HIV antibody is the 2F5 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable regions of the 2F5 antibody.

[0064] In one embodiment, the anti-HIV antibody is the VRC01 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable regions of the VRC01 antibody.

[0065] In one embodiment, the anti-HIV antibody is the 10-1074 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable regions of the 10-1074 antibody.

[0066] In one embodiment, the anti-HIV antibody is the 3BNC117 antibody, or an antibody or antigen binding antibody fragment comprising the CDR regions or variable regions of the 3BNC117 antibody.

[0067] In one embodiment, the antigen-binding antibody fragment is a Fab fragment (Fab), $F(ab')_2$ fragment, diabody, triabody, tetrabody, bispecific antibody, single-chain variable region fragment (scFv), or disulfide-stabilized variable region fragment (dsFv).

[0068] The anti-HIV antibody can be of any isotype (e.g., isotype IgA, IgD, IgE, IgG (e.g., IgG1, IgG2, IgG3, IgG4, or IgM). In one embodiment, the antibody is a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a plant, e.g., rice, or mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. In another embodiment, the antibody is a genetically-engineered antibody, e.g., a humanized antibody or a chimeric

antibody. The antibody can be in monomeric or polymeric form. Also, the antibody can have any level of affinity or avidity for HIV.

[0069] Methods of testing antibodies for the ability to bind to HIV are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay ("RIA"), enzyme-linked immunosorbent assay ("ELISA"), Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Murphy et al., *infra*, and U.S. Patent Application Publication No. 2002/0197266 A1).

Endosperm or Endosperm Extract

[0070] The endosperm can be from any plant engineered to express the anti-HIV lectin/anti-HIV antibody or antibody fragment. In some embodiments, the endosperm extract is from rice, sorghum, wheat, rye, triticale, oats, barley, spelt, soybean, maize, tobacco, or marshmallow endosperm. In particular embodiments, the endosperm extract is from rice or sorghum.

[0071] The endosperm can be in any form, and can be used, for instance, as a processed whole material (e.g., fresh, dried (air-, heat-, or freeze-dried), ground, pulverized, powdered, etc.), or as an extract. The endosperm extract can be provided by an extract of any portion of the plant that includes the endosperm. Thus, the endosperm extract can be provided, for instance, by a whole plant extract, a seed or embryo extract, or any other extract that also provides an extract of the endosperm. In other words, the composition comprises at least the endosperm extract, and may comprise extracts of other portions of the plant as well, although in some embodiments the composition comprises only the extract of the endosperm, seed, or embryo. In a particular embodiment, the composition comprises at least the globulin fraction of an endosperm extract, or comprises only the globulin fraction of an endosperm extract.

[0072] In some embodiements, the endosperm is from a transgenic plant that has been engineered to express an anti-HIV lectin or anti-HIV antibody. In a more particular embodiment, the endosperm is from a plant engineered to express one or more, two or more, or three or more of griffithsin (or a fragment or mutant thereof), cyanovirin-N (or a fragment or mutant thereof), scytovirn (or a fragment or mutant thereof), and an anti-HIV antibody or antigen-binding antibody fragment thereof. In a more specific example, the endosperm is

from a plant engineered to griffithsin (or a fragment or mutant thereof), cyanovirin-N (or a fragment or mutant thereof), and an anti-HIV antibody or antigen-binding antibody fragment thereof.

Pharmaceutcially Acceptable Carrier

[0073] The optional pharmaceutically acceptable carrier can be any of those conventionally used for introduction into a mammal or human (including by ingestion) and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active agent(s), and by the route of administration. As used herein, pharmaceutically acceptable includes carriers suitable for ingestion by people or animals. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0074] The choice of carrier will be determined in part by the particular inventive microbicidal composition, as well as by the particular method used to administer the inventive microbicidal composition. For instance, the composition can be formulated as a pill, capsule, suspension or solution (including liquid [e.g., spray], gel, or cream), for oral, parenteral, or topical administration. The composition can also be formulated to be released from a vaginal insert, such as a flexible plastic ring. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the embodiments of the invention. Preservatives may be used. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. A mixture of two or more preservatives optionally may be used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition.

[0075] Suitable buffering agents may include, for example, histidine or citrate-based buffers. Suitable buffering agents may includfe, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. A mixture of two or more buffering agents optionally may be used. The buffering agent or mixtures thereof are

typically present in an amount of about 0.001% to about 4% by weight of the total composition.

Transgenic Plants and Seeds

[0076] An embodiment of the invention provides a transgenic plant that expresses a plurality of anti-HIV lectins and/or anti-HIV antibodies/fragments. The anti-HIV lectins and antibodies/fragments can be any of those described above with respect to the microbicidal composition.

[0077] In one embodiment, the transgenic plant expresses two or more (e.g., three or more, or four or more) of (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, (c) scytovirin or a fragment or mutant thereof, (d) other anti-HIV lectin, or (e) an anti-HIV antibody or antigen-binding antibody fragment. The cyanovirin-N, griffithsin, scytovirin, fragments and mutants thereof, other lectins, and anti-HIV antibody or a fragment thereof, are as described above with respect to the microbicidal composition.

[0078] In one embodiment, the transgenic plant expresses scytovirin or a fragment or mutant thereof. The scytovirin and fragments and mutants thereof are as described above with respect to the microbicidal composition. In one embodiment, the transgenic plant expresses the scytovirin fragment SD1.

[0079] The transgenic plant can be any type of plant that has been genetically modified from the wild-type plant to express the anti-HIV lectins and/or anti-HIV antibody or antibody fragment. In an embodiment of the invention, the transgenic plant is a transgenic rice, sorghum, wheat, rye, triticale, oats, barley, spelt, soybean, maize, tobacco, or marshmallow plant. In two particular embodiments, the transgenic plant is transgenic sorghum or rice.

[0080] The transgenic plant can be engineered to express the anti-HIV lectins and antibodies/antibody fragments by transfecting the plant or a plant embryo with one or nucleic acids encoding the anti-HIV lectins and/or antibodies/antibody fragments. Each of the anti-HIV lectins and/or antibodies/antibody fragments can be encoded by a separate nucleic acid, or a single nucleic acid can encode two or more, or even all of the anti-HIV lectins and/or antibodies/antibody fragments. Thus, as additional aspects of the invention, there is provided

a nucleic acid encoding the plurality of anti-HIV lectins and/or anti-HIV antibodies/fragments, as well as transgenic plant comprising such a nucleic acid.

[0081] In one aspect, the transgenic plant comprises two or more (e.g., three or more or four or more) of (a) a nucleic acid encoding cyanovirin-N or a fragment or mutant thereof, (b) a nucleic acid encoding griffithsin or a fragment or mutant thereof, (c) a nucleic acid encoding scytovirin or a fragment or mutant thereof, (d) a nucleic acid encoding another anti-HIV lectin, or (e) a nucleic acid encoding an anti-HIV antibody or antigen-binding antibody fragment. In a more particular embodiment, the transgenic plant comprises (a) a nucleic acid encoding cyanovirin-N or a fragment or mutant thereof, (b) a nucleic acid encoding griffithsin or a fragment or mutant thereof, and (c) a nucleic acid encoding an anti-HIV antibody or antigen-binding antibody fragment. In another embodiment, the transgenic plant comprises a nucleic acid that encodes two or more (e.g., three or more or four or more) of (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, (c) scytovirin or a fragment or mutant thereof, (d) other anti-HIV lectin, or (e) an anti-HIV antibody or antigen-binding antibody fragment. In a more particular embodiment, the nucleic acid encodes (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, and (c) an anti-HIV antibody or antigen-binding antibody fragment. In a more particular embodiment, the transgenic plant comprises a nucleic acid that encodes (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, and (c) an anti-HIV antibody or antigen-binding antibody fragment. The nucleic acids, themselves, are considered to be additional embodiments of the invention. Thus, for instance, there is also provided a nucleic acid that encodes two or more (e.g., three or more or four or more) of (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, (c) scytovirin or a fragment or mutant thereof, (d) other anti-HIV lectin, or (e) an anti-HIV antibody or antigen-binding antibody fragment. In a more particular embodiment, the nucleic acid encodes (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, and (c) an anti-HIV antibody or antigen-binding antibody fragment. The anti-HIV lectin and anti-HIV antibody/fragment thereof can be any of those described with respect to the microbicidal composition.

[0082] There is also provided a nucleic acid that encodes scytovirin or a fragment or mutant thereof that comprises an endosperm-specific promoter. In an embodiment, the nucleic acid encodes the scytovirin fragment SD1.

[0083] The transgenic plant comprises a nucleic acid as described by being transfected or transformed with the nucleic acid. Thus, the nucleic acids encoding the anti-HIV lectins and/or anti-HIV antibody/fragments thereof may be in a vector suitable for introducing the nucleotide sequences into the plant genome or otherwise expressing the gene products.

[0084] Accordingly, each of the one or more nucleic acids optionally includes a promoter suitable for plant expression. It is within the knowledge of one of skill in the art to select a suitable promoter, many of which are known. Preferably, the nucleic acids comprise an endosperm-specific promoter operably linked to the nucleotide sequences encoding the anti-HIV lectins and/or anti-HIV antibodies/fragments thereof. Examples of endosperm-specific promoters include, for instance, rice glutelin-1 promoter (SEQ ID NO: 20), maize γ-zein promoter (SEQ ID NO: 21), rice prolamin RP5 promoter (SEQ ID NO: 22), barley hordein promoter (SEQ ID NO: 23), or a combination thereof.

[0085] Thus, the transgenic plant can comprise, for instance, two or more (e.g., three or more or four or more) of (a) a nucleic acid encoding cyanovirin-N or a fragment or mutant thereof, (b) a nucleic acid encoding griffithsin or a fragment or mutant thereof, (c) a nucleic acid encoding scytovirin or a fragment or mutant thereof, (d) a nucleic acid encoding another anti-HIV lectin, or (e) a nucleic acid encoding an anti-HIV antibody or antigen-binding antibody fragment, wherein each of (i)-(v) comprises an endosperm-specific promoter operably linked to the sequence encoding the anti-HIV lectin or anti-HIV antibody or a fragment thereof. The endosperm specific promoter can be, for example, a rice-endosperm specific promoter, such as a promoter selected from rice glutelin-1 promoter, maize γ-zein promoter, rice prolamin RP5 promoter, or barley hordein promoter.

[0086] When a single nucleic acid encodes multiple anti-HIV proteins, the nucleic acid comprises, for instance, two or more (e.g., three or more or four or more) of: (i) a nucleotide sequence encoding anti-HIV antibody, (ii) a nucleotide sequence encoding griffithsin or a fragment or mutant thereof, (iii) a nucleotide sequence encoding cyanovirin-N or a fragment

or mutant thereof, (iv) a nucleotide sequence encoding scytovirin or a fragment or mutant thereof, and (v) a nucleotide sequence encoding another anti-HIV lectin, wherein each nucleotide sequence of (i)-(v) is operatively linked to an endosperm-specific promoter, for example, a rice-endosperm specific promoter, such as a promoter selected from rice glutelin-1 promoter, maize γ-zein promoter, rice prolamin RP5 promoter, and barley hordein promoter. In a particular embodiment, the nucleic acid comprises two or more (e.g., three or more or four or more) of: (i) a nucleotide sequence encoding anti-HIV antibody operatively linked to rice glutelin-1 promoter or barley hordein promoter, (ii) a nucleotide sequence encoding griffithsin or a fragment or mutant thereof operatively linked to maize γ-zein promoter, (iii) a nucleotide sequence encoding cyanovirin-N or a fragment or mutant thereof operatively linked to rice prolamin RP5 promoter, (iv) a nucleotide sequence encoding scytovirin or a fragment or mutant thereof, or other anti-HIV lectin operatively linked to rice prolamin RP5 promoter.

[0087] An embodiment of the invention provides a transgenic plant comprising (i) a nucleotide sequence encoding anti-HIV antibody (e.g., 2G12) operatively linked to rice glutelin-1 promoter, (ii) a nucleotide sequence encoding griffithsin or a fragment or mutant thereof operatively linked to maize γ-zein promoter, and (iii) a nucleotide sequence encoding cyanovirin-N or a fragment or mutant thereof operatively linked to rice prolamin RP5 promoter.

[0088] "Nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In some embodiments, the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions. In some embodiments, the nucleic acid may encode additional amino acid sequences that do not affect

the function of the polypeptide or protein and which may or may not be translated upon expression of the nucleic acid by a host cell. In an embodiment of the invention, the nucleic acid is complementary DNA (cDNA). In an embodiment of the invention, the nucleic acid comprises a codon-optimized nucleotide sequence.

[0089] The nucleic acids can consist essentially of the specified nucleotide sequence or sequences described herein, such that other components, e.g., other nucleotides, do not materially change the biological activity of the encoded protein, functional portion, or functional variant.

[0090] The nucleic acid can comprise any isolated or purified nucleotide sequence which encodes any of the proteins, or functional portions or functional variants thereof.

[0091] The transgenic plant can be used for any purpose, but is particularly useful as a source for the microbial composition described herein. The plant can be used whole for this purpose, or any part of the plant expressing the anti-HIV lectin and/or anti-HIV antibody/fragment thereof can be used, such as the seed, embryo, or endosperm.

[0092] In this respect, an embodiment of the invention provides a seed or endosperm from a transgenic plant as described herein. In an embodiment, the seed or endosperm is from transgenic rice, sorghum, wheat, rye, triticale, oats, barley, spelt, soybean, maize, tobacco, or marshmallow plant. In a more particular embodiment, the seed is from transgenic sorghum or rice.

[0093] An embodiment of the invention provides a seed or endosperm that expresses two or more (e.g., three or more or four or more) of (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, (c) scytovirin or a fragment or mutant thereof, (d) other anti-HIV lectin, or (e) an anti-HIV antibody or antigen-binding antibody fragment. A particular embodiment provides a seed that expresses (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, and (c) anti-HIV antibody 2G12 or an antigen-binding antibody fragment.

[0094] The seed or endosperm can be in any form. Thus, for instance, the seed or part thereof comprising the endosperm can be milled to reduce the size of the grain, e.g., to

produce a powder or flour. Thus, in another embodiment, there is provided flour milled from a seed or endosperm of a transgenic plant as described herein.

[0095] Also provided is an extract of any portion of the plant that includes the endosperm. The extract can be provided, for instance, by a whole plant extract, a seed or embryo extract, or any other extract that also provides an extract of the endosperm. In other words, the composition comprises at least the endosperm extract, and may comprise extracts of other portions of the plant as well, although in some embodiments the composition comprises only the extract of the endosperm, seed, or embryo. In a particular embodiment, the composition comprises at least the globulin fraction of an endosperm extract, or comprises only the globulin fraction of an endosperm extract.

Method of Preparation

[0096] The microbicidal composition can be prepared by any suitable method. For example, the anti-HIV lectins and/or anti-HIV antibody/fragment thereof can be combined with the endosperm extract (or other extract or composition that comprises the endosperm extract) and the pharmaceutically acceptable carrier. The components can be combined by any of various methods known in the art, which will depend in part upon the desired final form of the composition.

[0097] The anti-HIV lectins and/or anti-HIV antibody/fragment thereof can be provided by any method. For instance, they can be expressed (together or separately) in cell culture and purified prior to adding the components to the composition. Conveniently, however, they can be provided along with the endosperm extract by a transgenic plant as described herein. Thus, also provided herein is a method of preparing the microbicidal composition comprising extracting at least the globulin fraction of a seed or endosperm from a transgenic plant as provided herein, which contains the anti-HIV lectins and/or anti-HIV antibody/fragment thereof. Any suitable method of preparing the extract (method of extraction) can be used. The extraction can be performed by contacting the endosperm, seed, or any other part of the transgenic plant comprising the endosperm (including the whole plant) with a suitable solvent. Solvents include polar or non-polar solvents, including, without limitation, water, salt solution, alcohol (e.g., methanol or ethanol), acetone, chloroform, dichloromethane,

organic acids (e.g., acetic acid), and the like. Mixtures of solvents can be used, and/or multi-step extraction wherein each step uses the same or different solvents or combinations of solvents. The solvent can be used hot (e.g., above 20 °C, such as about 50 °C or more, 70 °C or more, or even 100 °C or more) or cold (e.g., below 20 °C, such as about 10 °C or less, 5 °C or less, or even 0 °C or less). Extraction can be performed at atmospheric pressure, or under a pressure of greater than 1 atm. Supercritical or subcritical conditions can be used. The method may include maceration, infusion, percolation and decoction. In one embodiment, the method comprises extracting at least the globulin fraction, for instance, by using a salt solution.

[0098] Also provided is a method of providing a transgenic plant as described herein, which method comprises transfecting the plant with one or more nucleic acids as described herein, e.g., encoding two or more (e.g., three or more or four or more) of (a) cyanovirin-N or a fragment or mutant thereof, (b) griffithsin or a fragment or mutant thereof, (c) scytovirin or a fragment or mutant thereof, (d) other anti-HIV lectin, or (e) an anti-HIV antibody or antigen-binding antibody fragment. The method of providing a transgenic plant can be used in combination with the method of preparing the microbicidal composition.

[0099] All other aspects of the methods of preparation are as described with respect to the other aspects of the invention.

Methods of Prevention

[0100] The compositions described herein, including transgenic plants and parts thereof, can be used used for any purpose, but are believed to be of particular use for the prevention of an HIV infection.

[0101] Accordingly, an embodiment of the invention provides a method for preventing an HIV infection in a human comprising administering the microbicidal composition of any one of embodiments of the invention, plant of any one of the embodiments of the invention, seed of any one of the embodiments of the invention, endosperm of any one of the embodiments of the invention, or any extract thereof or product (e.g., foodstuff) made therefrom, to the human. Similarly, the invention provides a microbicidal composition of any one of the embodiments of the invention, plant of any of the embodiments of the invention, seed of any

one of the embodiments of the invention, or endosperm of any one of the embodiments of the invention, for use in a method of preventing an HIV infection, or for use in the preparation of a medicament for preventing an HIV infection.

[0102] The microbicidal composition, transgenic plant, seed, or endosperm can be administered in any form. For example, the microbicidal composition can be formulated as a pharmaceutical composition as described herein. Similarly, the plant, seed, endosperm, or an extract thereof can be processed (ground, milled, extracted, etc.) and formulated into a pharmaceutical composition or foodstuff.

[0103] The term "prevent" as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete prevention. Rather, there are varying degrees of prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of prevention of HIV in a human. Furthermore, the prevention provided by the inventive method can include prevention of one or more conditions or symptoms of the infection, e.g., HIV, being prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the infection, or a symptom or condition thereof, or spread of an infection within the body.

[0104] An "effective amount" or "an amount effective to prevent" refers to a dose that is adequate to prevent an HIV infection to any degree over the course of administration, either as a single does or multiple doses over time. Amounts effective for a prophylactic use will depend on, for example, the virulence of the virus, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the lectin or antibody selected, method of administration, timing and frequency of administration, the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular active, and the desired physiological effect. It will be appreciated by one of skill in the art that prevention of an HIV infection could require multiple administrations, perhaps using the inventive microbicidal compositions in each or various rounds of administration. By way of example and not intending to limit the embodiments of the invention, the dose of the inventive microbicidal composition can be about 0.001 to about 1000 mg/kg body weight of the subject being

treated/day, from about 0.01 to about 10 mg/kg body weight/day, about 0.01 mg to about 1 mg/kg body weight/day.

[0105] For purposes of the embodiments of the invention, the amount or dose of the inventive microbicidal composition administered should be sufficient to effect a prophylactic response in the subject or animal over a reasonable time frame. For example, the dose of the inventive microbicidal composition should be sufficient to partially or completely prevent an HIV infection or neutralize the spread or severity of an HIV infection. The dose will be determined by the efficacy of the particular inventive microbicidal composition and the condition of the human), as well as the body weight of the human to be treated.

[0106] The delivery systems useful in the context of embodiments of the invention may include time-released, delayed release, and sustained release delivery systems such that the delivery of the inventive composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The inventive composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the inventive composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain composition embodiments of the invention.

[0107] Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like.

[0108] When the inventive microbicidal compositions are administered with one or more additional therapeutic agents, one or more additional therapeutic agents can be coadministered to the human. As used herein, “coadministering” refers to administering one

or more additional therapeutic agents and the inventive microbicidal compositions sufficiently close in time such that the inventive microbicidal compositions can enhance the effect of one or more additional therapeutic agents, or vice versa. In this regard, the inventive microbicidal compositions can be administered first and the one or more additional therapeutic agents can be administered second, or *vice versa*. Alternatively, the inventive microbicidal compositions and the one or more additional therapeutic agents can be administered simultaneously.

[0109] Any suitable route of administration can be used, including without limitation topical, rectal, or vaginal. In some embodiments, the compositions are administered topically to a mucosal membrane of the subject human.

[0110] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0111] Assays were used to determine if 2G12, cyanovirin-N, and griffithsin were active in crude extracts of rice endosperm. A series of ELISA experiments were conducted with gp120 as the immobilized target. The three microbicide components (pure recombinant proteins) were prepared as a dilution series in PBS and ELISAs were carried out using the individual components, all three pairwise combinations, and the triple combination to determine whether there was any evidence of interdependent binding, mutual inhibition or cooperative binding. Two different versions of gp120 were used to ensure that the results were not dependent on a specific HIV strain.

[0112] When different concentrations of griffithsin and cyanovirin-N were combined in a matrix and gp120 binding was detected using either an anti-griffithsin polyclonal antibody or an anti-cyanovirin-N polyclonal antibody, there was no evidence that either component influenced the ability of the other to bind gp120 as seen by the clear concentration-dependent binding of the primary lectin (higher OD values at higher concentrations) regardless of the concentration of the competing lectin (Figures 7A-7C). However, when the ELISAs were repeated by reconstituting the components in extracts of wild-type rice endosperm rather than PBS, a small inhibitory effect was observed on the binding of cyanovirin-N, with slightly

lower OD values in the presence of rice extract (see Figure 1A), but a remarkable increase in the binding activity of griffithsin, with the OD values doubling in the presence of the rice extract (see Figure 1B). The pH of the rice endosperm extract was the same as PBS, so the results were not due to pH-dependent effects on electrostatic interactions. The ELISA experiments suggested that griffithsin binding to gp120 was enhanced by the rice extract but there was no effect on the interaction between cyanovirin-N and gp120.

[0113] Additional ELISA experiments were carried out to test the binding of 2G12 alone or in the presence of different concentrations of single lectins and both lectins. Again, there was concentration-dependent binding of 2G12, and the presence of either individual or double lectins had no effect at any of the concentrations tested, including double lectins at increasing concentrations in concert or in opposing gradients (see Figures 6A-6H). Figures 6A and 6B show that GRFT binds to gp120 W61D at increasing concentrations of CV-N. Figures 6C and 6D show that CV-N binds at increasing concentrations of GRFT. Figures 6E-6H show that 2G12 binds at increasing concentrations of CV-N and GRFT.

[0114] There was an increase in 2G12 binding when PBS was replaced with rice seed extract when 2G12 was presented alone (see Figure 1C) or in the presence of individual lectins (see Figures 1D and 1E) and both lectins (see Figure 1F). Overall, this example indicated that components in the crude rice endosperm extract doubled the binding activity of 2G12 and griffithsin compared with the same components at the same concentrations in PBS, but had no effect on cyanovirin-N.

[0115] The assays were conducted as follows: The specific antigen-binding activity of each protein was determined by coating the wells of ELISA plates with 100 ng recombinant gp120 IIIB or gp120 W61D (MRC Centralized Facility for AIDS Reagents, Potters Bar, UK). After washing with PBST and blocking with 2.5% BSA in PBST, serial dilutions of rice 2G12, griffithsin, or cyanovirin-N were added and the amount of bound protein determined using antibodies. Combinations of the standards at known concentrations were compared to single components to determine whether there was any competition for gp120 binding or any evidence of assisted binding. For the recapitulation experiments, each ELISA was carried out three times with the 2G12 and lectins diluted in PBS, and another three times with the 2G12 and lectins diluted in extracts of wild-type rice endosperm prepared in the same manner as

the transgenic extracts. The three components were tested alone, in all three pairwise combinations, and as a mixture of all three components at different concentrations. A randomized complete analysis of variance (ANOVA) was applied to each component (2G12, CV-N, and GRFT) at each concentration, and statistically significant differences between crude rice seed extracts (REX) and PBS, and between different endosperm extracts, were determined using the Tukey test in JMP-PRO v12.0.1 (SAS institute Inc., Cary, NC, US). Statistical significance was expressed as follows: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS = nonsignificant. A non-linear regression function was also used to determine the performance of each component at different concentrations, a quadratic non-linear regression curve was fitted and statistically significant differences were determined by comparing the fitted curve parameters. Model fitting and parameter estimations were carried out using GraphPad Prism v7.03 (GraphPad Software, Inc., San Diego, CA, USA). No statistical differences were found for Figures 1A and 1B ($p > 0.05$). Segments in each data point represent the SEM from three replications.

EXAMPLE 2

[0116] The functional impact of the enhanced binding apparently promoted by rice extracts was determined by conducting TZM-bl cell HIV-neutralization assays. Recombinant griffithsin, cyanovirin-N, and 2G12 were tested against the laboratory-adapted NL4.3-pseudotyped virus. The three components individually reconstituted in PBS showed neutralizing activity with IC_{50} values of 0.3, 1.6, and 795 ng/ml, respectively. Under these conditions (PBS), cyanovirin-N combined with griffithsin showed an increased effect over griffithsin alone or cyanovirin-N alone (based on IC_{50} values). Adding 2G12 to griffithsin or the griffithsin/cyanovirin-N combination had no significant effect on neutralization activity (see Table 1). However, when the three components were reconstituted in rice endosperm extract, it was observed that the increased effect of cyanovirin-N with griffithsin was reduced, but more potent neutralization was observed for the griffithsin, cyanovirin-N, and 2G12 triple combination than for griffithsin alone or in combination with cyanovirin-N or 2G12 (see Table 1). Therefore, the rice endosperm extract appeared to modulate the interaction between the different components without reducing their neutralization activity.

Table 1

	IC_{50} (ng/ml)			
	GRFT	CV-N+GFRT	2G12+GFRT	CV-N+2G12+GFRT
PBS	0.3 ± 0.026	0.1 ± 0.034	0.37 ± 0.111	0.18 ± 0.055
Crude rice endosperm extract	1.1 ± 0.049	1.03 ± 0.125	1.12 ± 0.164	0.77 ± 0.05

[0117] In greater detail, the assay referenced above was conducted as follows. HIV-1 pseudoviruses (NL4.3 and SVPB16 isolates) were generated by the co-transfection of 293T cells with Env-expressing plasmids and the PSG3 vector (see Sanchez-Palomino, *et al.*, A cell-to-cell HIV transfer assay identifies humoral responses with broad neutralization activity, *Vaccine,* (29): 5250-5259 (2011)). The supernatants were harvested 24 hours post-transfection, passed through a 0.45-μm filter and the viral stocks were frozen at –80 °C. Cell-free virus neutralization was tested using a standard TZM-bl based assay. Briefly, serially diluted samples and controls were pre-incubated with 200 $TCID_{50}$ of pseudovirus stock for 1 hour at 37 °C, in 96-well culture plates. 10,000 TZM-bl luciferase-reporter target cells were then added to each well. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 hours. The TZM-bl cells were treated with dextran (Sigma-Aldrich) to enhance infectivity. The luciferase substrate Britelite Plus (PerkinElmer Inc., Waltham, MA, USA) was used for the read out. Percent neutralization was determined by calculating the difference in average relative light units (RLU) between virus control (cells and virus) and test wells (cells and sample and virus), dividing this result by the difference in average RLU between the virus control and cell control wells and multiplying the result by 100. Neutralizing titers are expressed as the reciprocal dilution necessary to reduce the RLU by 50% (IC_{50}). Alternatively, the actual inhibitor concentration was calculated for each dilution to determine the apparent inhibitory concentration 50 (IC_{50}). Failure to score at least a 50% reduction of RLU at any dilution was considered a negative test. All data were fitted to an inhibitor versus response (three parameters) model using the GraphPad Prism v6 (GraphPad Software Inc., San Diego, CA, USA).

EXAMPLE 3

[0118] The following example illustrates the preparation of expression constructs for 2G12, cyanovirin-N (CV-N), and griffithsin (GRFT).

[0119] The 2G12 transformation constructs were based on the binary vector pTRA, a derivative of pPAM (GenBank accession no. AY027531). The vector contains two tobacco RB7 scaffold attachment regions flanking the expression cassette (obtained from Dr T. Rademacher, Fraunhofer IME, Aachen, Germany). The coding regions of the 2G12 heavy and light chains (obtained from Polymun, Vienna, Austria) included N-terminal signal sequences targeting the secretory pathway. The expression cassette comprised the endosperm-specific rice glutelin-1 promoter, the maize ubiquitin-1 first intron, the Tobacco etch virus 5' leader which acts as a translational enhancer, the coding region, and the Cauliflower mosaic virus 35*S* terminator, resulting in final constructs pTRAgtiGH and pTRAgtiGL.

[0120] The *CV-N* (N30Q/P51G) gene in vector pET30b was amplified by PCR in a 50-µL reaction comprising 1.25 units of GOTAQ polymerase in the appropriate buffer (Promega Corp., Fitchburg, WI, USA), 1 µM each of forward primer 5'-**GGG ATC C**AT GCT TGG TAA ATT CTC CCA G-3' (SEQ ID NO: 24) and reverse primer 5'-CC**G AAT TC**T TAT TCG TAT TTC AGG GTA CCG-3' (SEQ ID NO: 25) (bolded nucleotides represent BamHI and EcoRI restriction sites), 0.2 mM of each dNTP and 250 ng template DNA. The reaction was heated to 94 °C for 3 minutes followed by 35 cycles at 94 °C for 45 seconds, 60 °C for 45 seconds, and 72 °C for 3 minutes, and a final extension step at 72 °C for 10 min. The products were transferred to the shuttle vector pGEM-T Easy (Promega) and introduced into competent *E. coli* cells, which were incubated overnight at 37 °C under ampicillin selection. The integrity of the plasmid DNA was confirmed by sequencing (Universidad Autónoma de Barcelona, Barcelona, Spain) before digestion with BamHI and EcoRI to release the expression cassette, which was then inserted into vector pRP5 containing the endosperm-specific rice prolamin RP5 promoter and the rice α-amylase 3A signal peptide sequence (GenBank CAA39776).

[0121] The *GRFT* gene in vector pET-28a(+) was prepared by PCR in the same manner as in Example 2 using 1 μM each of forward primer 5'-T**GC ATG C**AT GGG CAG CAG CCA TCA T-3' (SEQ ID NO: 26) and reverse primer 5'-GGG **GAG CTC** TTA GTA CTG TTC ATA GTA G-3' (SEQ ID NO: 27) (the bolded nucleotides are SphI and SacI restriction sites introduced to facilitate cloning). The verified pGEM-T Easy shuttle vector was digested with SphI and SacI to release the expression cassette, which was then inserted into vector pgZ63 containing the endosperm-specific maize γ-zein promoter which also functions in rice, the rice α-amylase 3A signal peptide sequence and a sequence encoding a N-terminal His_6 affinity tag.

EXAMPLE 4

[0122] Having established that all three microbicide components remain active in a cocktail but that their interactions differ significantly according to whether they are reconstituted in PBS or rice endosperm extract, transgenic lines were generated that express all three proteins in order to characterize the behavior of the components in transgenic extracts compared to the reconstituted systems.

[0123] Rice embryos were co-transformed with four constructs containing the coding sequences for the 2G12 heavy and light chains, griffithsin, and cyanovirin-N, each controlled by endosperm-specific promoters (the rice glutelin-1 promoter for the 2G12 chains, the maize γ-zein promoter for griffithsin, and the rice prolamin RP5 promoter for cyanovirin-N) (see Example 3). A fifth construct containing the selectable marker *hpt* (encoding hygromycin B phosphotransferase) was controlled by the constitutive Cauliflower mosaic virus 35S promoter. Embryo-derived callus was selected on hygromycin-supplemented medium and 20 independent transformants were regenerated. The plantlets were transferred to the greenhouse. Leaves and T1 seeds from these plants were analyzed by PCR to confirm the presence of the transgenes, and the T1 seed extracts were analyzed by sandwich ELISA to confirm the presence of correctly-assembled 2G12 and the two lectins.

[0124] Nineteen transgenic lines were fertile, eight of which expressed a single microbicidal component (see Table 2 below). Eight lines expressed two components, with all three possible pairwise combinations represented, and three further lines were recovered

expressing all three components simultaneously. The double and triple component transgenic lines were renamed lines 1-11 (see Table 2). The analysis of individual and combinatorial expression revealed no specific relationship between the presence of each transgene and their expression levels, which made it possible to identify lines expressing any combination of the proteins at adequate levels for further analysis, even among a small population. T1 seeds from the single, double and triple transgenic lines were propagated to produce T1 populations, and T2 homozygous seeds were used for more detailed analysis (see Example 6).

Table 2

Microbicide component(s)	**Transgenic line**	**2G12 concentration** (μg/g dry seed weight)	**CV-N concentration** (μg/g dry seed weight)	**GRFT concentration** (μg/g dry seed weight)
2G12	2G12-1	14.4		
2G12	2G12-2	11.6		
2G12	2G12-3	14.8		
CV-N	CV-N-1		10	
CV-N	CV-N-2		2.4	
CV-N	CV-N-3		0.8	
GRFT	GRFT-1			32
GRFT	GRFT-2			26
CV-N + GRFT	1		4.4	37.6
CV-N + GRFT	2		2	1.6
CV-N + GRFT	3		0.4	3.2
2G12 + CV-N	4	11.6	0.8	
2G12 + CV-N	5	2	0.8	

2G12 + CV-N	6	12	0.8	
2G12 + GRFT	7	4		17.6
2G12 + GRFT	8	14.8		12.8
2G12 + CV-N + GRFT	9	16.4	0.4	2.8
2G12 + CV-N + GRFT	10	9.6	0.8	8.4
2G12 + CV-N + GRFT	11	17.2	1.2	6.4
WT	WT			

[0125] The details of the study are as follows. Seven-day-old mature rice zygotic embryos (*Oryza sativa* cv. Nipponbarre) were transferred to osmotic medium (4.4 g/L Murashige & Skoog (MS) powder supplemented with 0.3 g/L casein hydrolysate, 0.5 g/L proline, 72.8 g/L mannitol and 30 g/L sucrose) 4 hours before bombardment with 10 mg gold particles coated with the four constructs and the selectable marker *hpt*. A 6:6:3:3:1 ratio was used with the 2G12 heavy and light chains represented at twice the molar ratio of the lectins to ensure the recovery of plants expressing all four transgenes, and the *hpt* marker gene as the minority component. The embryos were returned to osmotic medium for 12 hours before selection on MS medium (4.4 g/L MS powder, 0.3 g/L casein, 0.5 g/L proline and 30 g/L sucrose) supplemented with 50 mg/L hygromycin and 2.5 mg/L 2,4-dichlorophenoxyacetic acid in the dark for 2 to 3 weeks. Transgenic plantlets were regenerated and hardened off in soil. Plants were grown in the greenhouse or growth chamber at 28/20 °C day/night temperature with a 10 hour photoperiod and 60–90% relative humidity for the first 50 days, followed by maintenance at 21/18 °C day/night temperature with a 16 hour photoperiod thereafter in a growth chamber.

[0126] Crude extracts from the T2 seeds were analyzed to determine their ability to bind gp120 *in vitro* using an ELISA in which the plates were coated with gp120IIIB. The three components were able to bind to gp120 as detected using different antibodies. To ensure that

the assay was calibrated correctly and that the three proteins were correctly folded and able to bind their target in the context of rice endosperm extracts, the recombinant proteins were compared with extracts from the transgenic lines expressing the individual components, and extracts from the 11 double and triple transgenic lines. gp120 binding was found in all cases (see Figures 2A-2C and 7A-7C).

[0127] Mature rice seeds were ground in three volumes of PBS and centrifuged twice at 13,000 x *g* for 10 min at 4 °C to remove debris. The wells of ELISA plates were coated with 100 ng recombinant gp120 IIIB or gp120 W61D, both provided by the MRC Centralized Facility for AIDS Reagents, Potters Bar, UK. After washing and blocking with 2.5% bovine serum albumin (BSA) in PBS containing 0.1% Tween-20 (PBST), serial dilutions of each seed extract were added and the three proteins were detected. The presence of bound 2G12 was detected using a horseradish peroxidase (HRP)-conjugated sheep anti-human kappa-chain antiserum (The Binding Site, Birmingham, UK) at a 1:1000 dilution. Griffithsin and cyanovirin-N were detected using a primary rabbit anti-GRFT and anti-cyanovirin-N polyclonal antisera (The Binding Site) and a secondary horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody (The Binding Site), each diluted 1:1000. HRP was detected by adding the substrate 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma-Aldrich, St. Louis, MO, USA) and reading the absorbance at 450 nm.

EXAMPLE 5

[0128] Having established that a crude extract of rice endosperm can increase the gp120-binding activity and HIV-neutralization activity of 2G12 with and without griffithsin and/or cyanovirin-N, the extract was fractionated into broad components in order to determine the source of the active principle. A further series of gp120 sandwich ELISAs were then run with 2G12 as the binding antibody in the presence or absence of the lectins. The efficiency of gp120-2G12 binding in different fractions was compared to a reconstituted PBS control.

[0129] Protein fractions were prepared by the overnight precipitation of rice endosperm extracts with ammonium sulfate followed by centrifugation at 4500 x *g* for 30 min. The protein pellet was resuspended in PBS and the supernatant was used as the protein-free fraction. The seed protein fractions were prepared by mixing 2 g of rice flour with 10 mL of

solvent for 90 min followed by centrifugation at 14,000 x g for 15 min at 4 °C (see Lang, et al., Evaluation of extraction solutions for biochemical analyses of the proteins in rice grains, *Biosci. Biotechnol. Biochem.*, (77): 126–131 (2013) and Balindong, et al., Optimization and standardization of extraction and HPLC analysis of rice grain protein, *J. Cereal Sci.,* (72): 124–130 (2016)). Albumins were extracted with water, globulins with PBS, glutelins with 0.1 M NaOH, and prolamins with 70% ethanol. The supernatant in each case was used directly in the ELISA. The supernatants were tested for their impact on gp120–2G12 binding – water, PBS and 70% ethanol preserved the interaction but no binding was observed in 0.1M NaOH.

[0130] As explained in Example 1, a randomized complete analysis of variance (ANOVA) was applied to each component (2G12, CV-N, and GRFT) at each concentration, and statistically significant differences between REX and PBS, and between different endosperm extracts, were determined using the Tukey test in JMP-PRO v12.0.1 (SAS institute Inc., Cary, NC, US). Statistical significance was expressed as follows: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. A non-linear regression function was also used to determine the performance of each component at different concentrations, a quadratic non-linear regression curve was fitted and statistically significant differences were determined by comparing the fitted curve parameters. Model fitting and parameter estimations were carried out using GraphPad Prism v7.03 (GraphPad Software, Inc., San Diego, CA, USA).

[0131] When a total protein extract and protein-depleted extract were prepared, it was observed that only the protein extract increased the binding activity of 2G12 whereas the depleted extract was similar in performance to the PBS control (see Figure 5A-5D). The signals in Figures 5A-5D were corrected for background (equivalent extraction solvent without components). Data are presented as a fitted quadratic regression model to compare the different curve parameters and establish significant differences among extracts at different concentrations of the three components. The curve comparison revealed no significant difference between the original REX (extracted in PBS, $p > 0.05$) and the PBS extract prepared using the protocol recommended for the isolation of globulins, indicating both extracts were functionally equivalent. Asterisks represent statistically significant differences (ANOVA) at different concentrations of the three components (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, NS = nonsignificant). Segments in each data set represent the SEM from three replications.

[0132] The rice endosperm was extracted again using the traditional approach to distinguish among different classes of seed storage proteins: the albumins (water extract), globulins (PBS extract), glutelins (alkaline extract) and prolamins (ethanolic extract). ELISAs with these fractions (used directly without buffer exchange), in addition to the reconstituted PBS control, the protein extract, the protein-depleted extract, and the original complete extract, provided strong evidence that the active principle segregates with the globulin fraction of the endosperm. The effect of each solvent on gp120–2G12 binding was tested and it was determined that PBS had no impact (as anticipated) and binding was still efficient in water, whereas ethanol and NaOH interfered with binding. Therefore, the active principle appears to segregate with the globulins rather than the albumins.

EXAMPLE 6

[0133] The crude T2 seed extracts in the TZM-bl cell HIV-neutralization assays using the NL4.3-pseudotyped virus were analyzed to determine whether the components remained functional.

[0134] The extracts from T2 seeds expressing single components achieved HIV-1 neutralization with titers of up to $>10^4$ (ID_{50} reciprocal dilution). The best apparent IC_{50}

values for each component were >119, 2.3, and 0.7 ng/mL for 2G12, cyanovirin-N, and griffithsin, respectively. Combinations of 2G12 and cyanovirin-N were no more effective than the corresponding single-component extracts, but all combinations containing griffithsin showed high neutralization activity. Remarkably, substantially lower apparent IC_{50} values were observed when all three components were present, suggesting synergic interactions (see Table 3 below). The lowest overall IC_{50} values were observed in extracts from the triple transgenic lines 9, 10, and 11, with IC_{50} ranges of 0.14–0.77 ng/mL for 2G12, 0.01–0.05 ng/mL for cyanovirin-N and 0.09–0.29 ng/mL for griffithsin, respectively (Table 3).

[0135] Assuming that griffithsin achieved the highest neutralizing activity (Table 3, single transgenic lines), the apparent IC_{50} values for griffithsin in single, double and triple transgenic lines, were compared which revealed significantly lower values for the extracts containing all three components than for the single and double extracts (see Figure 3A). To confirm this observation, similar concentrations of cyanovirin-N or 2G12 single extracts were mixed with a griffithsin extract and calculated the griffithsin apparent IC_{50} values. Again, dose response curves shifted in the presence of all three components and apparent IC_{50} values in the triple combination were lower than for single components or double combinations (see Figure 3B). The IC_{50} values for GRFT in single, double and triple transgenic lines, revealed significantly lower values for the extracts containing all three components than for the single and double extracts. These data suggest that the components may interact in pairwise combinations but that profound synergy occurs in the triple combination.

[0136] The extracts from all three triple transgenic lines were then compared against the single transgenic line expressing griffithsin in TZM-bl cell HIV-neutralization assays using the laboratory-adapted NL4.3 and the primary isolate SVPB16 pseudotyped viruses. In both cases, lower apparent IC_{50} values were observed for griffithsin in the triple transgenic lines compared to the single transgenic line, indicating that synergy occurred between the microbicide components to increase the efficacy of the combinatorial microbicide at lower concentrations, particularly against a primary HIV-1 isolate (see Figure 4).

Table 3

Lines	Crude ID_{50} (Reciprocal dilution)	Apparent IC_{50} (ng/mL) for individual components		
		2G12	CV-N	GRFT
2G12-1	<25	>144		
2G12-2	<25	>119		
2G12-3	<25	>148		
CV-N-1	<25		>220	
CV-N-2	234		2.50	
CV-N-3	86		2.30	
GRFT-1	11376			0.70
GRFT-2	4808			1.35
1	8892		0.12	1.06
2	755		0.66	0.53
3*	-	-	-	-
4	<25	>116	>8	
5	<25	>20	>8	
6	<25	>120	>8	
7	4178	0.24		0.24
8	1734	2.13		1.85
9	7482	0.55	0.01	0.09
10	17061	0.14	0.01	0.12
11	5585	0.77	0.05	0.29
WT	<25			

* Cell line was compromised during transit and was not subsequently tested.

EXAMPLE 8

[0137] This example demonstrates that rice is a suitable production platform for scytovirin (full length lectin and truncated). Scytovirin maintains its antiviral activity and therefore can be combined in rice endosperm with other antiviral compounds to produce a more potent microbicide.

[0138] *Transformation vectors*

[0139] The coding sequences for the fusion protein scytovirin/thioredoxin and SD1/thioredoxin contained in vector pET-32b (+) were amplified by PCR in a 50 µL reaction volume comprising 0.5 µL Go Taq G2 Flexi DNA polymerase (5U/µL) in the appropriate buffer (Promega, Madison, WI, USA), 0.5 µL each of forward primer 5'-**GGA TCC** ATG AGC GAT AAA ATT ATT C-3' (SEQ ID NO: 28) (10 µM, the bolded nucleotide represents *Bam*HI restriction site for linking pRP5 vector) and reverse primer 5'-**GAA TCC** GCA GCC GGA TCT CAG TGG TG-3' (SEQ ID NO: 29) (10 µM, the bolded nucleotides represent *Eco*RI restriction site for linking pRP5 vector) for scytovirin, forward primer 5'-**GGA TCC** ATG AGC GAT AAA ATT ATT C-3' (SEQ ID NO: 30) (10 µM) and reverse primer 5'-**GAA TCC** TCA GTG GTG GTG GTG GTG GTG-3' (SEQ ID NO: 31) (10 µM) for SD1, 2 µL dNTPs (10 µM) and 2 µL template DNA (100 ng/µL). The reaction temperature was raised to 94 °C for 4 minutes followed by 40 cycles of 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. The PCR products were digested with *Bam*HI and *Eco*RI to release the expression cassettes and insert them into pRP5 vectors containing the endosperm-specific rice prolamin promoter (see Naqvi, et al., *Proceedings of the National Academy of Sciences*, 106: 7762-7767 (2009)), and then sequenced. The *hpt* selectable marker was provided by a different vector containing the hygromycin resistant gene under the control of CaMV 35S promoter (see Christou, et al., The impact of selection parameters on the phenotype and genotype of transgenic rice callus and plants, *Transgenic Research,* 4: 44-51 (1995)).

[0140] *Transformation, selection, and regeneration of transgenic lines*

[0141] Rice seeds (*Oryza sativa* cv. Nipponbare) were germinated on MSP medium (4.4 g/L Murashige & Skoog powder [MSP] supplemented with 30 g/L sucrose, 2.5 mg/L 2,4-dichlorophenozyacetic acid and 6 g/L phytagel) in the dark for 5 days. Then, the 5-day mature rice zygotic embryos were isolated and transferred to osmoticum medium (MSP supplemented with 78.2 g/L mannitol) for 4 hours before being bombarded with 10 mg of gold particles coated with scytovirin or SD1 constructs and the selectable marker *hpt* at a 3:1 molar ratio (see Christou et al., Genotype-independent stable transformation of rice (Oryza sativa) plants, *Bio/Technology*, 9: 957-962 (1991)). The embryos were maintained on osmoticum medium for 12 hours, and then transferred to MSP for 2 days. Embryogenic callus selection, amplification and plant regeneration were performed (see Valdez et al., Transgenic Central American, West African and Asian elite rice varieties resulting from particle bombardment of foreign DNA into mature seed-derived explants utilizing three different bombardment devices, *Annals of Botany*, 82: 795-801 (1998)). Regenerated plants were hardened off in soil and grown in a controlled environment chamber.

[0142] *DNA blot analysis*

[0143] Genomic DNA was isolated from fresh or freeze-dried leaves using a modification of a published procedure (see Edwards et al., A simple and rapid method for the preparation of plant genomic DNA for PCR analysis, *Nucleic acids research*, 19: 1349 (1991)). Samples were ground to a fine powder under liquid nitrogen and dissolved in 2 mL extraction buffer containing 150 µL 20% SDS. After vortexing for 10 min, the samples were heated to 65 °C for 10 min and protein was extracted with phenol. Nucleic acids were isolated by phenol:chloroform:iso-amylalcohol extraction (25:24:1, v/v) and the aqueous phase was treated with 3 µL of RNAse (10 mg/mL) at 37 °C for 1 h. DNA was precipitated with one volume of isopropanol, and centrifuged at 5,000 rpm for 10 min. Subsequently, the supernatant was discarded in order to allow the pellet to air dry. Finally, the pellet was dissolved in 50 µL of distilled water and the DNA concentration was determined by nanodrop spectrophotometry. Thirteen µg of isolated genomic DNA were digested with *Bam*HI to a final volume of 250 µL. The samples were vacuum concentrated to 40 µL. The quality of the digested DNA was checked in 0.9% TBE agarose gel. After the

electrophoresis, DNA was denatured and transferred to a nylon membrane. The membrane was probed with a digoxigenin-labelled scytovirin/SD1 gene fragment generated using forward primer 5'-AAG TGG GTG CAC TGT CTA AAG-3' (SEQ ID NO: 32) and reverse primer 5'-TTA CCC CGG GTC CGG TTT ACG AG-3' (SEQ ID NO: 33). After washing and immunological detection with an alkaline phosphatase-conjugated anti-DIG antibody (Roche, Basel, Switzerland) at 1:1,000 dilution, the luminescent signal produced from the CSPD subtract (Roche) was detected on CHEMIDOC MP Imaging System (Bio-Rad Laboratories, Hercules, CA).

[0144] *ELISA*

[0145] The accumulation of scytovirin/SD1 in transgenic callus and endosperm tissue was confirmed by ELISA. Two hundred mg of callus was ground in 500 µL of PBS and centrifuged twice at 13,000 rpm for 4 min at 4 °C. The same procedure was used for mature rice seeds, except 200 mg of rice seeds was ground in three volumes of PBS. The supernatant was removed to an ELISA plate previously coated with 100 ng recombinant gp120 from HIV-1 strain IIIB (MRC Centralised Facility for AIDS Reagents, Potters Bar, UK). The plates were blocked with 2.5% (w/v) bovine serum albumin (BSA) at 37 °C overnight, and then washed with PBST (PBS containing 0.1% (v/v) TWEEN-20). The callus extract was added and the protein detected using a primary rabbit anti-scytovirin polyclonal antiserum (received from NCBI, USA) and a secondary horseradish peroxidase (HRP)-conjugated antirabbit IgG antibody (The Binding Site Group Ltd., Birmingham, UK), each diluted 1:1000. HRP was detected by adding the substrate 3,30,5,50-tetramerthylbenzidine (TMB) (Sigma-Aldrich, St. Louis, MO) and reading the absorbance at 450 nm.

[0146] *Protein purification*

[0147] Two grams of transgenic mature rice seeds were ground into a fine powder and the proteins were extracted at 4 °C in two volumes of protein extraction buffer (0.2 M Tris-HCl pH=7.5, 5 mM EDTA pH=8, 0.1% TWEEN-20). The insoluble particles were removed by centrifuging twice at 13,000 rpm for 10 min. The supernatant was filtered with a 0.45 µm filter and loaded into a HITRAP Chelating HP column (GE Healthcare, Little Chalfont, UK), previously prepared with 1 mL 0.1M $NiSO_4$. The column was washed with 0.1 M sodium

phosphate buffer pH 8.0 containing 17.55 g/L NaCl and 10 mM imidazole. Two elution steps were performed: (1) 0.1 M sodium phosphate buffer pH 8.0 containing 17.55 g/L NaCl and 100 mM imidazole, and (2) 0.1 M sodium phosphate buffer pH 8.0 containing 17.55 g/L NaCl and 600 mM imidazole. The protein containing fractions were pooled together and concentrated using VIVASPIN 6 centrifugal concentrator, MWCO 10,000 Da (Sigma-Aldrich).

[0148] *SDS/PAGE and Western Blot*

[0149] The purified fractions were separated by 12.5% reducing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS/PAGE). For immunoblotting, the samples were transferred to a PVDF membrane (GE Healthcare) and blocked with 3% BSA in Tris-buffered saline (TBS). After three washes with TBST (TBS containing 0.1% (v/v) TWEEN-20), the primary rabbit anti-scytovirin polyclonal antiserum (received from NCBI, USA) and subsequently, the secondary goat antirabbit IgG (whole molecule)-alkaline phosphatase antibodies (Sigma-Aldrich) were incubated at 1:2000 dilution. The signal was developed with SIGMAFAST BCIP/NBT tablet (Sigma-Aldrich).

[0150] *Molecular characterization of transgenic rice plants*

[0151] As described above, the mature rice embryos were transformed by particle bombardment using two different plasmid combinations. One contained the coding sequence of the scytovirin/thioredoxin fusion protein, under the control of the rice prolamin promoter (FIG. 8a) together with a second construct containing the selectable marker *hpt*. The other contained SD1/thioredoxin coding sequence (FIG. 8b). Callus was selected on hygromicin-supplemented medium and 20 independent transformants were regenerated per experiment and transferred to the growth chamber. Fertile lines were subjected to DNA analyses to confirm transgene integration. Genomic DNA was digested with *Bam*HI and hybridized with 300 bp DIG-labelled gene fragment, which was obtained by PCR as described in materials and methods. DNA blots showed unique band patterns for both scytovirin and SD1, indicating that their origin was independent.

[0152] A limited number of seeds were obtained for analysis. Seeds were amplified to generate more material. For every independent line, five seeds were germinated. DNA blot

analysis was used to screen transgene segregation in the T1 generation. Positive lines were kept for subsequent analysis and negative lines were used as negative controls. Genomic DNA blots from the T_1 population demonstrated stable transmission of the parental pattern from T_0 to T_1.

[0153] *Scytovirin/SD1 Detection*

[0154] The presence of soluble scytovirin/SD1 in rice callus was confirmed by ELISA. The crude extracts were tested for binding activity against HIV gp120 and both positive (500 ng/mL of purified scytovirin/SD1 from *E. coli*) and negative controls (wild-type rice callus extract) were used. The scytovirin/SD1 presence was detected by a primary rabbit anti-scytovirin polyclonal antiserum and a secondary horseradish peroxidase (HRP)-conjugated antirabbit IgG antibody. This analysis also indicated that rice was able to produce the properly folded form of the lectin, which maintained *in vitro* the binding activity against HIV gp120.

[0155] The level of protein in the callus was measured (using three technical replicates). The callus lines that regenerated into fertile plants showed a wide range of recombinant protein accumulation, from zero to values comparable to the positive control (Figures 9A-9B).

[0156] Ten seeds per plant were used for protein extraction, and the presence of properly folded lectins in T_1 rice endosperm was confirmed using the sandwich ELISA procedure described above. The concentrations were determined through standard curves based on positive controls (Figure 10A). In order to detect possible cross-reactions between scytovirin or SD1 and the proteins present in rice endosperm, an additional positive control was used. This was a non-transformed rice endosperm extract supplemented with scytovirin/SD1 at a concentration of 500 ng/mL. By comparing both positive controls, it was determined that no interactions took place between the recombinant lectins and endogenous rice endosperm proteins (Figure 10B). Extracts from some lines show higher binding activity compared to wild-type, confirming that scytovirin/SD1 remains functional in rice endosperm. For scytovirin, the highest expressing line was Line 2-10 accumulating 122 ng/g seed weight and for SD1 Line 2-21 with a concentration of 237 ng/g seed weight.

[0157] A western blot was performed on lines with fifty or more seeds. Mature rice seeds were ground into a fine powder and the proteins were extracted at 4 ºC in two volumes of protein extraction buffer. The protein concentration was checked using Bradford assay and 50 µg of total protein were loaded into 12.5% SDS-PAGE. Subsequent immunoblotting showed nonspecific bands, with no bands corresponding to scytovirin or SD1. This result may be due to the lectins being too diluted to detect using this method.

[0158] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0159] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0160] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as

specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A microbicidal composition comprising:

(a) an endosperm or endosperm extract; and

(b) an anti-HIV lectin, an anti-HIV antibody or antigen-binding antibody fragment, or combination thereof;

wherein the composition optionally comprises a pharmaceutically acceptable carrier.

2. The microbicidal composition of claim 1, wherein the anti-HIV lectin comprises scytovirin, griffithsin, cyanovirin-N, a fragment or mutant thereof, or a combination thereof.

3. The microbicidal composition of claim 1 or 2 comprising two or more of scytovirin, griffithsin, cyanovirin-N, a fragment or mutant thereof, or an anti-HIV antibody or antigen-binding antibody fragment.

4. The microbicidal composition of any one of claims 1-3, wherein the anti-HIV antibody or antigen-binding fragment thereof is an anti-HIV envelope antibody or antigen-binding fragment thereof.

5. The microbicidal composition of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof comprises the CDRs or variable regions of 4E10, 2F5, 2G12, VRC01, 10-1074, or 3BNC117.

6. The microbicidal composition of any one of claims 1-3, wherein the antibody or antigen-binding fragment thereof comprises the CDRs of 2G12.

7. The microbicidal composition of claim 6, wherein the antibody comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 2.

8. The microbicidal composition of any one of claims 1-7, wherein the endosperm or endosperm extract is from a transgenic plant that expresses two or more of griffithsin, cyanovirin, and an anti-HIV antibody or antigen-binding antibody fragment, or

that expresses all three of griffithsin, cyanovirin, and an anti-HIV antibody or antigen-binding antibody fragment.

9. The microbicidal composition of any one of claims 1-8, wherein the endosperm or endosperm extract is from rice, sorghum, wheat, rye, triticale, oats, barley, spelt, soybean, maize, tobacco, or marshmallow.

10. The microbicidal composition of any one of claims 1-8, wherein the endosperm is rice endosperm.

11. The microbicidal composition of any one of claims 1-10, further comprising wild-type plant proteins.

12. The microbicidal composition of any one of claims 1-11, wherein the endosperm extract comprises a globulin fraction of the endosperm.

13. The microbicidal composition of any one of claims 1-12, wherein the endosperm extract is provided by an extract of a plant embryo.

14. A transgenic plant that expresses two or more of:
(a) cyanovirin-N or a fragment or mutant thereof;
(b) griffithsin or a fragment or mutant thereof;
(c) scytovirin or a fragment or mutant thereof;
(d) other anti-HIV lectin; or
(e) an anti-HIV antibody or antigen-binding antibody fragment.

15. A transgenic plant that expresses scytovirin or a fragment or mutant thereof.

16. The transgenic plant of claim 14 or 15 wherein the scytovirin fragment is SD1.

17. The transgenic plant of any one of claims 14-16, wherein the transgenic plant is a transgenic rice, sorghum, wheat, rye, triticale, oats, barley, spelt, soybean, maize, tobacco, or marshmallow plant.

18. The transgenic plant of any one of claims 14-17, wherein the plant is rice.

19. The transgenic plant of any one of claims 14 and 17-18, wherein the antibody or antigen-binding fragment thereof is an anti-HIV envelope antibody or antigen-binding fragment thereof.

20. The transgenic plant of any one of claims 14 and 17-19, wherein the antibody or antigen-binding fragment thereof comprises the CDRs or variable regions of 4E10, 2F5, 2G12, VRC01, 10-1074, or 3BNC117.

21. The transgenic plant of any one of claims 14 and 17-19, wherein the antibody or antigen-binding fragment thereof comprises the CDRs of 2G12.

22. The transgenic plant of claim 21, wherein the antibody comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 2.

23. A seed or endosperm from a plant of any of claims 1-22.

24. The seed or endosperm of claim 23 in the form of milled flour.

25. A method of preventing an HIV infection in a human comprising administering the microbicidal composition of any one of claims 1-13, plant of any of claims 14-22, or seed or endosperm of claim 23 or 24, to the human.

26. The microbicidal composition of any one of claims 1-13, plant of any of claims 14-22, or seed or endosperm of claim 23 or 24 for use in a method of preventing an HIV infection, or for use in the preparation of a medicament for preventing an HIV infection.

27. A nucleic acid encoding two or more of

(a) cyanovirin-N or a fragment or mutant thereof;
(b) griffithsin or a fragment or mutant thereof;
(c) scytovirin or a fragment or mutant thereof,
(d) other anti-HIV lectin, or
(e) an anti-HIV antibody or antigen-binding antibody fragment.

28. A nucleic acid encoding scytovirin or a fragment or mutant thereof comprising an endosperm-specific promoter.

29. The nucleic acid of claim 27 encoding griffithsin or a fragment or mutant thereof, cyanovirin-N or a fragment or mutant thereof, scytovirin or a fragment or mutant thereof, and an anti-HIV antibody.

30. The nucleic acid of claim 26 or 27, further comprising an endosperm-specific promoter.

31. The nucleic acid of claim 28 or 30, wherein the endosperm-specific promoter is rice glutelin-1 promoter, maize γ-zein promoter, rice prolamin RP5 promoter, or barley hordein promoter.

32. The nucleic acid of claim 31, wherein the nucleic acid comprises two or more of:

(i) a nucleotide sequence encoding anti-HIV antibody operatively linked to rice glutelin-1 promoter,

(ii) a nucleotide sequence encoding griffithsin or a fragment or mutant thereof operatively linked to maize γ-zein promoter,

(iii) a nucleotide sequence encoding cyanovirin-N or a fragment or mutant thereof operatively linked to rice prolamin RP5 promoter,

(iv) a nucleotide sequence encoding scytovirin or a fragment or mutant thereof, or other anti-HIV lectin operatively linked to rice prolamin RP5 promoter.

33. The nucleic acid of any one of claims 27 and 29-32, wherein the anti-HIV antibody or antigen-binding fragment thereof is an anti-HIV envelope antibody or antigen-binding fragment thereof.

34. The nucleic acid of any one of claims 27 and 29-32, wherein the anti-HIV antibody or antigen-binding fragment thereof comprises at least the antigen binding domain of 4E10, 2F5, 2G12, VRC01, 10-1074, or 3BNC117.

35. The nucleic acid of any one of claims 27 and 29-32, wherein the anti-HIV antibody or antigen-binding fragment thereof comprises the CDRs of 2G12.

36. The nucleic acid of any one of claims 27 and 29-32, wherein the anti-HIV antibody or antigen-binding fragment thereof comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 2.

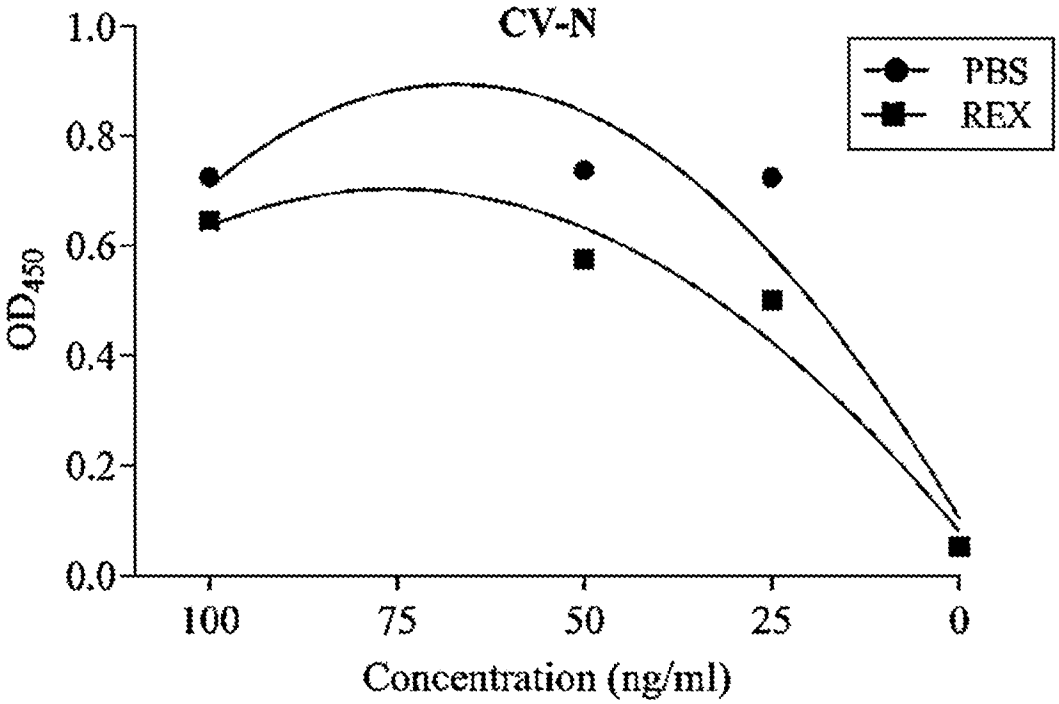

CV-N
PBS
REX
1.0
0.8
0.6
0.4
0.2
0.0
OD450
100
75
50
25
0
Concentration (ng/ml)

FIG. 1A

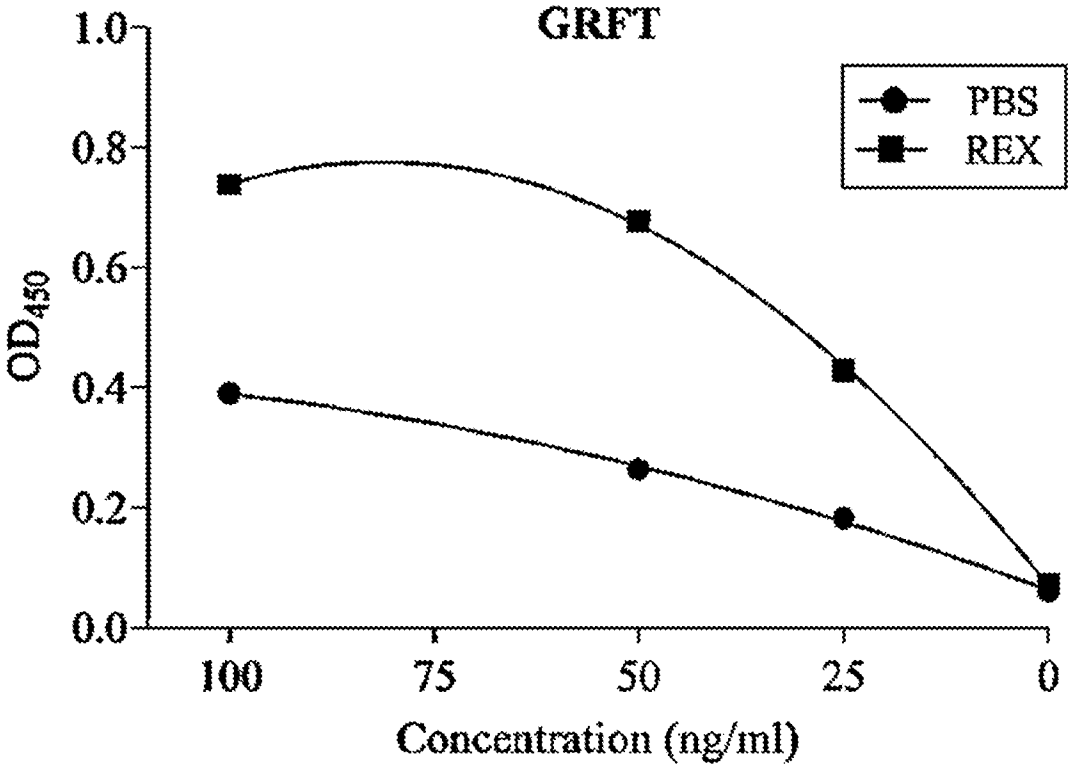

GRFT
PBS
REX
1.0
0.8
0.6
0.4
0.2
0.0
OD450
100
75
50
25
0
Concentration (ng/ml)

FIG. 1B

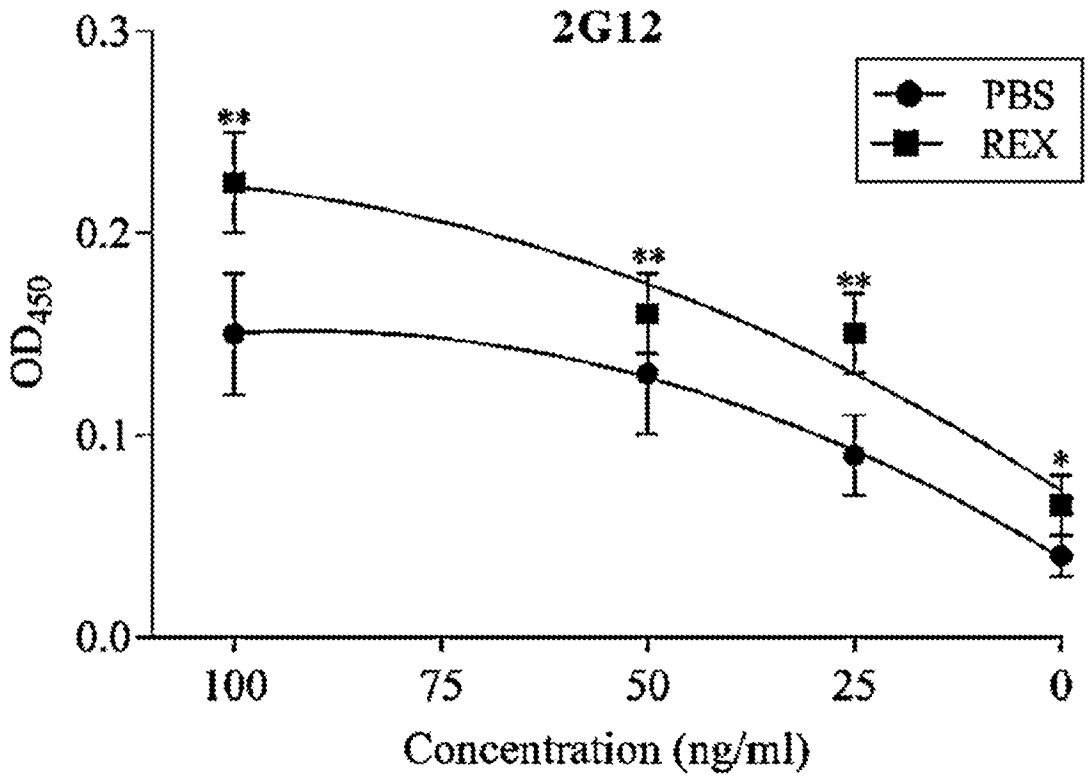

2G12
PBS
REX
0.3
0.2
0.1
0.0
OD_{450}
**
**
**
*
100
75
50
25
0
Concentration (ng/ml)

FIG. 1C

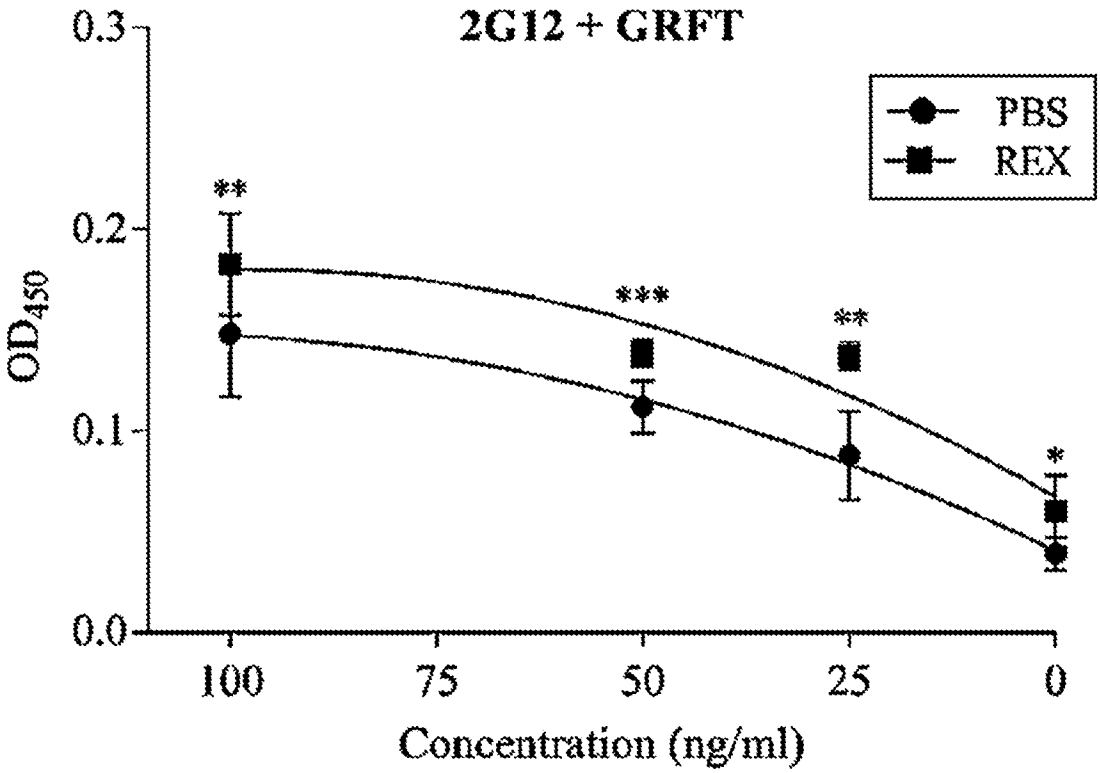

2G12 + GRFT
PBS
REX
0.3
0.2
0.1
0.0
OD_{450}
**
***
**
*
100
75
50
25
0
Concentration (ng/ml)

FIG. 1D

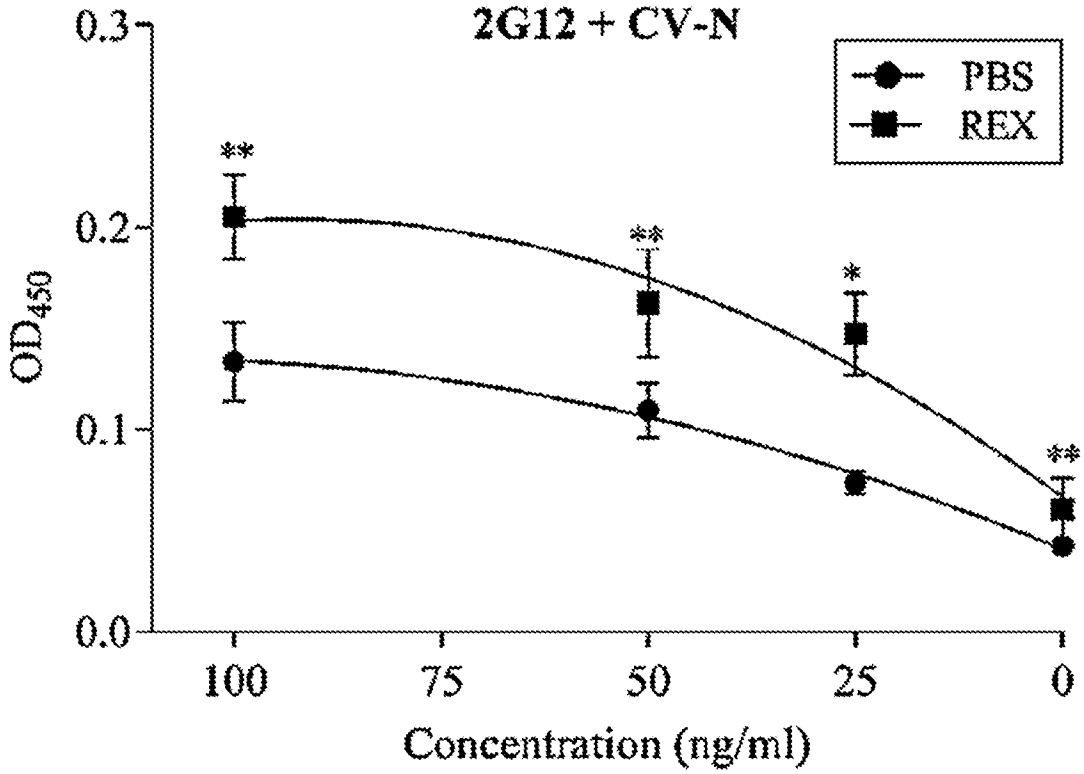

2G12 + CV-N
PBS
REX
0.3
0.2
0.1
0.0
OD_{450}
**
**
*
**
100
75
50
25
0
Concentration (ng/ml)

FIG. 1E

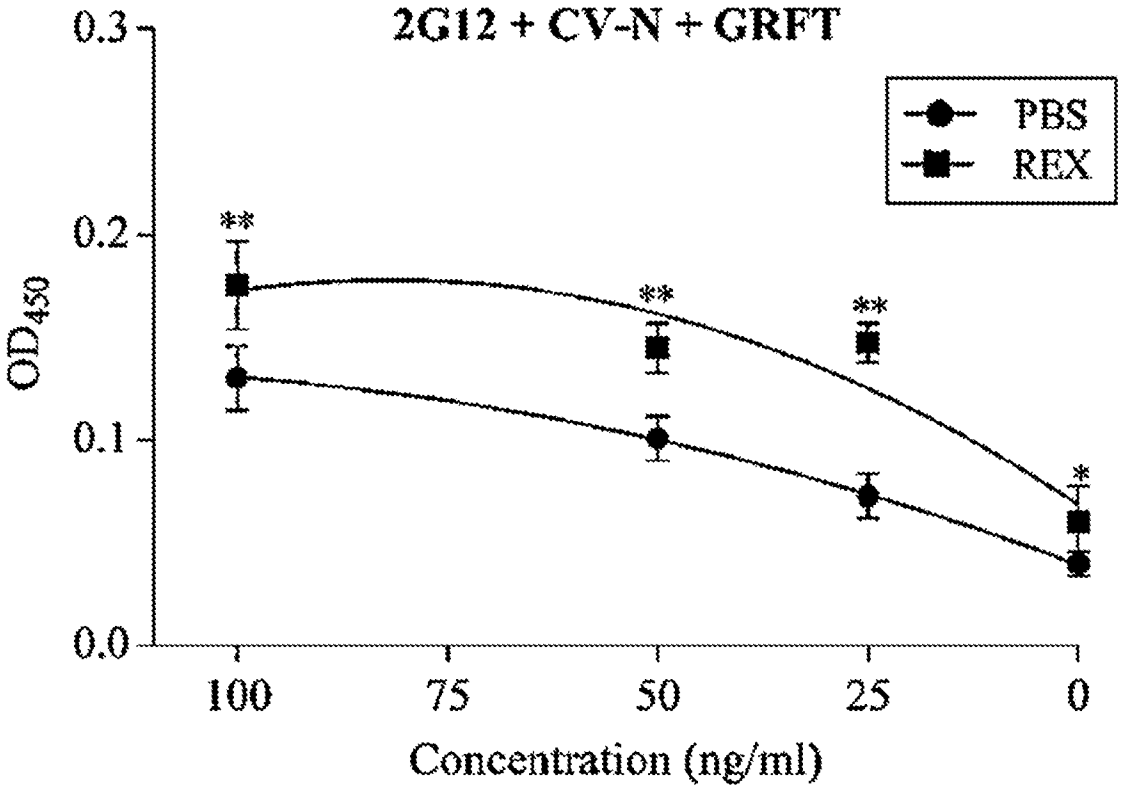

2G12 + CV-N + GRFT
PBS
REX
0.3
0.2
0.1
0.0
OD_{450}
**
**
**
*
100
75
50
25
0
Concentration (ng/ml)

FIG. 1F

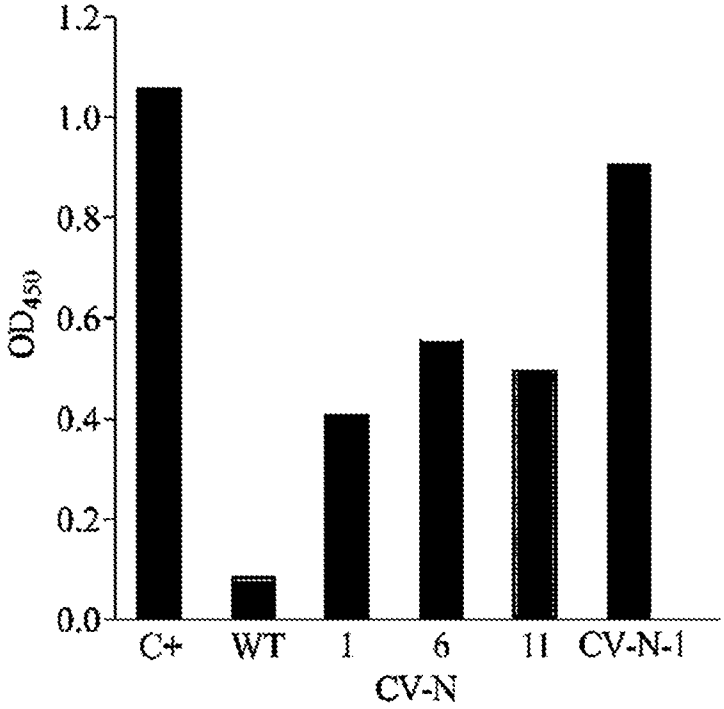

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
C+
WT
1
6
11
CV-N-1
CV-N
Independent events

FIG. 2A

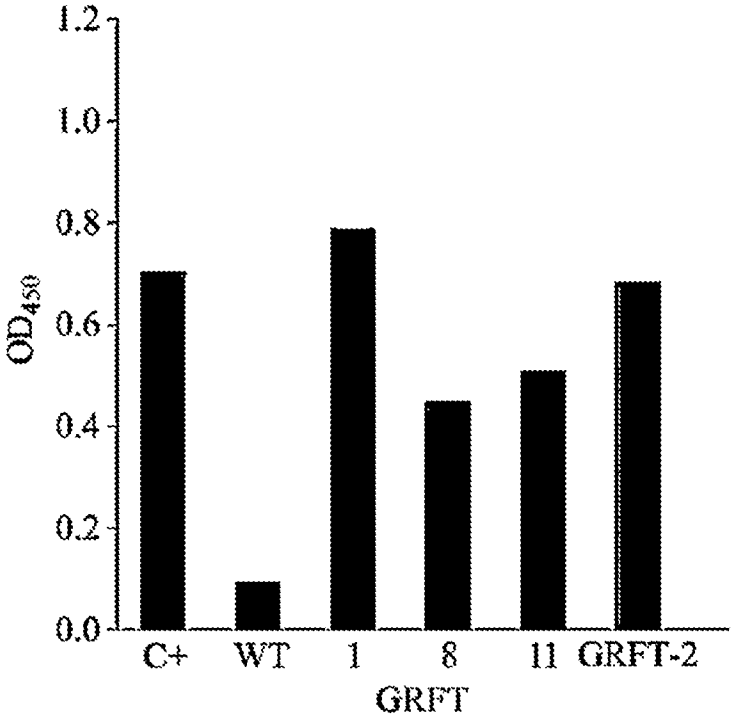

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
C+
WT
1
8
11
GRFT-2
GRFT
Independent events

FIG. 2B

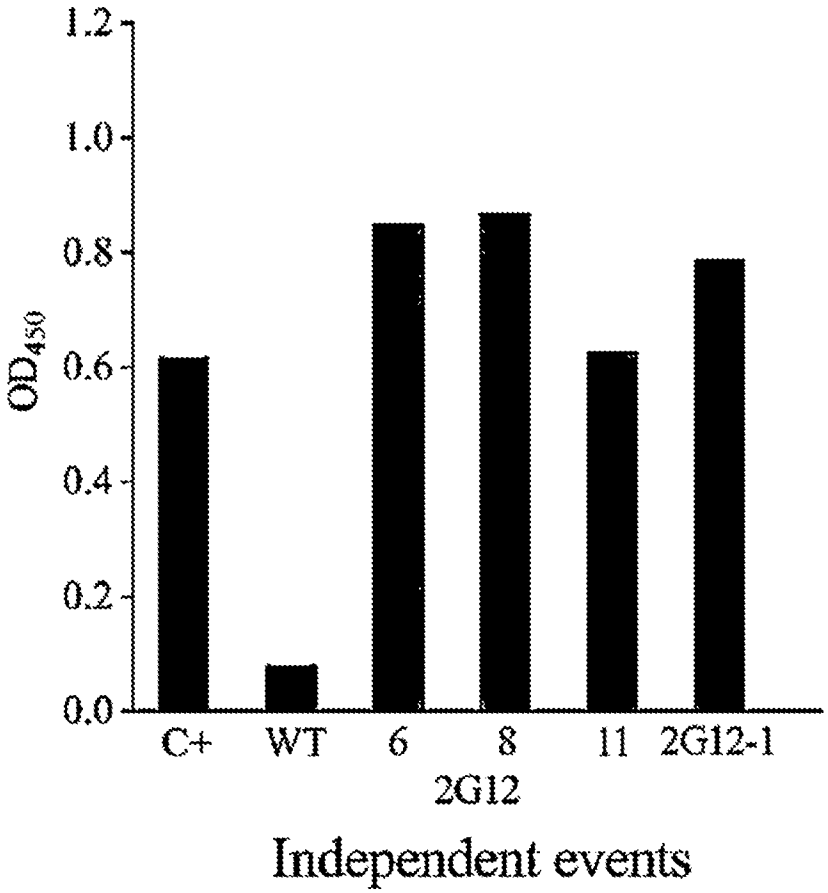

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
C+
WT
6
8
11
2G12-1
2G12
Independent events

FIG. 2C

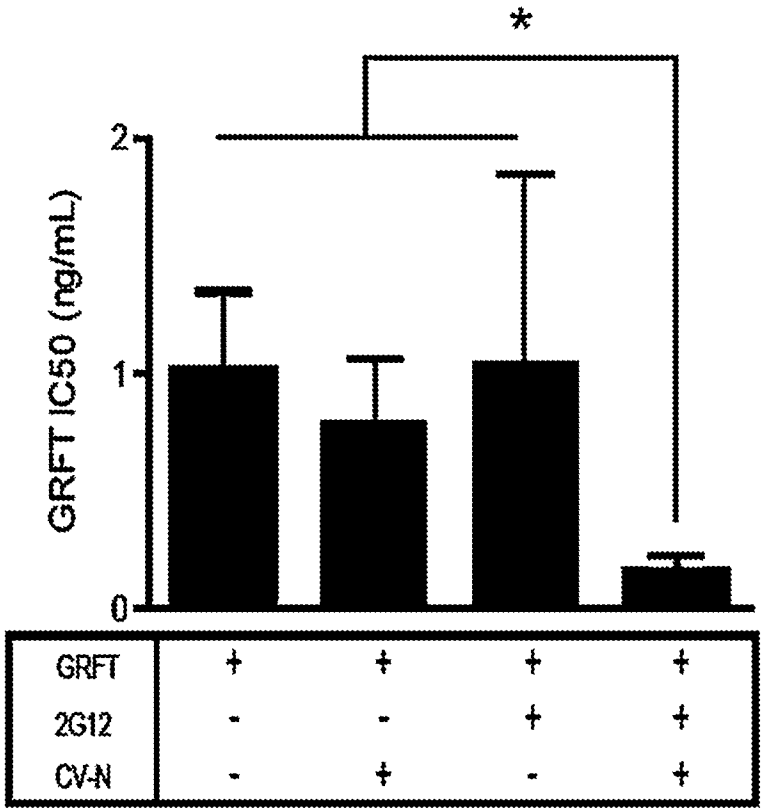

*
GRFT IC50 (ng/mL)
2
1
0
GRFT + + + +
2G12 - - + +
CV-N - + - +

FIG. 3A

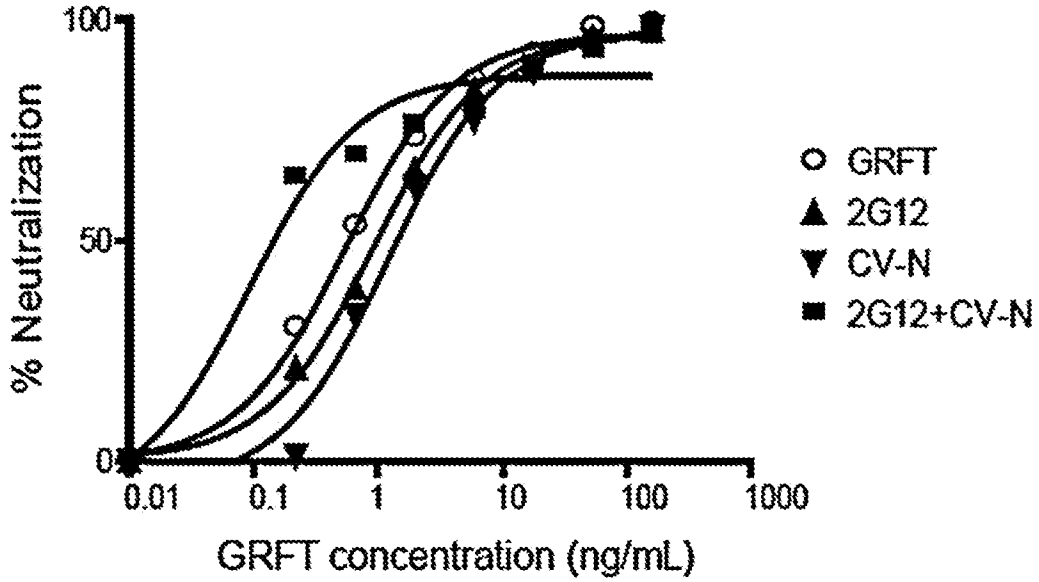

% Neutralization
100
50
0
0.01
0.1
1
10
100
1000
GRFT concentration (ng/mL)
GRFT
2G12
CV-N
2G12+CV-N

FIG. 3B

7/18

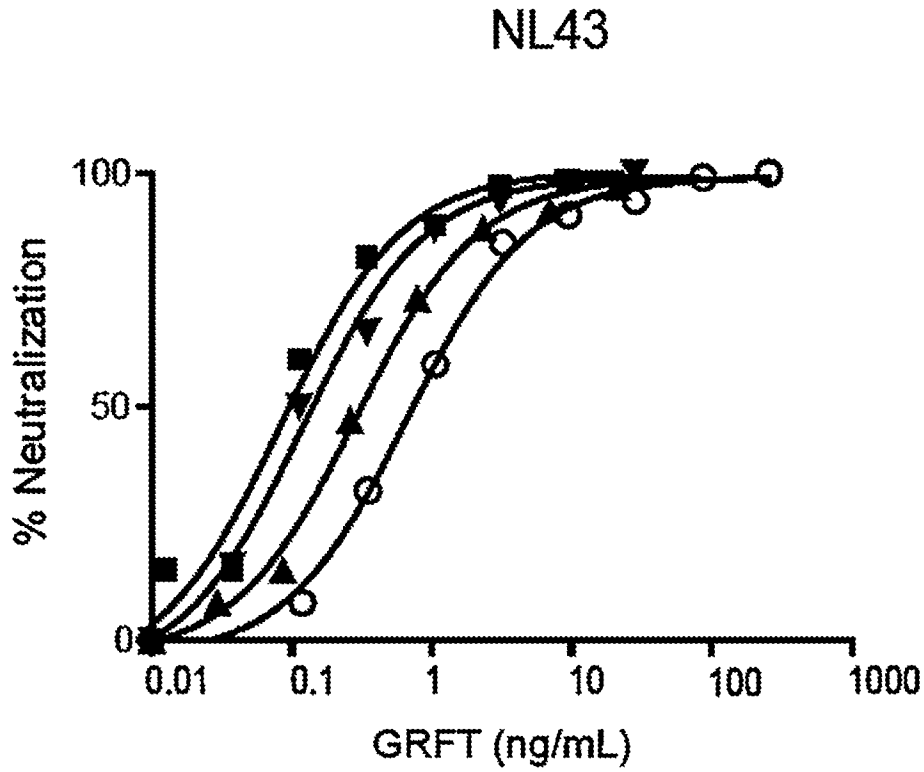


FIG. 4A

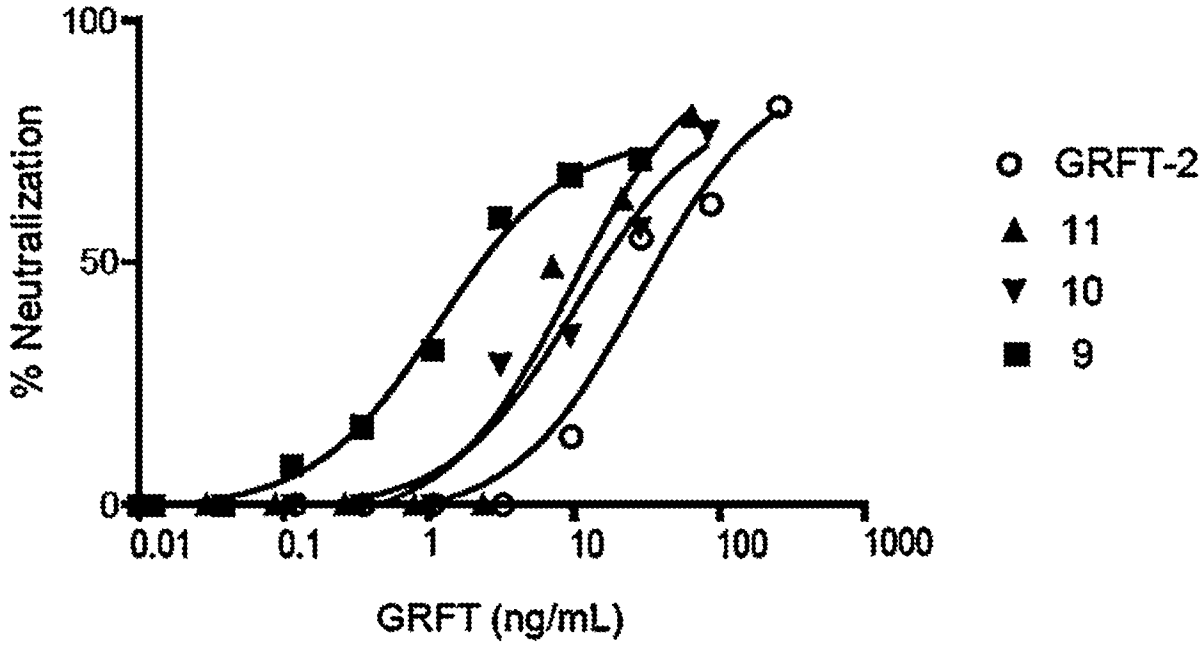


FIG. 4B

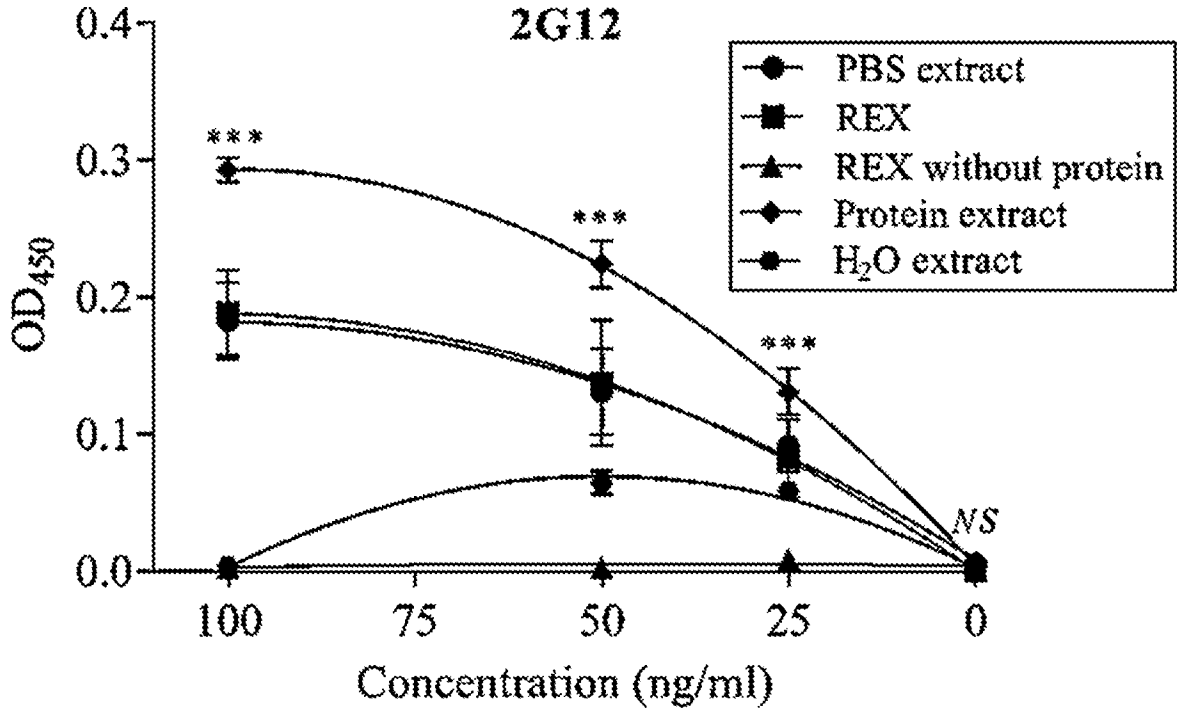

2G12
PBS extract
REX
REX without protein
Protein extract
H2O extract
OD450
0.4
0.3
0.2
0.1
0.0
***
***
***
NS
100
75
50
25
0
Concentration (ng/ml)

FIG. 5A

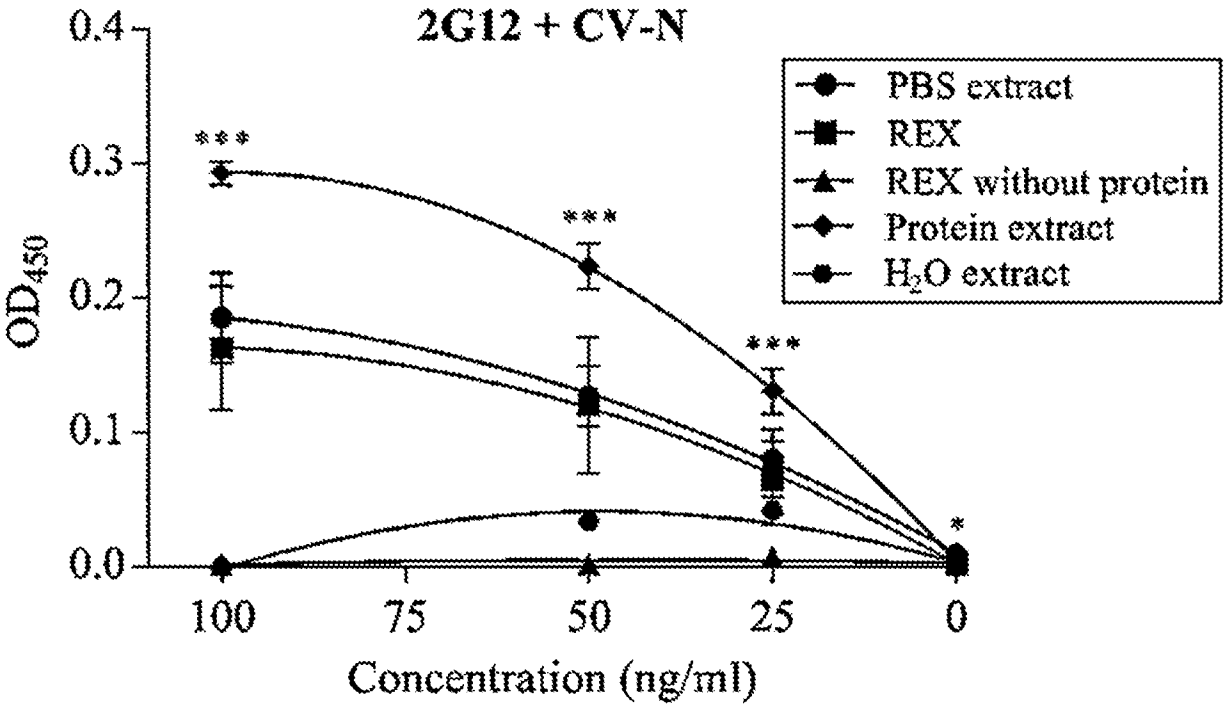

2G12 + CV-N
PBS extract
REX
REX without protein
Protein extract
H2O extract
OD450
0.4
0.3
0.2
0.1
0.0
***
***
***
*
100
75
50
25
0
Concentration (ng/ml)

FIG. 5B

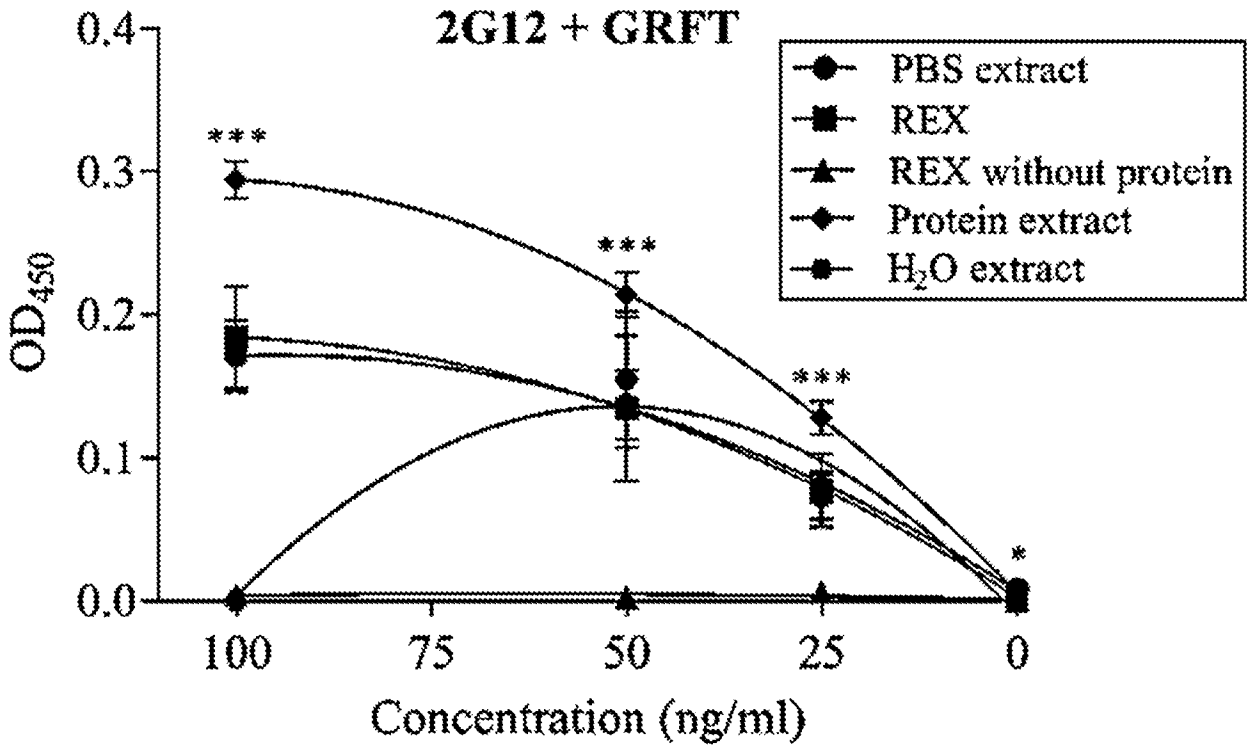

2G12 + GRFT
PBS extract
REX
REX without protein
Protein extract
H2O extract
OD450
0.4
0.3
0.2
0.1
0.0
***
***
***
*
100
75
50
25
0
Concentration (ng/ml)

FIG. 5C

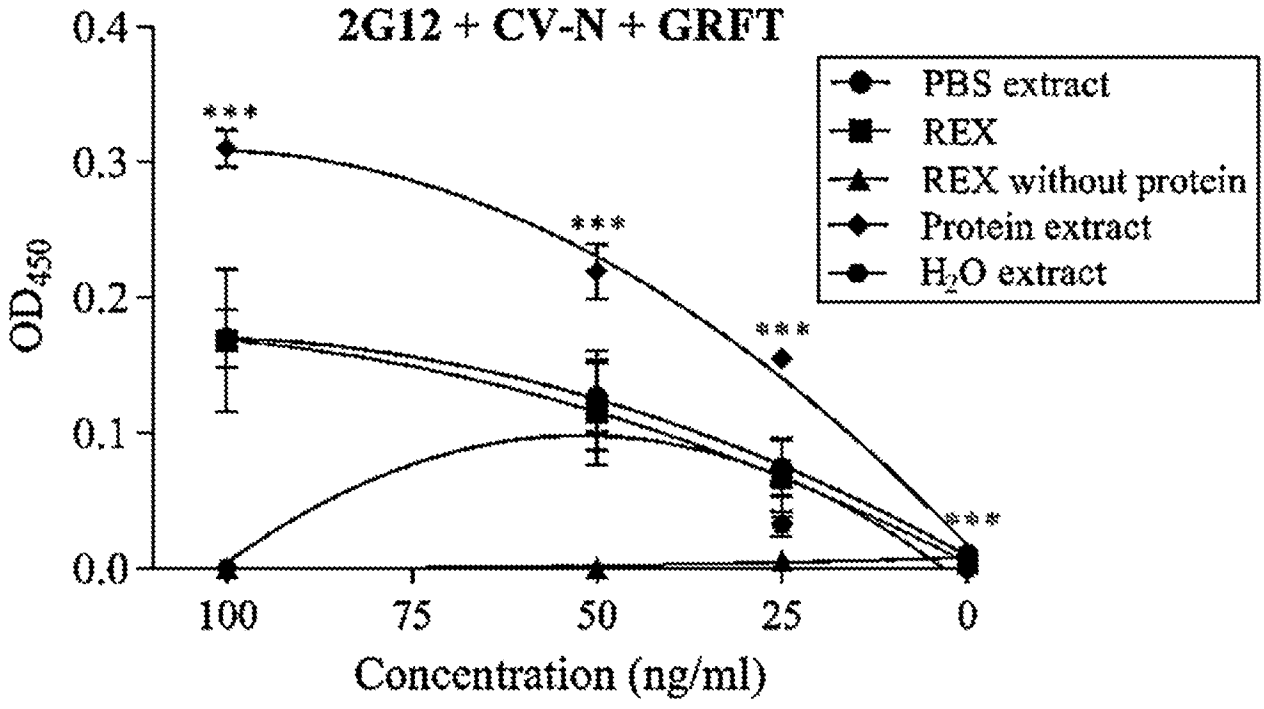

2G12 + CV-N + GRFT
PBS extract
REX
REX without protein
Protein extract
H2O extract
OD450
0.4
0.3
0.2
0.1
0.0
***
***
***
***
100
75
50
25
0
Concentration (ng/ml)

FIG. 5D

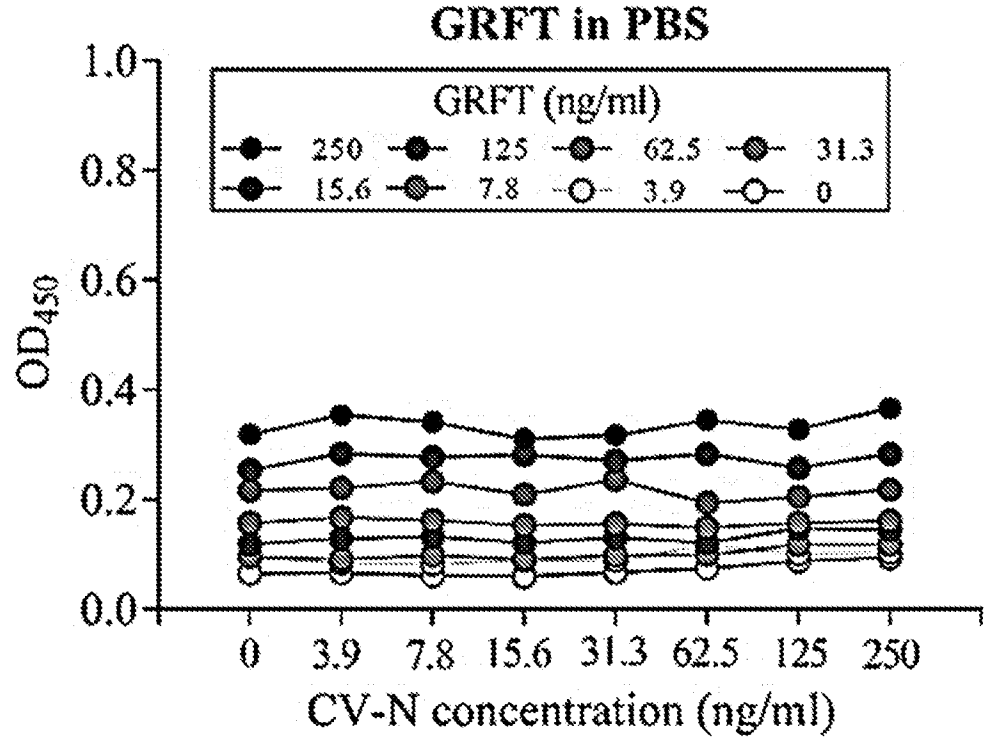

GRFT in PBS
GRFT (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
1.0
0.8
0.6
0.4
0.2
0.0
OD450
0
3.9
7.8
15.6
31.3
62.5
125
250
CV-N concentration (ng/ml)

FIG. 6A

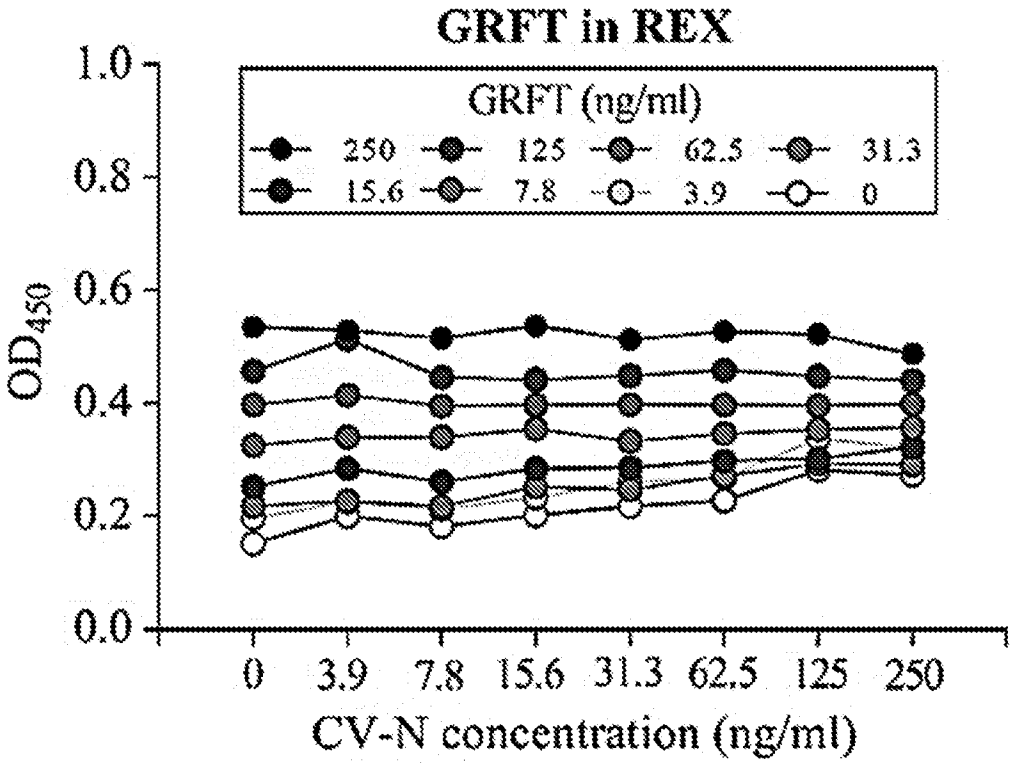

GRFT in REX
GRFT (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
1.0
0.8
0.6
0.4
0.2
0.0
OD450
0
3.9
7.8
15.6
31.3
62.5
125
250
CV-N concentration (ng/ml)

FIG. 6B

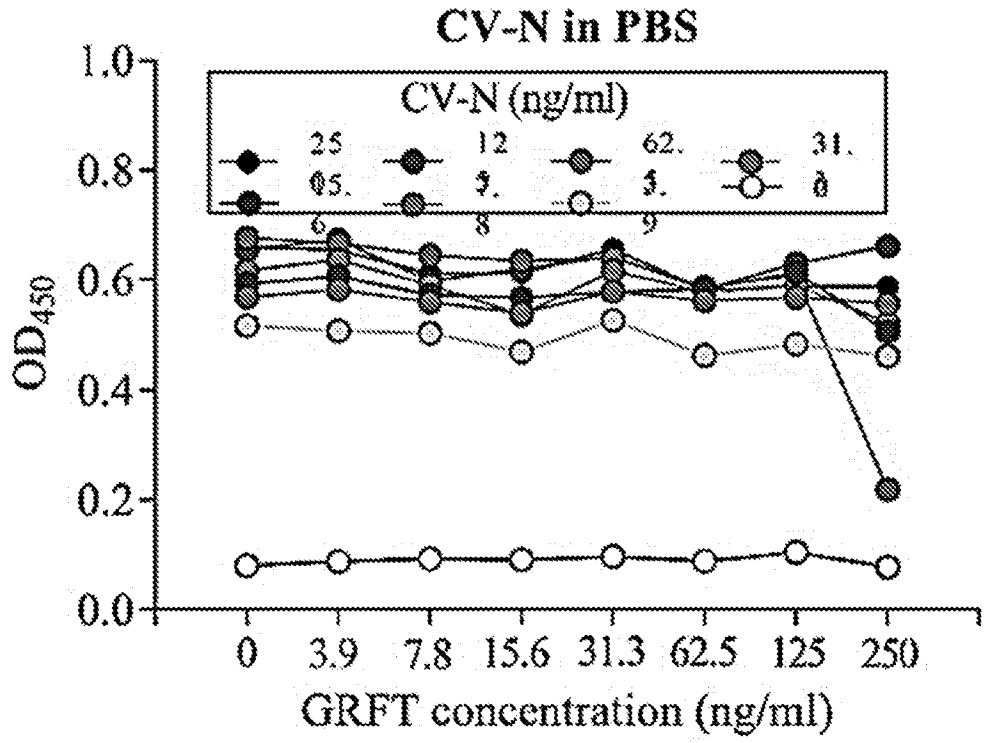

CV-N in PBS
CV-N (ng/ml)
25
12
62.
31.
OD450
1.0
0.8
0.6
0.4
0.2
0.0
0
3.9
7.8
15.6
31.3
62.5
125
250
GRFT concentration (ng/ml)

FIG. 6C

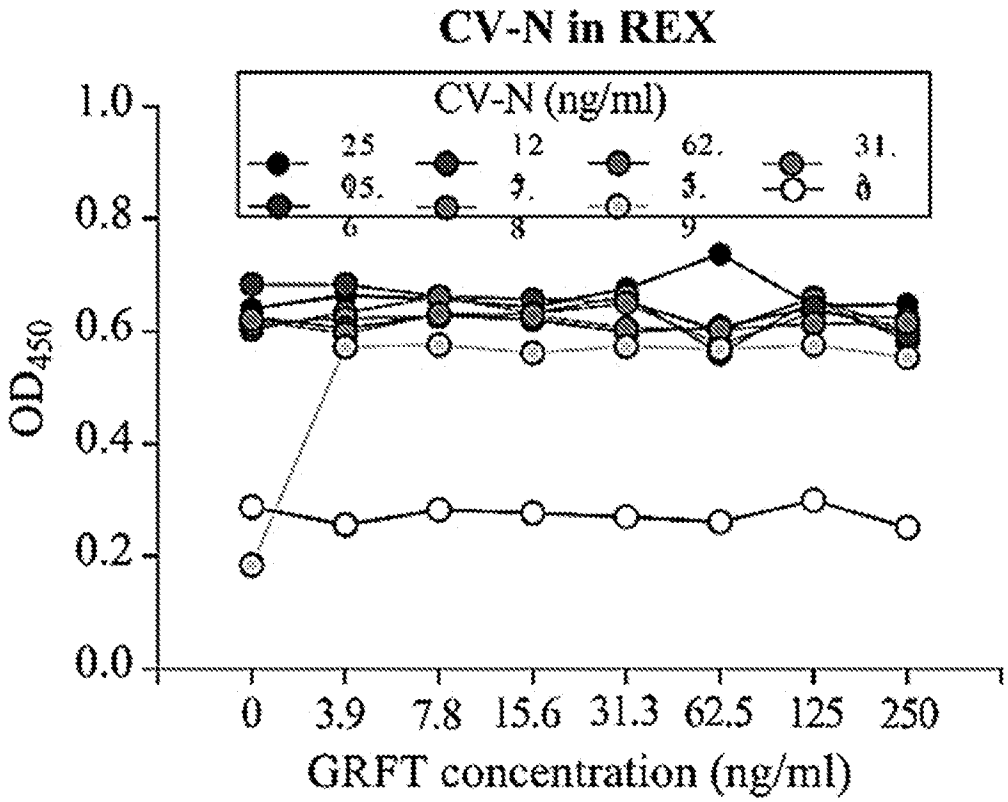

CV-N in REX
CV-N (ng/ml)
25
12
62.
31.
OD450
1.0
0.8
0.6
0.4
0.2
0.0
0
3.9
7.8
15.6
31.3
62.5
125
250
GRFT concentration (ng/ml)

FIG. 6D

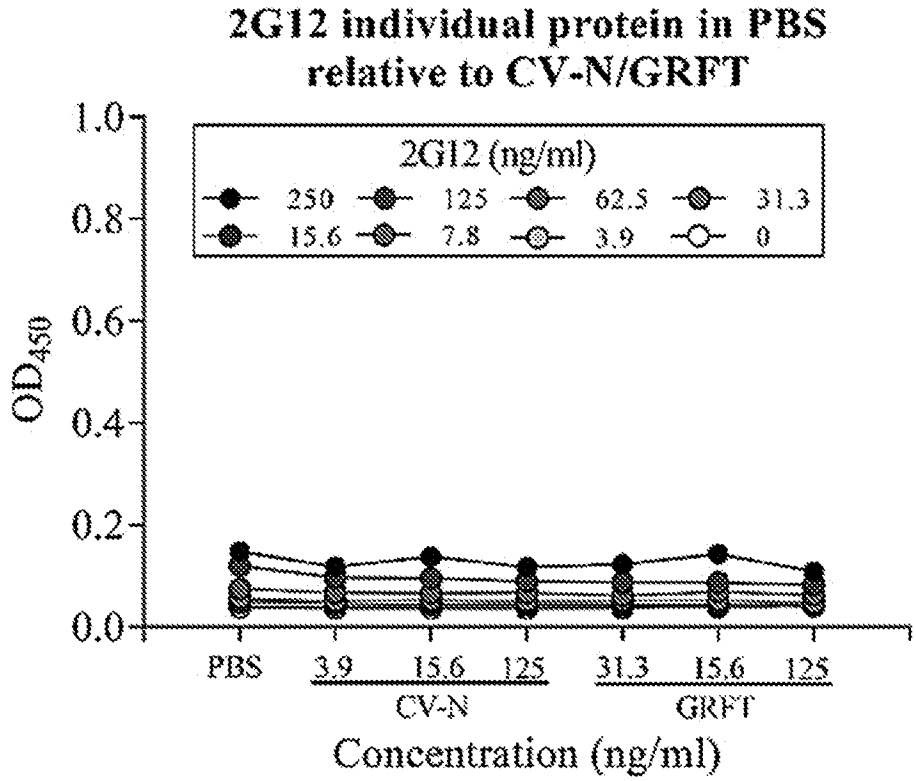

2G12 individual protein in PBS
relative to CV-N/GRFT
2G12 (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
OD450
1.0
0.8
0.6
0.4
0.2
0.0
PBS
3.9
15.6
125
31.3
15.6
125
CV-N
GRFT
Concentration (ng/ml)

FIG. 6E

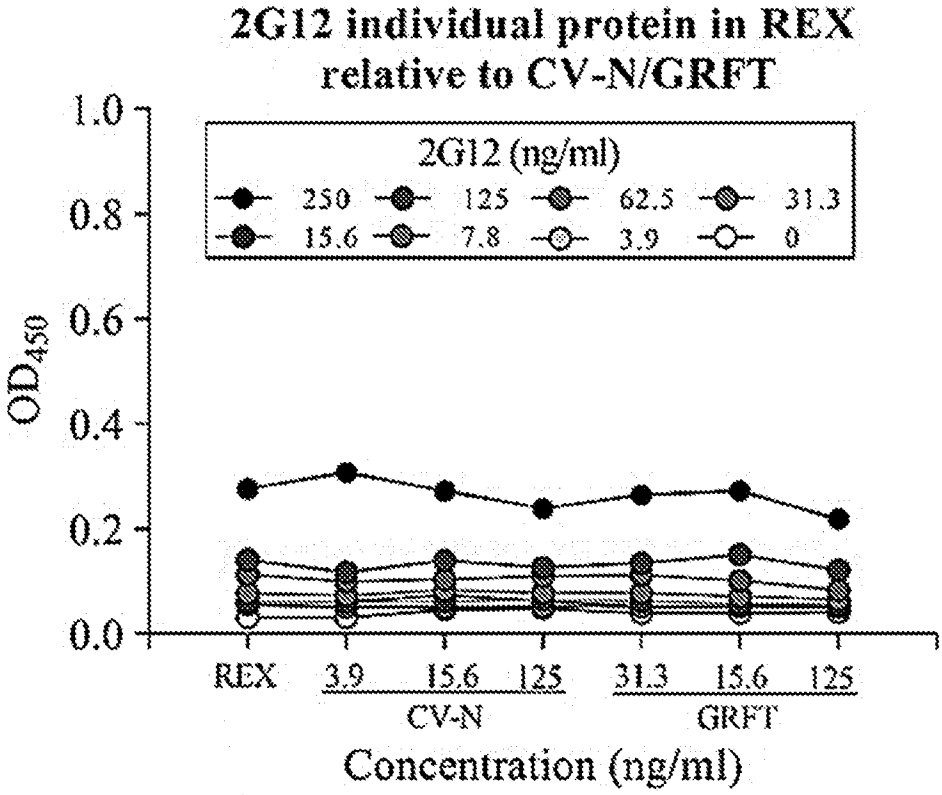

2G12 individual protein in REX
relative to CV-N/GRFT
2G12 (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
OD450
1.0
0.8
0.6
0.4
0.2
0.0
REX
3.9
15.6
125
31.3
15.6
125
CV-N
GRFT
Concentration (ng/ml)

FIG. 6F

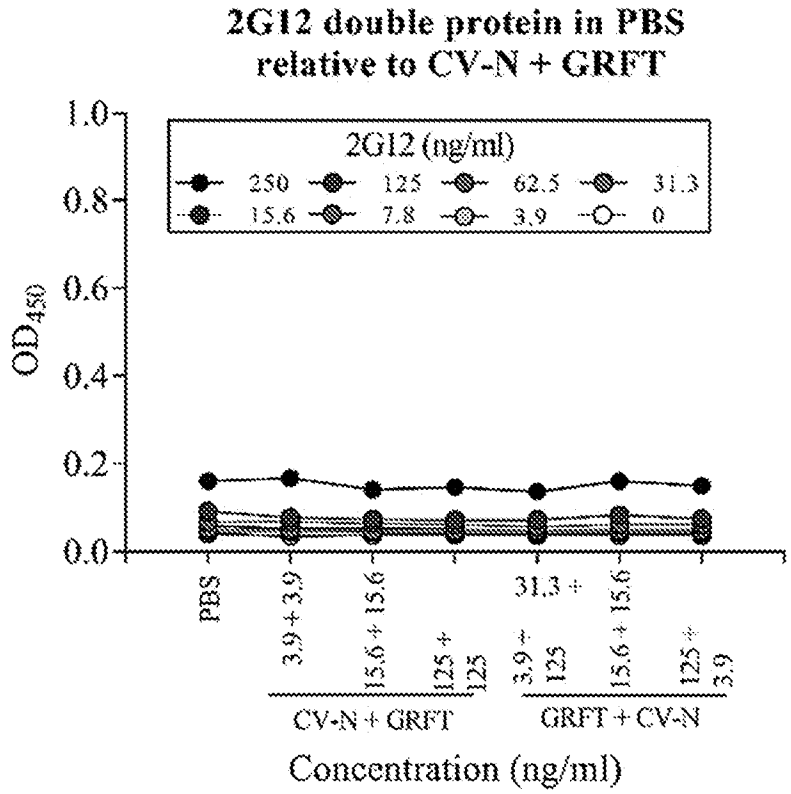

2G12 double protein in PBS
relative to CV-N + GRFT
2G12 (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
OD450
1.0
0.8
0.6
0.4
0.2
0.0
PBS
3.9 + 3.9
15.6 + 15.6
125 + 125
31.3 + 3.9
15.6 + 15.6
125 + 3.9
CV-N + GRFT
GRFT + CV-N
Concentration (ng/ml)

FIG. 6G

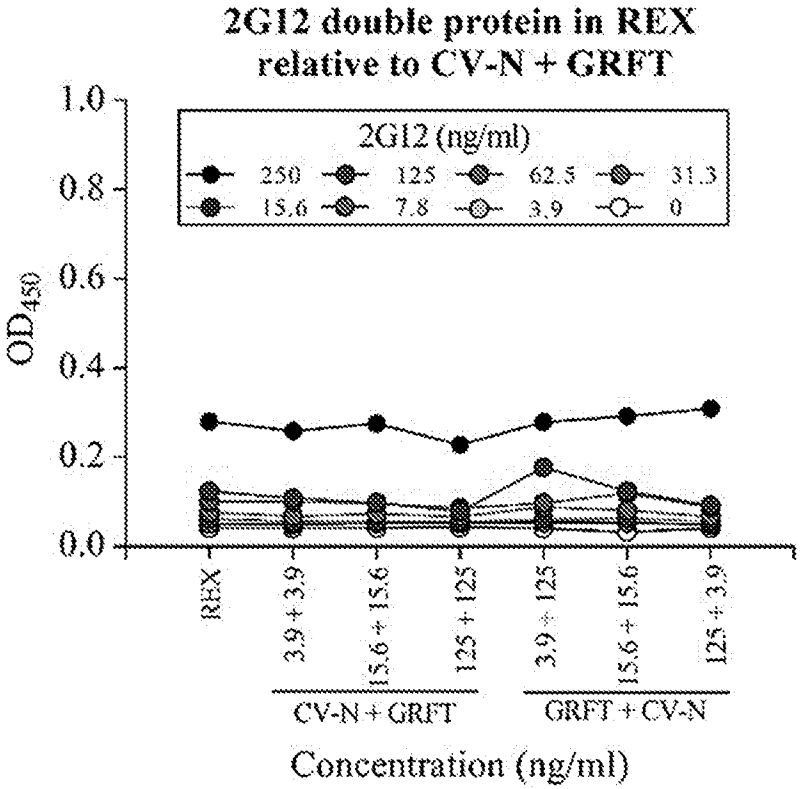

2G12 double protein in REX
relative to CV-N + GRFT
2G12 (ng/ml)
250
125
62.5
31.3
15.6
7.8
3.9
0
OD450
1.0
0.8
0.6
0.4
0.2
0.0
REX
3.9 + 3.9
15.6 + 15.6
125 + 125
3.9 + 125
15.6 + 15.6
125 + 3.9
CV-N + GRFT
GRFT + CV-N
Concentration (ng/ml)

FIG. 6H

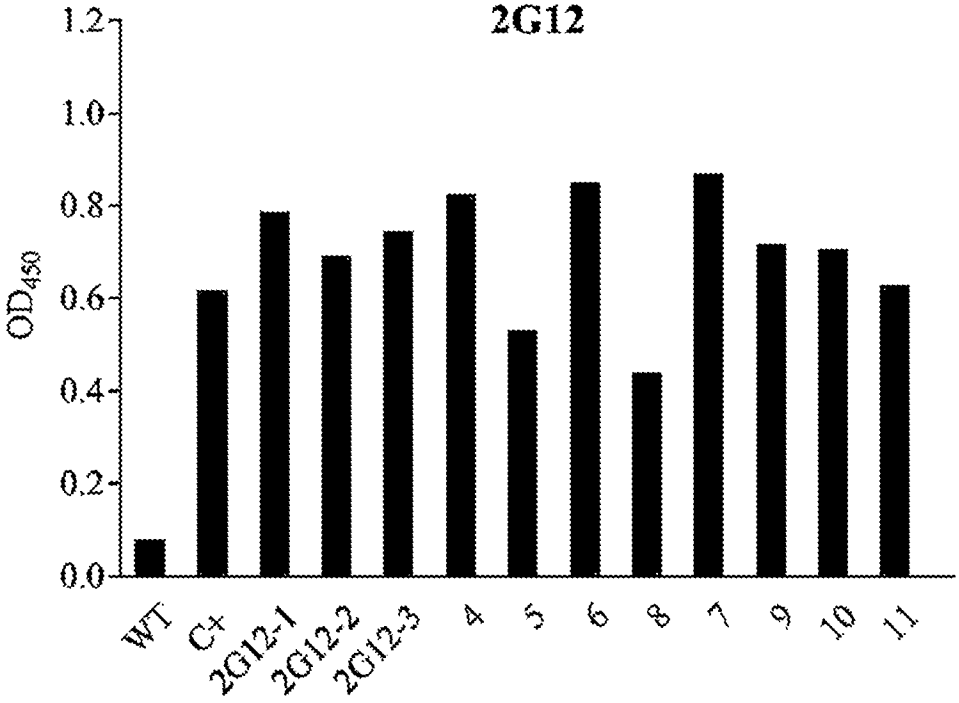

2G12
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
WT
C+
2G12-1
2G12-2
2G12-3
4
5
6
8
7
9
10
11
Independent events

FIG. 7A

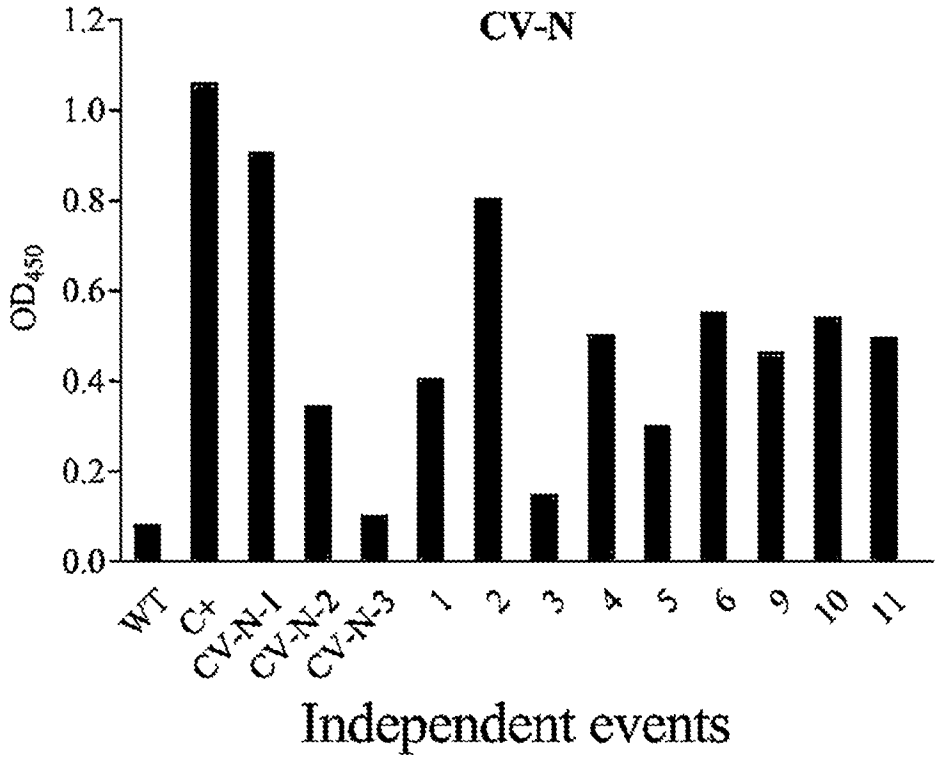

CV-N
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
WT
C+
CV-N-1
CV-N-2
CV-N-3
1
2
3
4
5
6
9
10
11
Independent events

FIG. 7B

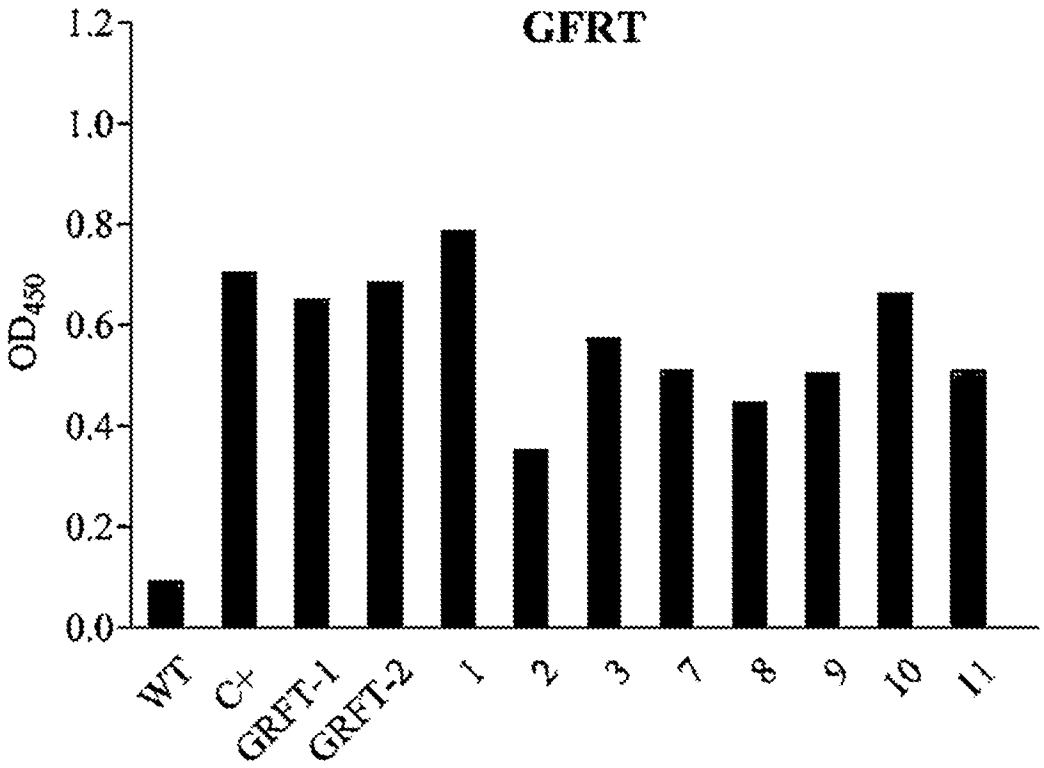

GFRT
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD450
WT
C+
GRFT-1
GRFT-2
1
2
3
7
8
9
10
11
Independent events

FIG. 7C


HindIII (2)
BamHI (1083)
EcoRI (1839)
XhoI (2743)
Rice prolamin promoter
Thioredoxin
His6
EK
Scytovirin
t-nos
pRP5-SVN

FIG. 8A


HindIII (2)
BamHI (1083)
EcoRI (1737)
XhoI (2591)
Rice prolamin promoter
Thioredoxin
His6
EK
SD1
t-nos
pRP5-SD1

FIG. 8B

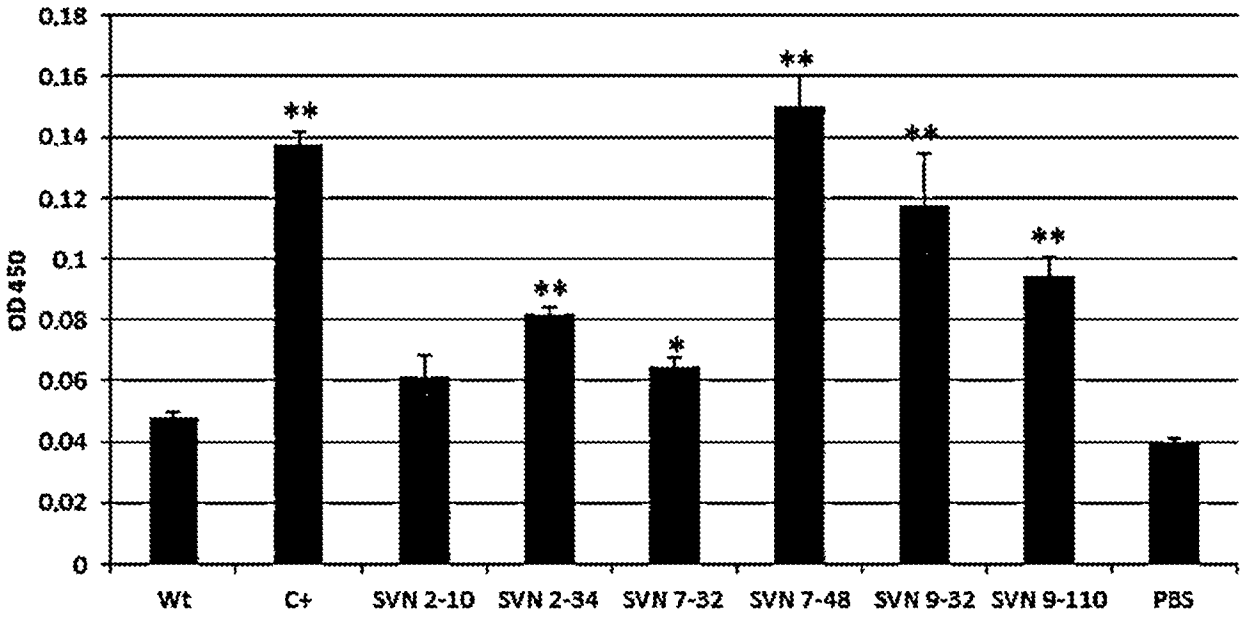

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
OD450
**
**
**
*
**
**
Wt
C+
SVN 2-10
SVN 2-34
SVN 7-32
SVN 7-48
SVN 9-32
SVN 9-110
PBS

FIG. 9A

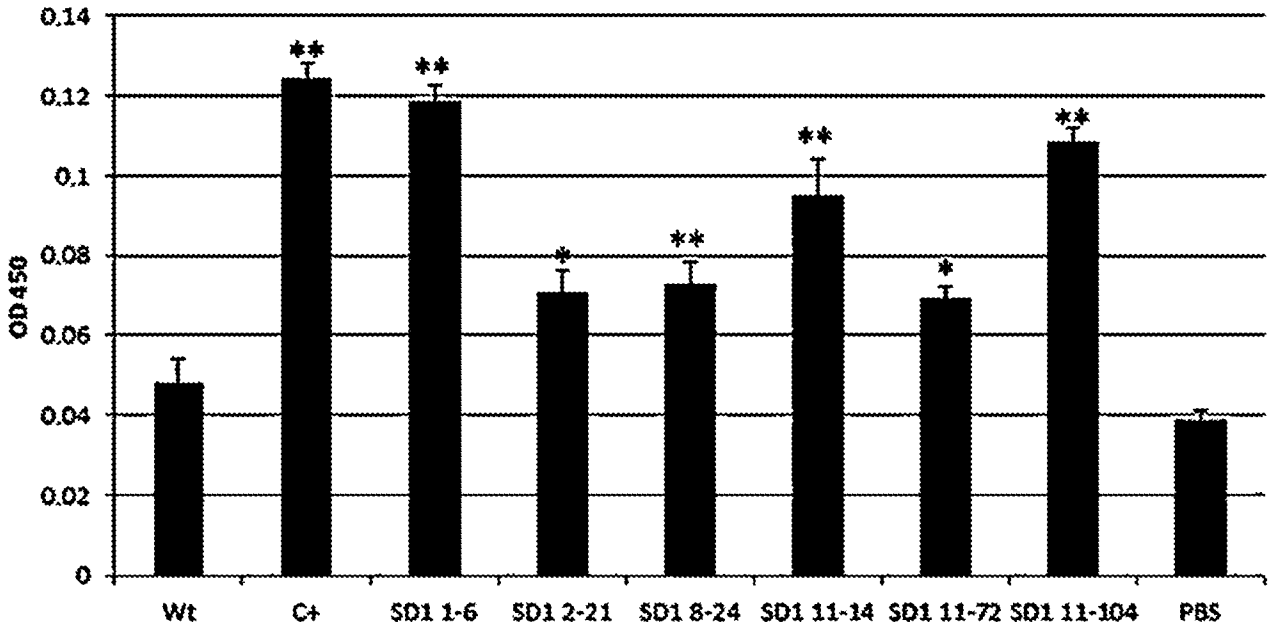

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
OD450
**
**
*
**
**
*
**
Wt
C+
SD1 1-6
SD1 2-21
SD1 8-24
SD1 11-14
SD1 11-72
SD1 11-104
PBS

FIG. 9B

18/18

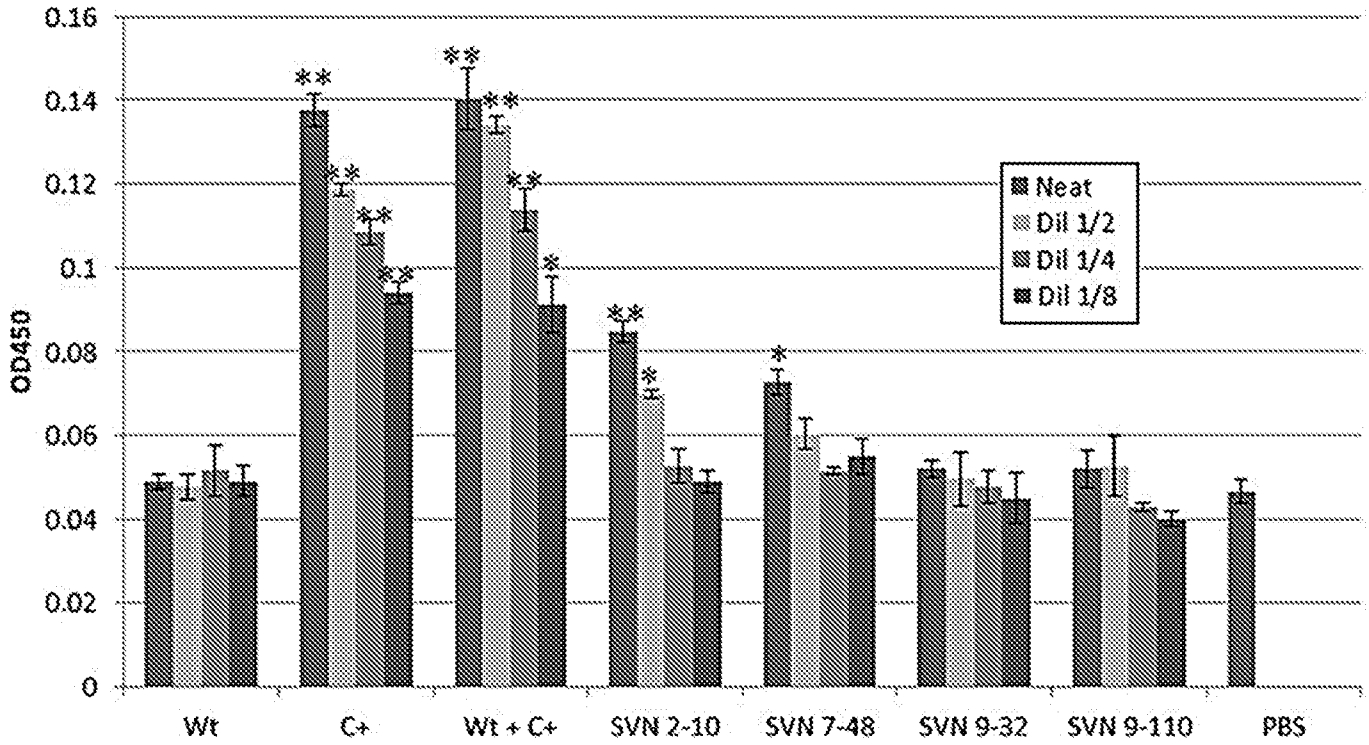


FIG. 10A

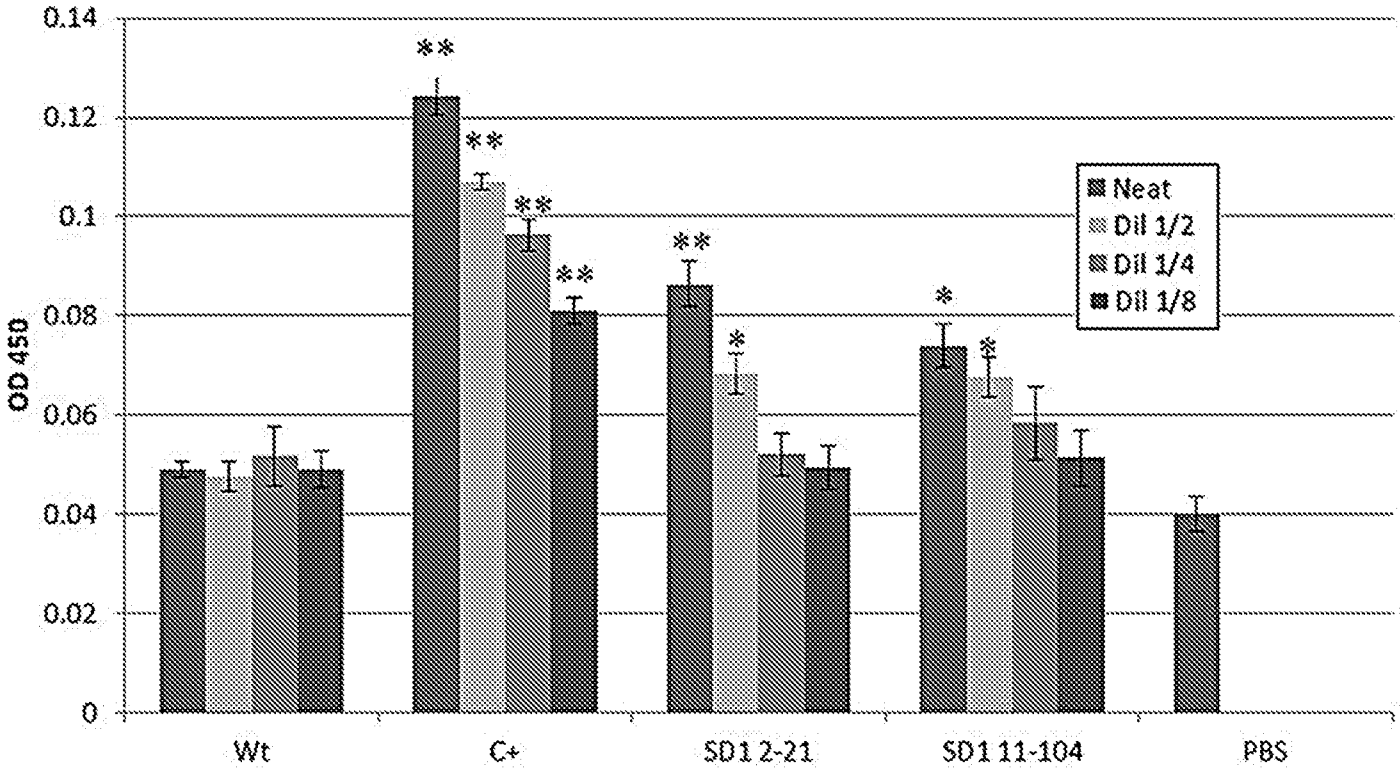


FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/062857

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10 A61K38/16 C07K14/405 C12Q1/6895 C12N15/82
A61K39/00 A61P31/18
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K C12N C12Q A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EVANGELIA VAMVAKA ET AL: "Rice endosperm is cost-effective for the production of recombinant griffithsin with potent activity against HIV", PLANT BIOTECHNOLOGY JOURNAL, vol. 14, no. 6, 1 June 2016 (2016-06-01), pages 1427-1437, XP055555372, GB ISSN: 1467-7644, DOI: 10.1111/pbi.12507 abstract ----- -/--	1-14, 16-27, 29-36

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 2019	14/05/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Turri, Matteo

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/062857

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. VAMVAKA ET AL: "Cyanovirin-N produced in rice endosperm offers effective pre-exposure prophylaxis against HIV-1BaL infection in vitro", PLANT CELL REPORTS, vol. 35, no. 6, 1 June 2016 (2016-06-01), pages 1309-1319, XP055555375, ISSN: 0721-7714, DOI: 10.1007/s00299-016-1963-5 abstract -----	1-14, 16-27, 29-36
X	EVANGELIA VAMVAKA ET AL: "Rice endosperm produces an underglycosylated and potent form of the HIV-neutralizing monoclonal antibody 2G12", PLANT BIOTECHNOLOGY JOURNAL, vol. 14, no. 1, 1 January 2016 (2016-01-01), pages 97-108, XP055555671, GB ISSN: 1467-7644, DOI: 10.1111/pbi.12360 abstract -----	1-14, 16-27, 29-36
X,P	EVANGELIA VAMVAKA ET AL: "Unexpected synergistic HIV neutralization by a triple microbicide produced in rice endosperm", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 115, no. 33, 14 August 2018 (2018-08-14), pages E7854-E7862, XP055555369, US ISSN: 0027-8424, DOI: 10.1073/pnas.1806022115 the whole document -----	1-14, 16-26, 29-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/062857

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 19-27, 29, 30, 32-36(completely); 16-18, 31(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 19-27, 29, 30, 32-36(completely); 16-18, 31(partially)

   All subject-matter relating to a combination of endosperm, anti-HIV lectin and anti-HIV antibody

   ---

2. claims: 15, 28(completely); 16-18, 31(partially)

   All subject-matter relating to the expression of scytovirin in a transgenic plant

   ---