encoding region of the HA is modified in such a way that protein production in bird cells achieves maximal yield. The main modification is codon optimisation for chicken and deletion of the proteolysis sensitive region of HA.

[0013] WO 2013/044390 describes a method of producing a virus like particle (VLP) in a plant with modified hemagglutinin (HA) wherein the modified HA protein comprises a modified proteolytic loop. The modified HA is expressed in the presence of the regulatory region Cowpea mosaic virus (CPMV) HT and the geminivirus amplification element from Bean Yellow Dwarf Virus (BeYDV).

[0014] US 2008/0069821 by Yang et al. discloses polypeptides and polynucleotides variants of influenza HA for use in the production of influenza viruses as vaccines. Reassortant influenza viruses are obtained by introducing a subset of vectors corresponding to genomic segments of a master influenza virus, in combination with complementary segments derived from the variants of influenza HA. Typically, the master strains are selected on the basis of desirable properties relevant to vaccine administration. For example, for vaccine production, e.g., for production of a live attenuated vaccine, the master donor virus strain may be selected for an attenuated phenotype, cold adaptation and/or temperature sensitivity.

SUMMARY OF THE ~~INVENTION~~DISCLOSURE

[0015] This ~~invention~~ disclosure relates to producing virus like particles and modified HA proteins in plants.

[0016] It is an object of the ~~invention~~ disclosure to provide an improved production of virus like particles and HA proteins in plants.

[0017] Described herein is a nucleic acid comprising an expression enhancer active in a plant and operatively linked to a nucleotide sequence encoding a modified influenza hemagglutinin (HA) comprising a modified proteolytic loop.

[0018] Furthermore described herein is a method (A) of producing a virus like particle (VLP) in a plant comprising,

a) introducing a nucleic acid comprising an expression enhancer active in a plant and operatively linked to a nucleotide sequence encoding a modified influenza

virus like particle (VLP) may comprise plant-specific N-glycans, or modified N-glycans.

[0025] The present disclosure also provides a composition comprising an effective dose of the VLP for inducing an immune response, and a pharmaceutically acceptable carrier.

[0025a] The present disclosure also provides the use of the VLP as disclosed herein in the manufacture of a medicament for inducing immunity to an influenza virus infection in a subject.

[0026] Also described herein is a nucleic acid comprising a nucleotide sequence encoding an influenza hemagglutinin (HA), the nucleotide sequence operatively linked with a regulatory region that is active in a plant, wherein the HA comprises a modified proteolytic loop sequence. The nucleic acid may encode an HA comprising a modified proteolytic loop, where in the protein has hemagglutinin (HA) activity. A plant comprising the nucleic acid is also provided. Also included is a virus like particle (VLP) produced in a plant, the VLP comprising an influenza virus hemagglutinin (HA) encoded by the nucleic acid and one or more than one lipid derived from a plant.

[0027] This summary of the ~~invention~~ disclosure does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0029] **Figure 1** shows components used to prepare A-2X35S/CPMV-HT/H5 Indonesia/NOS (Construct number 489). **Figure 1A** shows primer IF-H5A-I-05.s1+3c (SEQ ID NO: 2). **Figure 1B** shows primer IF-H5dTm.r (SEQ ID NO: 3). **Figure 1C** shows a schematic representation of construct 1191. **Figure 1D** shows Construct 1191 (SEQ ID NO 4). **Figure 1E** shows expression cassette number 489 (SEQ ID NO 5). **Figure 1F** shows amino acid sequence of H5 from influenza A/Indonesia/5/2005 (H5N1) (SEQ ID NO: 6). **Figure 1G** shows a nucleotide sequence encoding H5 from influenza A/Indonesia/5/2005 (H5N1) (SEQ ID NO: 42).

[0030] **Figure 2** shows components used to prepare B-2X35S/CPMV HT/M2 New Caledonia/NOS (Construct number 1261). **Figure 2A** shows primer IF-S1-

WHAT IS CLAIMED IS:

1. A nucleic acid comprising a regulatory region active in a plant and an expression enhancer active in a plant, the regulatory region and the expression enhancer operatively linked to a nucleotide sequence encoding a modified influenza hemagglutinin (HA) comprising a modified proteolytic loop.

2. The nucleotide acid of claim 1, wherein the expression enhancer is selected from the group consisting of CPMVX, CPMVX+ , CPMV-HT+ CPMV HT+[WT115] and CPMV HT+ [511].

3. The nucleotide acid of claim 1, wherein the expression enhancer is not CPMV HT.

4. The nucleotide acid of claim 1, wherein the nucleotide acid does not comprise a Bean Yellow Dwarf Virus long intergenic region (BeYDV LIR), and a BeYDV short intergenic region (BeYDV SIR).

5. The nucleotide acid of claim 1, wherein the modified proteolytic loop comprises one or more protease cleavage sites exhibiting reduced or abolished cleavage by a protease, when compared to the cleave of one or more cleavage sites of a native HA.

6. The nucleotide acid of claim 5, wherein the protease is Clara-like, Furin-like or Trypsin.

7. The nucleotide acid of claim 1, wherein the modified proteolytic loop comprises a linker sequence.

8. The nucleotide acid of claim 7, wherein the linker sequence has the amino acid sequence GG, TETQ or TETR.

9. The nucleotide acid of claim 1, wherein the HA encoded by the nucleotide sequence is selected from the group consisting of HA from influenza types B, C, A, and subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, and H16.

10. The nucleotide acid of claim 1, wherein the nucleotide sequence encoding the influenza hemagglutinin (HA) protein has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID NO:. 17, 18, 20, 21, 41, 58, 77, 81, 85, 92, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 134, 143, 147, 154, 161, 168, 194 and 199.

11. The nucleotide acid of claim 1, wherein the HA is an HA0.

12. The nucleic acid of claim 1, wherein the modified HA comprises a native or a non-native signal peptide.

13. The nucleic acid of claim 1, wherein the nucleotide sequence encoding the modified HA comprises a chimeric nucleotide sequence encoding, in series, a modified HA ectodomain comprising a modified proteolytic loop, an influenza transmembrane domain, and a cytoplasmic tail, wherein the modified HA ectodomain is from a first influenza strain and the transmembrane domain and the cytoplasmic tail are from a second influenza strain.

14. A method of producing influenza virus like particle (VLP) in a plant comprising,

a) introducing the nucleotide acid of claim 1 into the plant or portion of the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acids, thereby producing the VLP.

15. The method of claim 14, wherein in step a) a second nucleotide acid is introduced into the plant or portion of the plant, the second nucleotide acid comprising a second regulatory region active in the plant and operatively linked to a nucleotide sequence encoding a proton channel protein.

16. The method of claim 15, wherein the proton channel protein is selected from influenza M2 or BM2.

17. A method of producing influenza virus like particles (VLPs) in a plant comprising:

a) providing a plant, or a portion of a plant, comprising the nucleic acid of claim 1, and

b) incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

18. The method of claim 14, further comprising the steps of

c) harvesting the plant, and

d) purifying the VLPs, wherein the VLPs range in size from 80-300 nm.

19. A VLP produced by the method of any one of claim 14.

20. The VLP of claim 19 further comprising one or more than one lipid derived from a plant.

21. The VLP of claim 20, wherein the modified HA protein comprises plant-specific N-glycans or modified N-glycans.

22. A method of producing a modified HA protein comprising a modified proteolytic loop comprising one or more protease cleavage sites exhibiting reduced or abolished cleavage in a plant comprising,

a) introducing the nucleotide acid of claim 1 into the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the HA protein, thereby producing the modified HA protein,

c) harvesting the plant and purifying the modified HA protein.

23. An HA protein produced by the method of claim 22, where in the protein has hemagglutinin (HA) activity.

24. An HA encoded by the nucleic acid of claim 1.

25. The method of claim 14, wherein in the step of introducing (step a), the nucleic acid is introduced in the plant in a transient manner, or the nucleic acid is introduced in the plant so that it is stable.

26. A plant comprising the nucleic acid of claim 1.

28. A composition comprising an effective dose of the VLP of claim 19 for inducing an immune response, and a pharmaceutically acceptable carrier.

29. A vaccine comprising an effective dose of the VLP of claim 19 for inducing an immune response.

30. A method of inducing immunity to an influenza virus infection in a subject, comprising administering the VLP of claim 19.

31. The method of claim 30, wherein the VLP is administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

32. A method of increasing the product quality of an HA protein expressed in a plant, comprising,

a) introducing the nucleotide acid of claim 1 into the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the HA protein, thereby producing a modified HA protein,

c) harvesting the plant and purifying the modified HA protein, wherein the modified HA protein has increased product quality, when compared to a native HA.

33. The method of claim 32, wherein the quality comprises increased product yield, stability of the product, consistency of the product or a combination thereof.

CLAIMS

1. A nucleic acid comprising a regulatory region active in a plant and an expression enhancer active in a plant, the regulatory region and the expression enhancer operatively linked to a nucleotide sequence encoding a modified influenza hemagglutinin (HA) comprising a modified proteolytic loop.

2. The nucleic acid of claim 1, wherein the expression enhancer is selected from the group consisting of CPMVX, CPMVX+ , CPMV-HT+ CPMV HT+[WT115] and CPMV HT+ [511].

3. The nucleic acid of claim 1, wherein the expression enhancer is not CPMV HT.

4. The nucleic acid of claim 1, wherein the nucleic acid does not comprise a Bean Yellow Dwarf Virus long intergenic region (BeYDV LIR), and a BeYDV short intergenic region (BeYDV SIR).

5. The nucleic acid of claim 1, wherein the modified proteolytic loop comprises one or more protease cleavage sites exhibiting reduced or abolished cleavage by a protease, when compared to the cleave of one or more cleavage sites of a native HA.

6. The nucleic acid of claim 5, wherein the protease is Clara-like, Furin-like or Trypsin.

7. The nucleic acid of claim 1, wherein the modified proteolytic loop comprises a linker sequence.

8. The nucleic acid of claim 7, wherein the linker sequence has the amino acid sequence GG, TETQ or TETR.

9. The nucleic acid of claim 1, wherein the HA encoded by the nucleotide sequence is selected from the group consisting of HA from influenza types B, C, A, and subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, and H16.

10. The nucleic acid of claim 1, wherein the nucleotide sequence encoding the influenza hemagglutinin (HA) protein has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID NO:. 17, 18, 20, 21, 41, 58, 77, 81, 85, 92, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 134, 143, 147, 154, 161, 168, 194 and 199.

11. The nucleic acid of claim 1, wherein the HA is an HA0.

12. The nucleic acid of claim 1, wherein the modified HA comprises a native or a non-native signal peptide.

13. The nucleic acid of claim 1, wherein the nucleotide sequence encoding the modified HA comprises a chimeric nucleotide sequence encoding, in series, a modified HA ectodomain comprising a modified proteolytic loop, an influenza transmembrane domain, and a cytoplasmic tail, wherein the modified HA ectodomain is from a first influenza strain and the transmembrane domain and the cytoplasmic tail are from a second influenza strain.

14. A method of producing influenza virus like particle (VLP) in a plant comprising,

a) introducing the nucleic acid of claim 1 into the plant or portion of the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acids, thereby producing the VLP.

15.The method of claim 14, wherein in step a) a second nucleic acid is introduced into the plant or portion of the plant, the second nucleic acid comprising a second regulatory region active in the plant and operatively linked to a nucleotide sequence encoding a proton channel protein.

16. The method of claim 15, wherein the proton channel protein is selected from influenza M2 or BM2.

17. A method of producing influenza virus like particles (VLPs) in a plant comprising:

a) providing a plant, or a portion of a plant, comprising the nucleic acid of claim 1, and

b) incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

18. The method of claim 14, further comprising the steps of

c) harvesting the plant, and

d) purifying the VLPs, wherein the VLPs range in size from 80-300 nm.

19. A VLP produced by the method of any one of claim 14.

20. The VLP of claim 19 further comprising one or more than one lipid derived from a plant.

21. The VLP of claim 20, wherein the modified HA protein comprises plant-specific N-glycans or modified N-glycans.

22. A method of producing a modified HA protein comprising a modified proteolytic loop comprising one or more protease cleavage sites exhibiting reduced or abolished cleavage in a plant comprising,

a) introducing the nucleic acid of claim 1 into the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the HA protein, thereby producing the modified HA protein,

c) harvesting the plant and purifying the modified HA protein.

23. An HA protein produced by the method of claim 22, where in the protein has hemagglutinin (HA) activity.

24. An HA encoded by the nucleic acid of claim 1.

25. The method of claim 14, wherein in the step of introducing (step a), the nucleic acid is introduced in the plant in a transient manner, or the nucleic acid is introduced in the plant so that it is stable.

26. A plant comprising the nucleic acid of claim 1.

27. A composition comprising an effective dose of the VLP of claim 19 for inducing an immune response, and a pharmaceutically acceptable carrier.

28. A vaccine comprising an effective dose of the VLP of claim 19 for inducing an immune response.

29. Use of the VLP of claim 19 in the manufacture of a medicament for inducing immunity to an influenza virus infection in a subject.

30. The use of claim 29, wherein the VLP is to be administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

31. A method of increasing the product quality of an HA protein expressed in a plant, comprising,

a) introducing the nucleic acid of claim 1 into the plant;

b) incubating the plant or portion of the plant under conditions that permit the expression of the HA protein, thereby producing a modified HA protein,

c) harvesting the plant and purifying the modified HA protein, wherein the modified HA protein has increased product quality, when compared to a native HA.

32. The method of claim 31, wherein the quality comprises increased product yield, stability of the product, consistency of the product or a combination thereof.