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 DISCLOSURE

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

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

[0016a]In one aspect, there is provided 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 mutant influenza hemagglutinin (HA) comprising a fully deleted proteolytic loop, 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).

[0016b] In one aspect, there is provided a method of producing influenza virus like particle (VLP) in a plant comprising,

a) introducing the nucleic acid as disclosed herein into the plant or portion of the plant; wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof;

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

[0016c] In one aspect, there is provided 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 as disclosed herein, wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof and

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

[0016d] In one aspect, there is provided a VLP produced by the method as disclosed herein, wherein the VLP comprises HA comprising the fully deleted proteolytic loop.

[0016e] In one aspect, there is provided a VLP comprising lipid derived from a plant and modified HA, wherein the modified HA comprise a fully deleted proteolytic loop and plant-specific N-glycans or modified N-glycans.

[0016f] In one aspect, there is provided a method of producing a modified HA protein comprising a fully deleted proteolytic loop in a plant or portion of a plant, wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof, the method comprising,

a) introducing the nucleic acid as disclosed herein 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 HA protein, thereby producing the modified HA protein,

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

[0016g] In one aspect, there is provided an HA protein produced by the method as disclosed herein, wherein the HA comprises a fully deleted proteolytic loop and has hemagglutinin (HA) activity.

[0016h] In one aspect, there is provided an HA protein comprising a fully deleted proteolytic loop.

[0016i] In one aspect, there is provided an HA encoded by the nucleic acid as disclosed herein.

[0016j] In one aspect, there is provided a plant comprising the nucleic acid as disclosed herein.

[0016k] In one aspect, there is provided a composition comprising an effective dose of the VLP as disclosed herein and a pharmaceutically acceptable carrier for use as a medicament for inducing an immune response.

[0016l] In one aspect, there is provided a vaccine comprising an effective dose of the VLP as disclosed herein for use in inducing an immune response.

[0016m] In one aspect, there is provided 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.

[0016n] In one aspect, there is provided a method of increasing product yield of an HA from B or H3 influenza strain protein expressed in a plant or portion of a plant wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof, the method comprising,

a) introducing the nucleic acid as disclosed herein 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.

[0016o] In one aspect, there is provided 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) from B or H3 influenza strain, the mutant HA comprising a partially or fully deleted proteolytic loop, 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).

[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

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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 mutant influenza hemagglutinin (HA) comprising a fully deleted proteolytic loop, 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).

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

CPMVX comprises X nucleotides of SEQ ID NO:93, where X=160, 155, 150, or 114 of SEQ ID NO:93, or a sequence that comprises between 80% to 100% sequence similarity with CPMVX, where X=160, 155, 150, or 114 of SEQ ID NO:93,

CPMVX+ comprises X nucleotides of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1, or a sequence that comprises between 80 to 100 % sequence similarity with CPMVX, where X=160, 155, 150, or 114 of SEQ ID NO:93, and a stuffer sequence comprises from 1-100 nucleotides fused to the 3' end of the CMPVX sequence,

CPMV-HT+ comprises a comovirus 5' untranslated region (UTR) and a modified, lengthened, or truncated stuffer sequence,

CPMV HT+ comprises the sequence of SEQ ID NO:189, and

CPMV HT+ comprises the sequence of SEQ ID NO:188.

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

4. The nucleic acid of claim 1, wherein the modified HA is maintained as a HA0 precursor.

5. The nucleic acid of claim 1, wherein the proteolytic loop is fully replaced with a linker sequence.

6. The nucleic acid of claim 5, wherein the linker sequence has the amino acid sequence GG, TETQ or TETR.

7. 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.

8. 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.

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

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

11. 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 the fully deleted 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 is from a second influenza strain.

12. 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; wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof;

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

13. The method of claim 12, 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 wherein the proton channel protein is selected from influenza M2 or BM2.

14. 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, wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof and

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

15. The method of claim 17, further comprising the steps of

c) harvesting the plant, and

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

16. A VLP produced by the method of claim 12, wherein the VLP comprises HA comprising the fully deleted proteolytic loop.

17. The VLP of claim 16 further comprising one or more than one lipid derived from a plant.

18. The VLP of claim 17, wherein the modified HA protein comprises plant-specific N-glycans or modified N-glycans.

19. A VLP comprising lipid derived from a plant and modified HA, wherein the modified HA comprise a fully deleted proteolytic loop and plant-specific N-glycans or modified N-glycans.

20. A method of producing a modified HA protein comprising a fully deleted proteolytic loop in a plant or portion of a plant, wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof, the method 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 HA protein, thereby producing the modified HA protein,

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

21. An HA protein produced by the method of claim 20, wherein the HA comprises a fully deleted proteolytic loop and has hemagglutinin (HA) activity.

22. An HA protein comprising a fully deleted proteolytic loop.

23. The HA of claim 22, wherein the HA further comprises plant-specific N-glycans or modified N-glycans

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

25. The method of claim 12, 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 16 or 19 and a pharmaceutically acceptable carrier for use as a medicament for inducing an immune response.

28. A vaccine comprising an effective dose of the VLP of claim 16 or 19 for use in inducing an immune response.

29. Use of the VLP of claim 16 or 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 product yield of an HA from B or H3 influenza strain protein expressed in a plant or portion of a plant wherein the portion of the plant includes plant cells, tissue, plant leaves, stems, fruit, roots or a combination thereof, the method 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. 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) from B or H3 influenza strain, the mutant HA comprising a partially or fully deleted proteolytic loop, 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).