[0076] As described herein, by co-expressing NSP4 along with VP6 and VP4 or VP7 , in a host or host cell, for example a plant, portion of the plant, or a plant cell, RLPs comprising increased levels of VP4, VP7, or both VP4 and VP7 are observed, when compared to the level of VP4 and VP7 in RLPs produced by a second host or host cell for example a plant, portion of a second plant, or second plant cell, that expresses the one or more nucleic acid that encodes VP6 and VP4 or VP7, and does not encode NSP4, the second host or second host cell for example a second plant, the second portion of plant, or the second plant cell, incubated or grown, under the same conditions as the host or host cell for example a plant, portion of the plant, or plant cell.

[0076a] In one aspect, there is provided a method of producing a rotavirus like particle (RLP) in a plant, portion of a plant or plant cell comprising:

a-i) providing the plant, portion of the plant or plant cell comprising one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein, and a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the plant, portion of the plant or plant cell; the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus structural protein VP6, the third nucleotide sequence encoding rotavirus structural protein VP2, and the fourth nucleotide sequence encoding rotavirus structural protein VP7;

or

a-ii) introducing into the plant, portion of the plant or plant cell the one or more nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleotide sequence encoding the second rotavirus protein, the third nucleotide sequence encoding the third rotavirus protein, and the fourth nucleotide sequence encoding the fourth rotavirus protein, the first, second, third and fourth nucleotide sequence being operatively linked to the one or more regulatory region active in the plant, portion of the plant or plant cell; and the first nucleotide sequence encoding the rotavirus protein NSP4, the second nucleotide sequence encoding the rotavirus structural protein VP6, the third nucleotide sequence encoding the rotavirus structural protein VP2, and the fourth nucleotide sequence encoding rotavirus structural protein VP7;

and

b) incubating the plant, portion of the plant or plant cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6, VP2, and VP7 are expressed, thereby producing the RLP, the RLP comprising rotavirus proteins, the rotavirus proteins consisting of rotavirus structural proteins;

and optionally

c) harvesting the plant, portion of the plant or plant cell, and

d) purifying the RLPs from the plant, portion of the plant or plant cell, wherein the RLPs range in size from 70-100 nm.

[0076b] In another aspect, there is provided a use of rotavirus protein NSP4 for increasing incorporation of rotavirus structural protein VP4, VP7, or both VP4 and VP7 on the surface of a rotavirus like particle (RLP) produced in a plant, portion of a plant or plant cells, the RLP comprising rotavirus proteins, the rotavirus proteins consisting of rotavirus structural proteins, wherein the rotavirus structural proteins comprise VP2, VP6 and VP7 or VP2, VP6, VP4 and VP7.

[0077] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078] 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:

Figure 1 shows rotavirus cell entry and replication. When rotavirus enters a cell, VP4 and VP7 are lost, forming a double layered particle (DLP). Transcription of the dsRNA commences resulting in translation of VP2, VP4, VP6 and VP7. Progeny cores with replicase activity are produced in virus factories (also called viroplasms). Late transcription occurs in these progeny cores. At the periphery of virus factories, these core are coated with VP6, forming immature DLPs that bud across the membrane of the endoplasmic reticulum, acquiring a transient lipid membrane which is modified with the ER resident viral glycoproteins NSP4 and VP7; these enveloped particles also contain VP4. As the particles move towards the interior of the ER cisternae, the transient lipid membrane and the nonstructural protein NSP4 are lost, while the virus surface proteins VP4 and VP7 rearrange to form the outermost virus protein layer, yielding mature infectious triple-layered particles (see Swiss Institute of Bioinformatics (ViralZone): viralzone.expasy.org/viralzone/all_by_species/107.html)

[0080] **Figure 2** shows rotavirus-like particle purification by ultracentrifugation on iodixanol density gradient. **Figure 2A** presents the percentage of iodixanol and

WHAT IS CLAIMED IS:

1. A method of producing a rotavirus like particle (RLP) in a plant, portion of a plant or plant cell comprising:

a-i) providing the plant, portion of the plant or plant cell comprising one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein, and a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the plant, portion of the plant or plant cell; the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus structural protein VP6, the third nucleotide sequence encoding rotavirus structural protein VP2, and the fourth nucleotide sequence encoding rotavirus structural protein VP7;

or

a-ii) introducing into the plant, portion of the plant or plant cell the one or more nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleotide sequence encoding the second rotavirus protein, the third nucleotide sequence encoding the third rotavirus protein, and the fourth nucleotide sequence encoding the fourth rotavirus protein, the first, second, third and fourth nucleotide sequence being operatively linked to the one or more regulatory region active in the plant, portion of the plant or plant cell; and the first nucleotide sequence encoding the rotavirus protein NSP4, the second nucleotide sequence encoding the rotavirus structural protein VP6, the third nucleotide sequence encoding the rotavirus structural protein VP2, and the fourth nucleotide sequence encoding rotavirus structural protein VP7;

and

b) incubating the plant, portion of the plant or plant cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6, VP2, and VP7 are expressed, thereby producing the RLP, the RLP comprising rotavirus proteins, the rotavirus proteins consisting of rotavirus structural proteins;

and optionally

c) harvesting the plant, portion of the plant or plant cell, and

d) purifying the RLPs from the plant, portion of the plant or plant cell, wherein the RLPs range in size from 70-100 nm.

2. The method of claim 1, wherein, the one or more nucleic acid further comprises a fifth nucleotide sequence encoding a fifth rotavirus protein, the fifth nucleotide sequence encoding rotavirus structural protein VP4, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the plant, portion of the plant or plant cell; and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

3. The method of claim 2, wherein the one or more nucleic acid comprises:

i) one nucleic acid comprising the first, second, third, fourth and fifth nucleotide sequence encoding the first, second, third, fourth and fifth rotavirus protein;

ii) two nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second, third, fourth and fifth nucleotide sequence encoding the second, third, fourth and fifth rotavirus protein;

iii) two nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein;

iv) three nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein;

v) three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and a third nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein;

vi) four nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and fifth nucleotide sequence encoding the fourth and fifth rotavirus protein;

or

vii) five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth

nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein.

4. The method of claim 1, claim 2 or claim 3, wherein the plant is *Nicotiana benthamiana*.

5. The method of any one of claims 1 to 4, wherein the one or more nucleotide sequence is operatively linked to one or more expression enhancer, CPMV HT, CPMV 160, CPMV 160+ or CPMV HT+.

6. The method of claim 2, wherein in the step of introducing (step a-ii), the one or more nucleic acid comprises:

A) two nucleic acids:

i) a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second, third, fourth and fifth nucleotide sequence encoding the second, third, fourth and fifth rotavirus protein;

or

ii) a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding a third, fourth and fifth rotavirus protein, and

wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid that is introduced into the plant, portion of the plant or plant cell being between 1:0.8 and 1:2;

B) three nucleic acids:

i) the first nucleic acid comprising the first and the second nucleotide sequence encoding the first and the second rotavirus protein, the second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and the fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, or

ii) the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and the third nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein,

wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid and to the amount of the third nucleic that is introduced into the plant, portion of the plant or plant cell is 1:1:1;

C) four nucleic acids,

the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and the fifth nucleotide sequence encoding the fourth and fifth rotavirus protein, wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid, to the amount of the third nucleic and to the amount of the fourth nucleic acid that is introduced into the plant, portion of the plant or plant cell is 1:1:1:1:

or

D) five nucleic acids,

the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid, to the amount of the third nucleic acid, to the amount of the fourth nucleic acid and to the amount of the fifth nucleic acid that is introduced into the plant, portion of the plant or plant cell is 1:1:1:1:1.

7. The method of claim 6, wherein the first rotavirus protein is NSP4, and the second, third, fourth and fifth rotavirus proteins are VP2, VP4, VP6 and VP7.

8. Use of rotavirus protein NSP4 for increasing incorporation of rotavirus structural protein VP4, VP7, or both VP4 and VP7 on the surface of a rotavirus like particle (RLP) produced in a plant, portion of a plant or plant cells, the RLP comprising rotavirus proteins, the rotavirus proteins consisting of rotavirus structural proteins, wherein the rotavirus structural proteins comprise VP2, VP6 and VP7 or VP2, VP6, VP4 and VP7.

9. The method of claim 1, wherein nucleotide sequences encoding the rotavirus structural proteins VP2, VP6 and VP7 are obtained from a rotavirus strain having a genotype selected from one of G1 to G27.