PATENTS

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Title	:	CRISPR-SYSTEMS FOR MODIFYING A TRAIT OF INTEREST IN A PLANT	

AMENDMENT AND RESPONSE

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Commissioner:

In response to the Non-Final Office Action mailed July 2, 2024 (the "Office Action"), please enter and consider the remarks provided herein.

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Reg. No. 54,875

AMENDMENT

In the claims:

Please amend the claims as shown below. This listing of claims will replace all previous listing of claims in the application.

1. (Currently amended) A plant modified to express

(a) a Type VI Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) effector protein, and

(b) ~~a~~ one or more guide RNAs (gRNAs), comprising a first and a second guide RNA, each having complementarity with a target ribonucleotide sequence of one or more plant virus RNA molecules, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence in said plant,~~comprising a guide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, wherein the gRNA is capable of forming a complex with the Type VI CRISPR effector protein in said plant, and wherein each of the gRNA in said complex is capable of binding to at least one target RNA molecule, wherein the at least one target RNA molecule is selected from the group consisting of~~

wherein the ribonucleotide sequence of the first guide RNAs does not comprise a ribonucleotide sequence of a first plant virus chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX),

wherein the ribonucleotide sequence of the second guide RNA comprises a ribonucleotide sequence of a second plant virus chosen from Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV),

sweet potato sunken vein closterovirus (SPSVV), Arabis mosaic virus (ArMV), and Rupestris stem pitting-associated virus (RSPaV),

~~(i) an RNA molecule that is part of a pathogen capable of infecting said plant or transcribed from a DNA molecule of a pathogen capable of infecting said plant;~~

~~(ii) an mRNA that encodes a target for an herbicide; and~~

~~(iii) an mRNA that encodes an enzyme involved in lignin biosynthesis;~~

wherein the Type VI CRISPR effector protein or gRNA, or both, is stably integrated into the plant's genome ~~of the plant~~, and

wherein the Type VI CRISPR effector protein is ~~selected~~ chosen from *Leptotrichia wadei* F0279, *Lachnospiraceae* bacterium MA2020, or *Listeriaceae* bacterium FSL M6-0635,

wherein the first and second plant viruses are different.

2. (Canceled)

3. (Currently amended) The plant of claim 1, wherein the one or more guide RNAs further comprise a third guide RNA, and wherein the ribonucleotide sequence of the third guide RNA comprises a ribonucleotide sequence of a third plant virus.~~comprising a polynucleotide that expresses the Type VI CRISPR effector protein in one or more cells of said plant; wherein said Type VI CRISPR effector protein is an RNA-guided RNase; and said polynucleotide is codon optimized for expression in said one or more plant cells.~~

4. (Currently amended) The plant of claim ~~1~~49, wherein the one or more guide RNAs (gRNAs) in a complex with the Type VI CRISPR can hybridize to the target ribonucleotide sequence of a plant virus RNA molecule~~said~~ which triggers~~complex in the plant is, upon binding to the~~ of the target RNA molecule, capable of

(i) ~~cleaving~~ cleavage of the~~said~~ target ribonucleotide sequence~~RNA molecule~~,

(ii) an increase~~ing the~~ in translation of ~~said~~ the target RNA molecule,

(iii) ~~reducing~~ a reduction in ~~the~~ translation of ~~said~~ the target RNA molecule, or

(iv) a change in ~~modulating the~~ splicing of ~~said~~ the target RNA molecule.

5. (Currently amended) The plant of claim ~~1~~49, wherein the target ribonucleotide sequence of the one or more guide RNAs comprises one or more ~~-~~target ribonucleotide sequences of

(i) an mRNA molecule of a pathogen susceptibility gene;

(ii) an mRNA of a herbicide resistance gene;

(iii) an mRNA of an enzyme required for lignin or volatile organic compound (VOC) biosynthesis~~wherein said binding is selective such that said gRNA binds with higher affinity to said target RNA molecule than to any RNA molecule expressed in said plant~~.

6. (Canceled)

7. (Currently amended) The plant of claim ~~1~~1, wherein said Type VI CRISPR effector protein cleaves ~~said~~ the target ribonucleotide sequence ~~RNA molecule~~ of ~~said~~ the first and second ~~pathogen~~ plant viruses~~in said plant~~ if ~~said~~ the ~~pathogen~~ viruses infect~~s~~ or ~~has~~ have infected said plant.

8. (Currently amended) The plant of claim ~~1~~49, wherein said Type VI CRISPR effector protein is expressed in said plant ~~under the control of~~from an inducible promoter.

9. (Currently amended) The plant of claim ~~1~~49, wherein the one or more guide RNA~~s~~ ~~is~~ are not expressed from a DNA sequence in said plant.

10. (Currently amended) The plant of claim ~~1~~5, wherein the pathogen susceptibility gene is chosen from translation initiation like factors elF4E and elF(iso)4E, Mildew-resistance locus (MLO) proteins, ERF transcription factor gene OSERF922, alcohol dehydrogenase and polyphenol oxidase (PPO).~~whereinthe is both the CRISPR effector protein and the guide RNA are expressed from DNA which is foreign DNA in said plant.~~

11. (Canceled)

12. (Canceled)

13. (Currently amended) The plant of claim ~~1~~ 49, wherein the plant is selected from the group consisting of *Oryza sativa, Solanum tuberosum*, *Solanum lycopersicum*, *Zea mays*, *Triticum spp.*, *Triticum aestivum*, *Sorghum bicolor*, *Dioscorea spp.*, *Musa spp.*, *Manihot esculenta*, *Glycine max*, *Gossypium hirsutum*, *Hordeum vulgare*, *Avena sativa*, *Secale cereale*, *Brassica rapa* and *Brassica napus*.

14. (Currently amended) The plant of claim ~~1~~49, wherein said plant is a cereal plant, a pseudocereal plant, or a vegetable plant.

15. (Currently amended) The plant of claim ~~21~~5, wherein the enzyme required for lignin biosynthesis is chosen from 4-coumarate 3–hydroxylase (C3H), phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), hydroxycinnamoyl transferase (HCT), caffeic acid O-methyltransferase (COMT), caffeoyl COA 3-O- methyltransferase (CCOAOMT), ferulate 5–hydroxylase (F5H), cinnamyl alcohol dehydrogenase (CAD), cinnamoyl CoA-reductase (CCR), 4-coumarate-CoA ligase (4CL), monolignol-lignin-specific glycosyl-transferase, and aldehyde dehydrogenase (ALDH), and the enzyme required for herbicide resistance is chosen from Resistance to Phytophora infestans (RPi) genes, 5-enolpyruvylshikimate-3-phosphate synthase, acetolactate synthase (ALS) and 15-cis-phytoene desaturase chloroplastic/chromoplastic, and wherein the gene required for volatile organic compound (VOC) biosynthesis is chosen from patchoulol synthase, linalool/nerolidol synthase and E-(β) caryophyllene synthase.

~~Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM).~~

16. (Canceled)

17. (Currently amended) ~~The~~ A plant part of the plant of claim ~~1~~49, wherein the plant part

comprises the Type VI CRISPR effector protein and ~~the~~ a ~~polynucleotide~~nucleotide sequence encoding the Type VI CRISPR effector protein.

18. (Currently amended) The plant part of claim 17, wherein said plant part is ~~selected~~ chosen from the group consisting of a plant cell, a somatic embryo, a pollen, gametophyte, ovule, a leave, a seedling, a stem, a callus, a stolon, a microtuber, a shoot, a seed, a fruit ~~and~~ and a spore.

19. (Previously presented) A composition comprising at least two plant parts of claim 18.

20. (Currently amended) A packaging comprising the plant of claim ~~1~~49.

21. (Withdrawn – Currently amended) An engineered, ~~or~~ non-naturally occurring Type VI Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system comprising ~~a first component which is~~

a Type VI CRISPR effector protein, and/or ~~—~~ a ~~poly~~nucleotide sequence encoding ~~the~~ a Type VI CRISPR effector protein ~~of (a1)~~; and

~~a second component which is~~

one or more ~~a~~ guide RNAs (gRNAs) and/or a nucleotide sequence encoding the one or more gRNAs, comprising a first and a second gRNA, each having complementarity with a target ribonucleotide sequence of a target RNA molecule of a plant pathogen, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence~~comprising a guide sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20~~,

wherein the target ribonucleotide sequence of the one or more guide RNAs does not comprise a ribonucleotide sequence of a plant virus chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX),

wherein the nucleotide sequences encoding the Type VI CRISPR effector protein

and the one or more guide RNAs are stably integrated into a genome of a plant, and

wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM). ~~gRNA is capable of forming a complex with the CRISPR effector protein in a plant, and wherein the gRNA in said complex is capable of binding to a target RNA molecule.~~

22. (Canceled)

23. (Withdrawn - Currently amended) The ~~A~~ vector system of claim 25, ~~comprising~~ wherein the ~~polynucleotide~~nucleotide sequence encoding the CRISPR effector protein ~~of claim 21~~ and ~~a~~ the ~~polynucleotide~~nucleotide sequence encoding the one or more gRNAs are on a same vector. ~~selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.~~

24. (Withdrawn - Currently amended) The vector of claim 23, wherein the vector comprises a promoter ~~that is selected from the group consisting of~~chosen from a plant actin promoter, a plant U6 promoter, ~~and~~ or a CaMV 35S promoter.

25. (Withdrawn - Currently amended) A vector system comprising

a first vector comprising ~~the~~ a ~~polynucleotide~~nucleotide sequence encoding ~~the~~ a Type VI CRISPR effector protein, ~~of claim 21~~ and

a second vector comprising ~~the~~ a ~~polynucleotide~~nucleotide sequence encoding ~~the guide sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.~~ one or more guide RNAs (gRNAs), comprising a first and a second gRNA, each having complementarity with a target ribonucleotide sequence of a target RNA molecule of a plant pathogen, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence,

wherein the target ribonucleotide sequence of the one or more guide RNAs does

not comprise a ribonucleotide sequence of a Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX), and

wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM),

26. **(Withdrawn – Currently amended)** The vector system of claim 25, wherein the first and/~~or~~ second vector ~~is~~ are ~~a~~ plant viral vectors.

27. **(Withdrawn – Currently amended)** A delivery system comprising the engineered, non-naturally occurring ~~or engineered~~CRISPR system of claim 21.

28. **(Withdrawn - Currently amended)** A method for producing a modified plant cell ~~wherein the amount of a target RNA or translation of a target RNA is modified~~, comprising delivering the engineered, non-naturally occurring ~~or engineered~~ CRISPR system of claim 21 to the plant cell.

29. **(Withdrawn - Currently amended)** A method for producing a modified plant wherein ~~the amount~~expression of a target RNA molecule or translation of a target RNA molecule is modified, comprising

(a) producing a modified plant cell according to the method of claim 28, and

(b) regenerating a modified plant from said plant cell.

30. **(Canceled)**

31. **(Canceled)**

32. **(Withdrawn - Currently amended)** A method for identifying a CRISPR system that is functional in a plant cell comprising ~~the steps~~

(a) expressing a Type VI CRISPR effector protein ~~candidate~~ in the plant cell;

(b) providing one or more candidate guide RNAs, each having complementarity with a target ribonucleotide sequence of a target RNA molecule, each capable of forming ~~a gRNA comprising a guide sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, to form~~ a complex with the Type VI CRISPR effector protein, and hybridizing to the target ribonucleotide sequence ~~candidate in (a)~~ in said plant cell;

(c) quantifying the target RNA molecule in the plant cell, ~~where said target RNA is an RNA to which the gRNA in said complex can bind in the plant cell~~; and

(d) selecting ~~said~~ a candidate guide RNA if ~~the~~ expression of the ~~quantity~~ target RNA molecule determined in c) is reduced as compared to a plant cell having no CRISPR effector protein and/or no gRNA,

wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM).

33. (Canceled)

34. (Currently amended) The plant of claim ~~1~~5, wherein the pathogen is ~~selected~~ chosen from ~~the group consisting of~~ Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), the RT virus Cauliflower mosaic virus (CaMV), Plum pox virus (PPV), Brome mosaic virus (BMV), Potato virus X (PVX), Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine fleck virus (GFkV), Grapevine leafroll-associated virus-1, -2, and -3, (GLRaV-

1, -2, and -3), Arabis mosaic virus (ArMV), or Rupestris stem pitting-associated virus (RSPaV).

35. (Currently amended) The ~~CRISPR system~~ plant of claim ~~1~~5, ~~wherein the CRISPR system comprises more than one gRNA,~~ wherein the one or more guide RNAs are capable of binding to ~~the~~ a same or ~~to~~ different target RNA molecules.

36. (Withdrawn - Currently amended) A method for improving a CRISPR system in a plant comprising ~~the steps~~

(a) expressing a Type VI CRISPR effector protein in a plant cell;

(b) providing ~~a~~ one or more guide RNAs capable of forming a complex with the CRISPR effector protein ~~comprising a guide sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, to form a complex with the CRISPR effector protein of (a)~~ in said plant cell;

(c) quantifying a target RNA molecule in the plant cell, where said target RNA molecule is an RNA to which the guide RNA in said complex can bind in the plant cell; and

(d) comparing the quantified target RNA in (c) with the target RNA molecule quantified in another plant cell that comprises the target RNA molecule, the CRISPR effector protein and the one or more guide RNAs, except that either the CRISPR effector protein or the one or more guide RNAs ~~has~~ have been modified compared to the CRISPR effector proteins or the one or more guide RNAs ~~versions~~ used in steps (a) and (b); and

(e) determining whether the ~~modification~~ modified CRISPR effector protein or the one or more guide RNAs in (d) results in a different amount of the target RNA molecule ~~amount~~ in said plant cell, wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM).

37. (Withdrawn – Currently amended) A method for treating, preventing or ameliorating a

~~plant~~ disease in a plant comprising delivering to the plant the Type VI CRISPR system of claim 21. ~~a guide RNA (gRNA) comprising a guide sequence to said plant, wherein said guide sequence is directed to a target RNA which is causative of said disease said plant comprises a CRISPR effector protein, and wherein the gRNA is capable of forming a complex with the CRISPR effector protein in said plant, and wherein the guide sequence is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.~~

38. (Withdrawn – Currently amended) The method according to claim 37, wherein said plant disease is caused by a plant pathogen and said one or more guide RNAs ~~is~~ are directed at a target RNA molecule ~~in~~of ~~~~ said plant pathogen.

39. (Withdrawn – Currently amended) The method according to claim 37, wherein said Type VI CRISPR effector protein is expressed in said plant ~~under the control of~~from an inducible promoter, and said method comprises contacting said plant with an agent ~~which induced~~that induces expression of said Type VI CRISPR effector protein.

40. (Withdrawn – Currently amended) The method according to claim 37, wherein said ~~CRISPR effector protein and said guide RNA are administered to said~~ plant pathogen is chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), the RT virus Cauliflower mosaic virus (CaMV), Plum pox virus (PPV), Brome mosaic virus (BMV), Potato virus X (PVX), Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine fleck virus (GFkV), Grapevine leafroll-associated virus-1, -2, and -3, (GLRaV-1, -2, and -3), Arabis mosaic virus (ArMV), or Rupestris stem pitting-associated virus (RSPaV).

41. **(Withdrawn – Currently amended)** The method according to claim ~~36~~37, further comprises administering to said plant a microorganism expressing ~~said~~ Type VI CRISPR effector protein and ~~said~~ one or more of said guide RNAs.

42. **(Canceled)**

43. **(Canceled)**

44. **(Canceled)**

45. **(Canceled)**

46. **(Canceled)**

47. **(Currently amended)** The plant of claim ~~15~~14, wherein said plant is a rice plant.

48. **(Previously presented)** The plant of claim 47, wherein said rice plant is *Oryza sativa.*

49. **(Currently amended)** A plant modified to express

(a) a Type VI Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) effector protein, and

(b) ~~a~~ one or more guide RNAs (gRNAs), each having complementarity with a target ~~target~~ ribonucleotide sequence of ~~a~~ a ~~plant virus~~ target RNA molecule~~chosen from Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Arabis mosaic virus (ArMV), or Rupestris stem pitting associated virus (RSPaV~~, and each ~~wherein the gRNA is~~ capable of forming a complex with the Type VI CRISPR effector protein ~~in said plant, wherein the gRNA in said complex is capable of~~and hybridizing to the target

ribonucleotide sequence in said plant,

wherein the ribonucleotide sequence of the one or more guide RNAs does not comprise a ribonucleotide sequence of a Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) or Potato virus X (PVX-),

wherein the Type VI CRISPR effector protein or gRNA, or both, is stably integrated into the plant's genome ~~of the plant~~, and

wherein the Type VI CRISPR effector protein is selected from *Leptotrichia wadei* F0279, *Lachnospiraceae* bacterium MA2020, or *Listeriaceae* bacterium FSL M6-0635.

50. (Withdrawn - Currently amended) A vector comprising

~~the~~ a ~~polynucleotide~~nucleotide sequence~~s~~ encoding a Type VI CRISPR effector protein, and/or a ~~polynucleotide~~nucleotide sequence encoding ~~a~~ one or more guide RNAs (gRNAs), comprising a first and a second guide RNA, each having complementarity with a target ribonucleotide sequence of one or more plant pathogens, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence in said plant,

wherein the target ribonucleotide sequence of the one or more guide RNAs does not comprise a ribonucleotide sequence of a first plant virus chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX), and

wherein the target ribonucleotide sequence of the second guide RNA comprises a ribonucleotide sequence of ~~having complementarity with a target ribonucleotide sequence of~~ a second plant virus ~~chosen from Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic~~

~~virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Arabis mosaic virus (ArMV), or Rupestris stem pitting-associated virus (RSPaV,~~

wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM),

wherein the first and second plant viruses are different.

~~, wherein the gRNA can form a complex with the CRISPR effector protein in a plant, and wherein the gRNA in said complex can hybridize with the target ribonucleotide sequence~~

51. (Withdrawn - Currently amended) A method for producing an engineered plant comprising delivering

a CRISPR effector protein, and/or a nucleotide sequence encoding the CRISPR effector protein, and

~~a~~ one or more guide RNAs (gRNAs) or a nucleotide sequence encoding the one or more guide RNAs (gRNAs), each gRNA having complementarity with a target ribonucleotide sequence of a plant virus, and each capable of forming a complex with a Type VI CRISPR effector protein, and hybridizing to the target ribonucleotide sequence in said plant,

wherein the target ribonucleotide sequence of the one or more guide RNAs does not comprise a ribonucleotide sequence of a plant virus chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX),

wherein the Type VI CRISPR effector protein is chosen from *Leptotrichia wadei* F0279 C2c2 (Lw2), *Listeriaceae* bacterium FSL M6-0635 or *Lachnospiraceae* bacterium MA2020 C2c2 (LbM)~~having complementarity with a target ribonucleotide sequence of a plant virus which is causative of said disease, wherein said plant virus is chosen from Citrus~~

~~tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Arabis mosaic virus (ArMV), or Rupestris stem pitting-associated virus (RSPaV).~~, ~~and~~

~~a CRISPR effector protein, and/or a polynucleotide encoding the CRISPR effector protein,wherein the gRNA can form a complex with the CRISPR effector protein in a plant, and wherein the gRNA in said complex can hybridize with the target ribonucleotide sequence.~~

52. **(Withdrawn - Currently amended)** A method for treating, preventing, or ameliorating a plant disease in a plant comprising delivering to a plant

a Type VI CRISPR effector protein, and/or a nucleotide sequence encoding the Type VI CRISPR effector protein,

one or more ~~a~~ guide RNAs (gRNAs), and/or a nucleotide sequence encoding the one or more gRNAs, comprising a first and a second guide RNA, each having complementarity with a target ribonucleotide sequence of one or more plant virus RNA molecules, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence in said plant,~~comprising a guide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, wherein the gRNA is capable of forming a complex with the Type VI CRISPR effector protein in said plant, and wherein each of the gRNA in said complex is capable of binding to at least one target RNA molecule, wherein the at least one target RNA molecule is selected from the group consisting of~~

wherein the ribonucleotide sequence of the first guide RNAs does not comprise a ribonucleotide sequence of a first plant virus chosen from Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY),

Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX),

wherein the ribonucleotide sequence of the second guide RNA comprises a ribonucleotide sequence of a second plant virus chosen from Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Arabis mosaic virus (ArMV), and Rupestris stem pitting-associated virus (RSPaV),

~~(i) an RNA molecule that is part of a pathogen capable of infecting said plant or transcribed from a DNA molecule of a pathogen capable of infecting said plant;~~

~~(ii) an mRNA that encodes a target for an herbicide; and~~

~~(iii) an mRNA that encodes an enzyme involved in lignin biosynthesis;~~

wherein the Type VI CRISPR effector protein or gRNA, or both, is stably integrated into the plant's genome ~~of the plant~~, and

wherein the Type VI CRISPR effector protein is ~~selected~~ chosen from *Leptotrichia wadei* F0279, *Lachnospiraceae* bacterium MA2020, or *Listeriaceae* bacterium FSL M6-0635, and

wherein the first and second plant viruses are different.

~~, and a CRISPR effector protein, and/or a polynucleotide encoding the CRISPR effector protein, wherein the gRNA can form a complex with the CRISPR effector protein in a plant, and wherein the gRNA in said complex can hybridize with the target ribonucleotide sequence.~~

REMARKS

Status of the Claims

Claims 1, 3–5, 7–10, 13–15, 17–21, 23–29, 32, 34–41, and 47–52 are pending, with claims 1, 21, 25, 32, 36, and 49–52 being independent. Claims 1, 3–5, 7–10, 13–15, 17–18, 20–21, 23–25, 27–29, 32, 34–41, 47, and 49–52 are amended. Claims 2, 6, 11–12, 16, 22, 30–31, 33, and 42–46 are canceled without prejudice or disclaimer as to the subject matter recited therein. Claims 21, 23–29, 32, 36–41, and 50–52 are withdrawn pursuant to the Restriction Requirement of March 20, 2020.

Written description for "one or more guide RNAs" of independent claims 1, 21, 25, 32, 36, and 49–52, and all claims dependent therefrom can be found, for example, in paragraph [0040] of the corresponding published U.S. Patent Application No. 2019/0352652, which states:

> In a preferred embodiment, the CRISPR system may comprise more than one gRNA for multiplexed use. ***The gRNAs may be different and may target different regions within the same target RNA or within different target RNAs***. In other words, the different gRNAs may differ from one another in their ability to hybridize to a specific target RNA. The different target RNAs may be from the same or from different organ[i]s[]ms, such as, for example, from the same plant pathogen or from ***different plant pathogens***. In a preferred embodiment[,] the CRISPR system comprises different gRNAs specific for different target sequences and the different target sequences are located in multiple regions of a target RNA from a plant pathogen; different target RNAs from the same plant pathogen; or ***different target RNAs from different plant pathogens***. (Emphasis added)
>
> Written description for the *proviso*:
>
> . . .wherein the target ribonucleotide sequence of the first guide RNA does **not** comprise a ribonucleotide sequence of a Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX). (Emphasis added)

can be found, for example, in paragraph [0017] of the corresponding published U.S. Patent Application No. 2019/0352652, which states:

> For example, the virus may be selected from the group comprising ***Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), the RT virus Cauliflower mosaic virus (CaMV), Plum pox***

> ***virus (PPV), Brome mosaic virus (BMV), Potato virus X (PVX),*** Citrus tristeza virus (CTV), Barley yellow dwarf virus (BYDV), Potato leafroll virus (PLRV), Tomato bushy stunt virus (TBSV), rice tungro spherical virus (RTSV), rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), maize rayado fino virus (MRFV), maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), Sweet potato feathery mottle virus (SPFMV), sweet potato sunken vein closterovirus (SPSVV), Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grape vine fleck virus (GFKV), Grapevine leafroll-associated virus-1,-2, and-3, (GLRaV-1,-2, and-3), Arabis mosaic virus (ArMV), or Rupestris stem pitting-associated virus (RSPaV). (Emphasis added)

Thus, the specification teaches ***alternative*** embodiments of the plant virus, which according to the Federal Circuit and the MPEP, is sufficient to fulfill the written description requirement of a negative *proviso* under 35 U.S.C. §112(a).

MPEP 2173.05(i) states:

> Any negative limitation or exclusionary proviso must have basis in the original disclosure. ***If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims***. See *In re Johnson*, 558 F.2d 1008, 1019 (CCPA 1977) ("[the] specification, having described the whole, necessarily described the part remaining."). (Emphasis added)

This position was recently upheld by the Federal Circuit in *Novartis Pharms. Corp. v. Accord Healthcare, Inc.,* 21 F.4th 1362 (Fed. Cir. 2022) in which the Court confirmed:

> "[w]hile a negative limitation need not be recited in the specification in *haec verba*, there generally must be something in the specification that conveys to a skilled artisan that the inventor intended the exclusion, such as a discussion of disadvantages or ***alternatives***." *Id*. (Emphasis added)

Accordingly, the Applicant submits the aforementioned amendments are fully supported by the specification as filed.

Response to Notice of Additional Fees Due

Applicant notes the issuance of a Notice of Additional Fees due on June 6, 2024. The Office confirmed this Notice issued in error because Applicant previously authorized deduction of additional claims fees from the Deposit Account 50-5193.

Change to Priority Date

The Examiner alleges the priority date of the claimed invention as previously presented should be December 8th, 2017, not December 9, 2016, because the provisional applications failed to include a sequence listing, or otherwise disclose SEQ ID NOs: 17-20.

Applicants traverse for the following reasons.

The objection is moot in view of the instant claim amendments.

Nevertheless, the Applicant wishes to enter into the record that SEQ ID NOs: 17-20 are indeed disclosed in TABLE 3 at paragraph [00510] on page 203 of the Provisional Application 62/432,543, filed 12/09/2016 and at paragraph [00616] on page 271 of the Provisional Application 62/567,959, filed 10/04/2017. Furthermore, according to MPEP 201.04, "[a] provisional application disclosing nucleotide and/or amino acid sequences is not required to include a separate sequence listing . .."

Correction of this error is respectfully requested.

Objections to the Claims

In the Office Action, claims 10, 15-17, 34-35 and 49 were objected to because of alleged informalities.

1) **Claim 10, line 2:** the "DNA" and the "foreign DNA" are missing articles.
   The objection is moot in view of the instant claim amendments.
2) **Claim 15 line 2:** the "selected from" is suggested to be —selected from the group consisting . ..
   The objection is moot in view of the cancellation of claim 15.
3) **Claim 16, line 2:** the "guide RNA" is missing an article.
   The objection is moot in view of the instant claim amendments.
4) **Claim 17, line 1:** recites a wrong particle. The "The plant part of the plant of claim 1" is suggested to be changed to --A plant part of the plant of claim 1--.
   The objection is moot in view of the instant claim amendments.
5) **Claim 17, line 2:** recites a wrong particle. The "the polynucleotide encoding the Type VI CRISPR effector protein" is suggested to be changed to --a polynucleotide encoding the Type VI CRISPR effector protein--.

The objection is moot in view of the instant claim amendments.

6) **Claim 34 , line 12:** the "or" should be changed to —and—because of the "the group consisting of" in lines 1-2.

   The objection is moot in view of the instant claim amendments.

7) **Claim 35, line 1:** the "wherein the CRSIPR system" is suggested to —the plant--.

   The objection is moot in view of the instant claim amendments.

8) **Claim 49 (b), line 7:** the "(RSPaV" should be —(RSPaV)--.

   The objection is moot in view of the instant claim amendments.

   Withdrawal of the objections is respectfully requested.

Claim Rejection under 35 U.S.C. § 112(b)

In the Office Action, the Examiner rejected claims 1, 3-5, 7-10, 13-16, 17-20, 34-35, and 47-49 under 35 U.S.C. § 112(b) as allegedly being indefinite. Applicant respectfully submits that the rejection does not apply to the claims as presently presented.

Withdrawal of the rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103

In the Office Action, the Examiner rejected claim 49 as allegedly being obvious over Severinov, et al., (WO2016/205764, filed as PCT/US2016/038258 on 6/17/2016, priority filing date: 6/18/2015) ("Severinov"), in view of Collins, et al., (Self-Cleaving RNA Associated with Rice Yellow Mottle Virus in the Smallest Viroid-like RNA," Virology 241, 269-275, 1998), ("Collins,") and Sanfaçon (Plant Translation Factor and Virus Resistance," Viruses, 7, 3392-3419, 2015), ("Sanfaçon").

According to the Examiner, Severinov teaches all the elements of the claimed invention including a Type VI CRISPR effector C2c2 in a plant cell except the limitation wherein the guide RNA (gRNA) has complementarity with a target ribonucleotide sequence of a particular plant viruses, like rice yellow mottle virus (RYMV), an RNA virus.

The Examiner cites Collins for the teaching of rice yellow mottle virus (RYMV) and Sanfaçon for the teaching of plant resistance to viruses.

The Examiner then alleges it would have been obvious at the time the invention was made to modify the teachings of Severinov in view of Collins and Sanfaçon and target the specific plant viruses of the claimed invention.

Applicant respectfully traverses the rejection.

1) The references cited by the Examiner do not teach every element of the claimed invention

Claim 49 is amended to require the following *proviso*:

> (b) one or more guide RNAs (gRNAs), each having complementarity with a target ribonucleotide sequence of a target RNA molecule, and each capable of forming a complex with the Type VI CRISPR effector protein and hybridizing to the target ribonucleotide sequence in said plant,
>
> wherein the ribonucleotide sequence of the one or more guide RNAs ***does not comprise*** a ribonucleotide sequence of a *Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) or Potato virus X (PVX), ...*(Emphasis added)

At paragraph [00400], Severinov states:

> [00412] The RNA targeting effector protein of the invention can further be used for an activity in plants, in particular against RNA viruses . . . Examples of viruses that can be counteracted in this way include, but are not limited to, *Tobacco mosaic virus (MA), Tomato spotted wilt virus (TSWV). Cucumber mosaic virus (CMV). Potato virus Y (PTO, Cauliflower mosaic virus (CalVIV) (RT virus), Plum pox virus Brome mosaic virus (BMV) and Potato virus X (PVX).* (Emphasis added)

Consequently, the references cited by the Examiner fail to teach or suggest every element of the claimed invention.

2) CRISPR/cas13a's activity in a plant cell is unpredictable

The Examiner's rationale for obviousness appears to hinge on the assumption that the activity of CRISPR/cas13a in a plant cell is predictable. The Applicant respectfully disagrees.

A number of factors can influence Cas13a activity.

In a 2016 publication (Abudayyeh, O. O. *et al.* C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science* **353**, aaf5573 (2016)), the inventors showed C2C2 cleavage depends on local target sequence and RNA secondary structure.

In a January 4, 2018, publication, Aman *et al*. (RNA virus interference via CRISPR/Cas13a system in plants, Genome Biology (2018) 19:1) confirmed the secondary structure of a viral RNA or the presence of RNA binding proteins can influence the accessibility and thus the activity of Cas13a in plant cells. Cas13a activity can cause off target effects and the expression of Cas13a and gRNAs may also vary in different plant tissues.

Thus, the showing of C2C2 cleavage in mammalian HEK293 cells is not predictive of Cas13a activity in plant cells.

3) Severinov teaches away from the claimed invention

The Examiner relies on Severinov (filed as PCT/US2016/038258 on **June 17, 2016)**, Collins and Sanfacon to suggest the modification of plant RNA viruses with C2C2/Cas13a was *prima facie* obvious. However, a person of ordinary skill in the art at the time of Applicant's filing on **October 4, 2017** would have known the ability of C2C2/Cas13 to modify plants was not established until the publication of the instant application.

In the Zaidi review (Engineering plant immunity: Using CRISPR/Cas9 to generate virus resistance. *Front. Plant Sci.* **7**, 1673 (2016)), that was accepted for publication on **24 October 2016** and was cited by the Examiner in the Office Action dated September 24, 2020, the authors state unequivocally that in plants "[c]urrently ***there is no report of directly targeting and cleaving RNA viruses***." (Emphasis added)

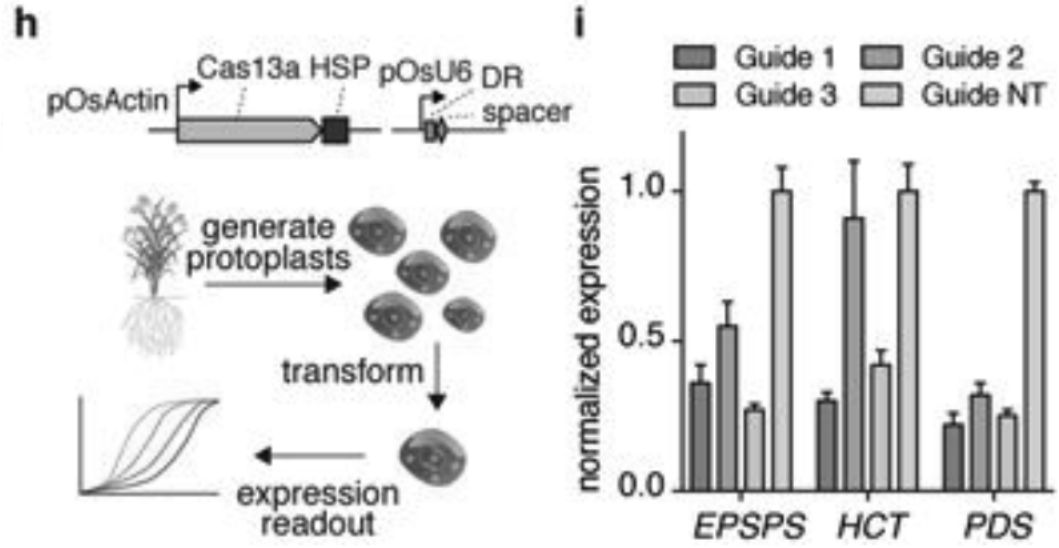
In a paper that published online on **June 2, 2016**, i.e., just 15 days before the filing of the Severinov PCT on June 17, 2016, Abudayyeh *et al.,* including inventors of the present application, (C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science* **353**, aaf5573 (2016)) characterized the Class 2 type VI-A CRISPR-Cas effector C2c2 from the bacterium *Leptotrichia shahii*. The authors showed that C2c2 cleaved target ssRNA in a crRNA sequence-specific manner in *E. coli*. However, the paper also reports the growth rate of these bacteria was substantially reduced. Subsequent experiments demonstrated the binding of the LshC2c2-crRNA complex to its target ribonucleotide sequence triggered ***a non-specific collateral RNAse activity in vitro and in vivo*** (in *E. coli*) suggesting such promiscuous RNA cleavage can cause significant cellular toxicity, resulting in the observed growth rate inhibition, and potentially

cell death. These observations are reported almost verbatim in the Severinov PCT (see Example 7 at paragraph [00995]).

Severinov does **not** provide any evidence that the activity of c2c2 in plants is ***not*** cytotoxic.

Consequently, a person of ordinary skill in the art reading Severinov would therefore have been disincentivized from using Cas13a for targeting plant RNA viruses. **E*ven if CRISPR/Cas13a was allegedly active in plants, studies in vitro and in bacteria as reported by Severinov suggested the effect of Cas13a's collateral RNAse activity on cell viability would be unpredictable and potentially cytotoxic in plants.*** This would be especially true where the modified protoplast must be able to develop into a whole plant.

The instant application and the accompanying paper (Abudayyeh *et al*. RNA targeting with CRISPR-Cas13a. Nature 550, 280 (2017)) that published on **October 12, 2017**, just 8 days after Applicant's filing on October 4, 2017, demonstrate c2c2/Cas13a from *Leptotrichia wadei* (LwaCas13a) can knockdown endogenous transcripts by as much as 78% in rice protoplasts without overt cytotoxicity (see FIGs. 1h and 1i reproduced below).



pOsActin: rice actin promoter; pOsU6: rice U6 promoter; rice 5-enolpyruvylshikimate-3-phosphate synthase (**OsEPSPS**) gene, which is the target of glyphosate-based herbicides, and the rice hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (**OsHCT**) gene, which is necessary for proper plant growth, 15-cis-phytoene desaturase chloroplastic/chromoplastic (**OsPDS**) gene is an essential plant carotenoid biosynthetic enzyme and a prominent target of certain inhibitors, such as norflurazon, acting as bleaching herbicides.

In view of the reports of cytotoxicity in bacteria, these results were surprising, and hence nonobvious.

The requirement of the *proviso* in the claimed invention and the reports on the potential for cytotoxicity caused by Cas13a's nonspecific collateral RNAse activity all teach away from the claimed invention.

According to the Federal Circuit, references that teach away from the claimed invention cannot serve to create a *prima facie* case of obviousness. As explained by the Federal Circuit Court of Appeals in *Spectralytics Inc. v. Cordis Corp*., 649 F.3d 1336, 1343 (Fed. Cir. 2011), "'teaching away' does not require that the prior art foresaw the specific invention that was later made and warned against taking that path." The court stated: "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference or would be led in a direction divergent from the path that was taken by the applicant."

Withdrawal of the rejection is respectfully requested.

No Waiver

All of Applicant's arguments and amendments are without prejudice or disclaimer. Applicant may not have addressed each specific rejection of the independent and dependent claims because Applicant submits that the independent claims are allowable, as discussed above. Applicant has not acquiesced to any such rejection and reserves the right to address the patentability of any additional claim features in the future.

CONCLUSION

Applicant submits the foregoing as a complete response to the Office Action. Applicant submits that this Amendment and Response places the application in condition for allowance and respectfully requests such action. If any issues can be resolved with an Examiner's Amendment or a telephone conference, please contact the Applicant's representative at 912-257-4864.

No additional fees are believed to be due for consideration of this paper. However, the Commissioner is hereby authorized to charge any fee deemed necessary for consideration of this paper, including any extension of time fees or additional claim fees, or to credit any overpayment, to Deposit Account Number 50-5193, reference number BROD-0951US.

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