[0006] However, this important crop is now severely threatened by Fusarium Wilt, also known as Panama Disease, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*).

[0007] Half of the commercial banana crop world-wide and even up to 90% of banana exports in some countries consist of a single group of cultivars, the Cavendish genotypes, which are propagated clonally. Also, most of the commercially traded bananas and many of the locally consumed bananas are clonally cultivated with a single crop in a given area, known as 'monoculture.' The monoculture has been widely practiced by farmers to mass-produce highly demanded crops such as banana, which is easily affected by a range of fungal, viral, bacterial and nematode diseases. Clearly, the current expansion of the Panama disease epidemic is particularly destructive due to the massive monoculture of susceptible Cavendish bananas.

[0008] Cavendish bananas are the fruits of one of a number of banana cultivars belonging to the Cavendish subgroup of the AAA banana cultivar group. The same term is also used to describe the plants on which the bananas grow. They include commercially important cultivars like 'Dwarf Cavendish' (1888) and 'Grand Nain' (the "Chiquita banana"). 'Williams' is a cultivar of the 'Giant Cavendish' type in the Cavendish subgroup. It is one of the most widely grown cultivars in commercial plantations. 'Formosana' is another name for the somaclonal variant 'GCTCV-218,' which has some resistance to Fusarium wilt TR4. Other representative commercial cultivars include 'Masak Hijau' and 'Robusta.' Since the 1950s, these cultivars have been the most internationally traded bananas. They replaced the Gros Michel banana (commonly known as *Kampala* banana in Kenya and *Bogoya* in Uganda) after it was devastated by Panama disease.

[0009] Thus, there is an urgent need in the art for bananas that are resistant to Fusarium Wilt or Panama Disease.

SUMMARY

[0010] The present disclosure solves the aforementioned Panama Disease problem by identifying the underlying genetic architecture giving rise to resistance. Furthermore, the disclosure teaches methodology by which this resistance genetic architecture can be imported into disease susceptible bananas and thus render these bananas disease resistant. The importation of this genetic architecture can take many forms, as elaborated upon herein, including: traditional plant breeding, transgenic genetic engineering, next generation plant breeding (CRISPR, base editing, MAS, etc.), and other methods.

[0010a] In one aspect, the present disclosure refers to a nucleic acid construct comprising a nucleic acid sequence conferring resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 18; or, alternatively, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 21, and SEQ ID NO: 24, and wherein the nucleic acid sequence is operably linked to a promoter capable of driving expression of the nucleic acid sequence.

[0010b] In another aspect, the present disclosure refers to a nucleic acid construct comprising a nucleic acid sequence conferring resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is the nucleic acid sequence of SEQ ID NO: 31.

[0010c] In another aspect, the present disclosure refers to a transgenic plant, plant part, plant cell, or plant tissue culture comprising a nucleic acid construct comprising a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 18; or, alternatively, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 21, and SEQ ID NO: 24, and wherein the nucleic acid sequence is operably linked to a promoter capable of driving expression of the nucleic acid sequence.

[0010d] In another aspect, the present disclosure refers to a method of transforming a plant cell comprising introducing the nucleic acid construct as disclosed herein into a plant cell, whereby the transformed plant cell expresses the nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4.

[0010e] In yet another aspect, the present disclosure refers to a transgenic plant, plant part, plant cell, or plant tissue culture comprising a nucleic acid construct comprising a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is the nucleic acid sequence of SEQ ID NO: 31.

[0011] In some examples as provided herein are isolated nucleic acid molecules comprising nucleic acid sequence SEQ ID NO: 14 coding for susceptibility to *Fusarium oxysporum* race 4 when expressed in a plant, wherein SEQ ID NO: 14 is modified by one, two, three or four nucleic acid substitutions so that the resulting nucleic acid sequence codes for resistance to *Fusarium oxysporum* race 4 when expressed in a plant. In some examples, the isolated nucleic acid molecule includes nucleic acid substitutions comprising replacing a T corresponding to position 148 of SEQ ID NO: 14 with a G (148T>G). In some examples, the isolated nucleic acid molecule includes nucleic acid substitutions comprising replacing a T corresponding to position 323 of SEQ ID NO: 14 with an A (323T>A). In some examples, the isolated nucleic acid molecule includes nucleic acid substitutions comprising replacing a G corresponding to position 344 of SEQ ID NO: 14 with a C (344G>C). In some examples, the isolated nucleic acid molecule includes nucleic acid substitutions comprising replacing an A corresponding to position 347 of SEQ ID NO: 14 with a T (347A>T). In some examples, the isolated nucleic acid molecule includes nucleic acid substitutions comprising replacing a T corresponding to position 323 with an A (323T>A), replacing a G corresponding to position 344 with a C (344G>C), and replacing an A corresponding to position 347 with a T (347A>T), and wherein all positions are based on SEQ ID NO: 14. In some examples the isolated nucleic acid molecule of SEQ ID NO: 14 codes for an amino acid sequence of SEQ ID NO: 15 and wherein the nucleic acid substitutions result in replacing a Leucine corresponding to position 50 of SEQ ID NO: 15 with a Valine (50L>V). In some examples, the isolated nucleic acid molecule includes SEQ ID NO: 14 which codes for an amino acid sequence of SEQ ID NO: 15 and wherein the nucleic acid substitutions result in replacing a Valine corresponding to position 108 of SEQ ID NO: 15 with a Glutamic Acid (108V>E). In some examples, the isolated nucleic acid includes a SEQ ID NO: 14 which codes for an amino acid sequence of SEQ ID NO: 15 and wherein the nucleic acid substitutions result in replacing an Arginine corresponding to position 115 of SEQ ID NO: 15 with a Proline (115R>P). In some examples, the isolated nucleic acid molecule includes a SEQ ID NO: 14 which codes for an amino acid sequence of SEQ ID NO: 15 and wherein the nucleic acid substitutions result in replacing an Aspartic Acid corresponding to position 116 of SEQ ID NO: 15 with a Valine (116D>V). In some examples, the isolated nucleic acid molecule includes a SEQ ID NO: 14 which codes for an amino acid sequence of SEQ ID NO: 15 and wherein the nucleic acid substitutions result in replacing a Valine corresponding to position 108 of SEQ ID

NO: 15 with a Glutamic Acid (108V>E), an Arginine corresponding to position 115 of SEQ ID NO: 15 with a Proline (115R>P), and an Aspartic Acid corresponding to position 116 of SEQ ID NO: 15 with a Valine (116D>V).

[0012] In some examples, the expression occurs in a plant cell, plant tissue, plant cell culture, plant tissue culture, or whole plant. In some examples the expression occurs in a *Musa* cell, tissue, cell culture, tissue culture, or whole plant. In some examples, the expression occurs in a *Musa acuminata* cell, tissue, cell culture, tissue culture or whole plant.

[0013] In some examples, a nucleic acid construct comprises the nucleic acid sequences of the present disclosure which are operably linked to a promoter capable of driving expression of the nucleic acid sequence. In some examples, the promoter is a plant promoter. In some examples, the promoter is a 35S promoter. In some examples, the promoter is coded by SEQ ID NO: 31.

[0014] In some examples, a transformation vector comprises the nucleic acid constructs of the present disclosure.

[0015] In some examples, provided herein is a method of transforming a plant cell comprising introducing the transformation vectors of the present disclosure into a plant cell, whereby the transformed plant cell expresses the nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4. In some examples, the method uses a plant cell which is a *Musa* plant cell. In some examples, the method uses a plant cell which is a *Musa acuminata* plant cell.

[0016] In some examples, the transformed plant tissue is produced from the transformed plant cell. In some examples, a transformed plantlet is produced from the transformed plant tissue. In some examples, a clone is produced from the transformed plantlet. In some examples, the method comprises growing the transformed plantlet or clone of the transformed plantlet into a mature transformed plant. In some examples, the mature transformed plant is a *Musa* plant and the mature transformed *Musa* plant is capable of producing fruit. In some examples, the methods of the present disclosure include further producing clones of the mature transformed *Musa* plant. In some examples, the mature transformed *Musa* plant or clone of the mature transformed *Musa* plant are used in breeding methods.

[0017] In some examples, the present disclosure provides an isolated amino acid molecule comprising an amino acid sequence of SEQ ID NO: 15 coding for a protein that when produced in a plant results in susceptibility to *Fusarium oxysporum* race 4, wherein SEQ ID NO: 15 is modified

by one, two, three or four amino acid substitutions so that it codes for a protein which when produced in a plant results in resistance to *Fusarium oxysporum* race 4. In some examples, the amino acid substitutions comprise replacing a Leucine corresponding to position 50 of SEQ ID NO: 15 with a Valine (50L>V). In some examples, the amino acid substitutions comprise replacing a Valine corresponding to position 108 of SEQ ID NO: 15 with a Glutamic Acid (108V>E). In some examples, the amino acid substitutions comprise replacing an Arginine corresponding to position 115 of SEQ ID NO: 15 with a Proline (115R>P). In some examples, the amino acid substitutions comprise replacing an Aspartic Acid corresponding to position 116 of SEQ ID NO: 15 with a Valine (116D>V). In some examples, the amino acid substitutions comprise replacing a Valine corresponding to position 108 of SEQ ID NO: 15 with a Glutamic Acid (108V>E), an Arginine corresponding to position 115 of SEQ ID NO: 15 with a Proline (115R>P), and an Aspartic Acid corresponding to position 116 of SEQ ID NO: 15 with a Valine (116D>V). In some examples, the protein production occurs in a plant cell, plant tissue, plant cell culture, plant tissue culture, or whole plant. In some examples, the protein production occurs in a *Musa* cell, tissue, cell culture, tissue culture, or whole plant. In some examples, the protein production occurs in a *Musa acuminata* cell, tissue, cell culture, tissue culture or whole plant.

[0018] In some examples, the nucleic acid constructs of the present disclosure comprise a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24 and SEQ ID NO: 29, and wherein the nucleic acid sequence is operably linked to a promoter capable of driving expression of the nucleic acid sequence. In some examples, the promoter is a plant promoter. In some examples, the promoter is a 35S promoter. In some examples, the promoter is coded by SEQ ID NO: 31. In some examples, a transformation vector comprises the nucleic acid constructs of the present disclosure. In some examples, the present disclosure provides methods of transforming a plant cell comprising introducing the transformation vector into a plant cell, whereby the transformed plant cell expresses the nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4. In some examples, the plant cell is a *Musa* plant cell. In some examples, the plant cell is a *Musa acuminata* plant cell. In some examples, the methods further comprise producing transformed plant tissue from the

transformed plant cell. In some examples, a transformed plantlet is produced from the transformed plant tissue. In some examples, the methods further comprise producing a clone of the transformed plantlet. In some examples, the methods further comprise growing the transformed plantlet or clone of the transformed plantlet into a mature transformed plant. In some examples, the mature transformed plant is a *Musa* plant and the mature transformed *Musa* plant is capable of producing fruit. In some examples, the methods further comprise producing clones of the mature transformed *Musa* plant. In some examples, the mature transformed *Musa* plant or clone of the mature transformed *Musa* plant is used in a breeding method.

[0019] In some examples, the disclosure provides a banana breeding method comprising crossing a first *Musa* plant comprising a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 with a second *Musa* plant that is susceptible to *Fusarium oxysporum* race 4 and selecting resultant progeny of the cross based on their resistance to *Fusarium oxysporum* race 4, wherein said nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24 and SEQ ID NO: 29. In some examples, the banana breeding methods further comprise producing clones of the resultant progeny of the cross wherein the clones are selected based on their resistance to *Fusarium oxysporum* race 4. In some examples, the first and second *Musa* plants are from different *Musa* species. In some examples, the first and second *Musa* plants are from the same *Musa* species. In some examples, the first and/or second *Musa* plant is a *Musa acuminata* plant. In some examples, the progeny of the cross that display resistance to *Fusarium oxysporum* race 4 are selected using molecular markers that are designed based on the nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 that is present in the first *Musa* plant used in the cross.

[0020] In some examples, the present disclosure provides methods for obtaining a *Musa acuminata* plant cell with a silenced endogenous gene coding for susceptibility to *Fusarium oxysporum* race 4, the method comprising introducing a double-strand break to at least one site in an endogenous gene coded by SEQ ID NO: 14 to produce a *Musa acuminata* plant cell with a silenced endogenous gene coding for susceptibility to *Fusarium oxysporum* race 4. In some examples, the methods further comprise generating a *Musa acuminata* plant from the *Musa acuminata* plant cell with a silenced endogenous gene coding for susceptibility to *Fusarium oxysporum* race 4 to produce a Musa *acuminata* plant with a silenced endogenous gene coding for

susceptibility to *Fusarium oxysporum* race 4. In some examples, the methods further comprise using the *Musa acuminata* plant with a silenced endogenous gene coding for susceptibility to *Fusarium oxysporum* race 4 in a banana breeding program. In some examples, the methods of the present disclosure utilize a plant cell that is the *Musa acuminata* plant cell with a silenced endogenous gene coding for susceptibility to *Fusarium oxysporum* race 4. In some examples, the double-strand break is induced by a nuclease selected from the group consisting of a TALEN, a meganuclease, a zinc finger nuclease, and a CRISPR-associated nuclease. In some examples, the double-strand break is induced by a CRISPR-associated nuclease and where a guide RNA is provided.

[0021] In some examples, the present disclosure provides methods for producing a plant cell resistant to *Fusarium oxysporum* race 4 comprising introducing at least one genetic modification into one or more endogenous nucleic acid sequences coding for susceptibility to *Fusarium oxysporum* race 4, wherein the genetic modification confers resistance to *Fusarium oxysporum* race 4 to the plant cell. In some examples, at least one genetic modification is introduced by a TALEN, a meganuclease, a zinc finger nuclease or a CRISPR-associated nuclease. In some examples, the at least one genetic modification is introduced by a CRISPR-associated nuclease and an associated guide RNA. In some examples, the at least one genetic modification is selected from the list consisting of replacing a T corresponding to position 148 of SEQ ID NO: 14 with a G (148T>G), replacing a T corresponding to position 323 of SEQ ID NO: 14 with an A (323T>A), replacing a G corresponding to position 344 of SEQ ID NO: 14 with a C (344G>C), and replacing an A corresponding to position 347 of SEQ ID NO: 14 with a T (347A>T). In some examples, the at least one genetic modification results in a change in an amino acid selected from the group consisting of replacing a Leucine corresponding to position 50 of SEQ ID NO: 15 with a Valine (50L>V), replacing a Valine corresponding to position 108 of SEQ ID NO: 15 with a Glutamic Acid (108V>E), replacing an Arginine corresponding to position 115 of SEQ ID NO: 15 with a Proline (115R>P), and replacing an Aspartic Acid corresponding to position 116 of SEQ ID NO: 15 with a Valine (116D>V). In some examples, the plant cell is a *Musa* plant cell. In some examples, the plant cell is a *Musa acuminata* plant cell. In some examples, the methods further comprise producing transformed plant tissue from the transformed plant cell. In some examples, the methods further comprise producing a transformed plantlet from the transformed plant tissue. In some examples, the methods further comprise producing a clone

of the transformed plantlet. In some examples, the methods further comprise growing the transformed plantlet or clone of the transformed plantlet into a mature transformed plant. In some examples, the mature transformed plant is a *Musa* plant and the mature transformed *Musa* plant is capable of producing fruit. In some examples, the methods further comprise producing clones of the mature transformed *Musa* plant. In some examples, the methods further comprise using the mature transformed *Musa* plant or clone of the mature transformed *Musa* plant in a breeding method.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 illustrates banana *FusR1* coding sequences aligned. Initiation (start) and termination (stop) codons are underlined.

[0023] *FusR1* nucleotide base substitutions between *Musa* species are **bolded**. Substitutions that code for replacement amino acid residues (*i.e.*, are nonsynonymous) are shown in **bolded** font with an asterisk (*); silent substitutions are shown in **bolded** font with a dot (•). The first 96 bases code for a leader peptide (shown in lower case) that is cleaved from the mature protein. This is known to be common for Bowman-Birk proteins (Barbosa et al., 2007). Inventor confirmed the extent of the leader sequence using two different bioinformatics tools, SignalP-5.0 (Armenteros et al, 2019), and PrediSi (Hiller et al., 2004), which both identified the same leader peptide. Using the bioinformatics tool DeepLoc-1.0 (Armenteros et al., 2017), inventor then established that the mature FUSR1 protein is localized to the cell cytoplasm (likelihood of 0.9732).

[0024] Bases shown in UPPER CASE code for the mature protein.

[0025] A missing base, shown as a dash (-), in the *M. balbisiana FusR1* sequence results in a premature stop codon (shown in *italicized, underlined lower case*), relative to the other *FusR1* sequences. As described in the text, *FusR1* mRNAs from all *M. balbisiana* accessions inventor examined have an unspliced (*i.e.*, expressed) intron; for clarity in the Figure and to focus on sequence similarities/differences in *FusR1* coding sequences from different banana species, the intron sequence has been removed here from *M. balbisiana*, even though inventor has not seen that happen. Thus SEQ ID NO: 27 is a 'hypothetical" coding sequence.

[0026] The *M. itinerans* FusR1 sequence was obtained from multiple accessions (ITC1526, ITC1571, and PT-BA-00223), all of which are FW-resistant. The *M. acuminata FusR1* sequence labeled 'FW-resistant' was obtained from multiple FW-resistant accessions, including ITC0896

CLAIMS

What is claimed is:

1. A nucleic acid construct comprising a nucleic acid sequence conferring resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 18; or, alternatively, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 21, and SEQ ID NO: 24, and wherein the nucleic acid sequence is operably linked to a promoter capable of driving expression of the nucleic acid sequence.

2. The nucleic acid construct of claim 1, wherein the promoter is a plant promoter.

3. The nucleic acid construct of claim 1, wherein the promoter is a 35S promoter.

4. A nucleic acid construct comprising a nucleic acid sequence conferring resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is the nucleic acid sequence of SEQ ID NO: 31.

5. A transgenic plant, plant part, plant cell, or plant tissue culture comprising a nucleic acid construct comprising a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 18; or, alternatively, wherein said nucleic acid sequence is selected from the group consisting of a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 21, and SEQ ID NO: 24, and wherein the nucleic acid sequence is operably linked to a promoter capable of driving expression of the nucleic acid sequence.

6. A method of transforming a plant cell comprising introducing the nucleic acid construct of claim 1 into a plant cell, whereby the transformed plant cell expresses the nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4.

7. The method of claim 6, wherein the plant cell is a *Musa* plant cell.

8. The method of claim 6, wherein the plant cell is a *Musa acuminata* plant cell.

9. The method of claim 6, further comprising producing a transformed plant tissue from the transformed plant cell.

10. The method of claim 9, further comprising producing a transformed plantlet from the transformed plant tissue.

11. The method of claim 10, further comprising producing a clone of the transformed plantlet.

12. The method of claim 10, further comprising growing the transformed plantlet into a mature transformed plant.

13. The method of claim 12, wherein the mature transformed plant is a *Musa* plant and the mature transformed *Musa* plant is capable of producing fruit.

14. The method of claim 13, further comprising producing clones of the mature transformed *Musa* plant.

15. The transgenic plant of claim 5, wherein the promoter is a plant promoter.

16. The transgenic plant of claim 5, wherein the promoter is a 35S promoter.

17. A transgenic plant, plant part, plant cell, or plant tissue culture comprising a nucleic acid construct comprising a nucleic acid sequence coding for resistance to *Fusarium oxysporum* race 4 when expressed in a plant, wherein said nucleic acid sequence is the nucleic acid sequence of SEQ ID NO: 31.