[0005] Visual observation of the symptoms is the current detection method of the disease. However, little can be done once the physical symptoms are observed, as the damage to the plant has already been done. It should be noted that though BSR is often associated with *G. boninense,* it may be possible for other Ganoderma species to also have pathogenic ability in oil palm, and it is also possible for different varieties of *G. boninense* to have varying degrees of pathogenicity.

[0006] Therefore, there is a need for a means of detecting the presence of pathogenic Ganoderma, in particular pathogenic strains of *G. boninense*, at an early stage of infection to enable early treatment of an affected plant, and to restrict spread of the disease. This enables planters to better manage the spread of disease and consequently reduce loss of fresh fruit bunch yield and plants before their full commercial value is realized.

[0007] There is also a need to detect the presence of pathogenic Ganoderma species, in particular pathogenic strains of *G. boninense,* directly from an environmental sample, such as but not limited to, a soil sample.

[0008] However, in the current state of the art, there are only methods to identify the Ganoderma isolate or strain but there are no known methods for identifying and differentiating between pathogenic and non-pathogenic Ganoderma isolates using any available biological markers.

[0009] Therefore, there is further need to provide molecular markers that are capable of differentiating between pathogenic and non-pathogenic Ganoderma species to enable detecting and identifying a pathogenic Ganoderma species.

SUMMARY

[0010] Disclosed is a method for determining the pathogenicity of a Ganoderma species, wherein the method comprises the step of detecting the presence or absence of one or more sequences selected from a group consisting of SEQ ID NOs: 1 to 123 or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 123 or a variant thereof indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 123 or a variant thereof indicates that the Ganoderma species is non-pathogenic.

[0010a] In one aspect, there is provided a method for determining the pathogenicity of Ganoderma species, wherein the method comprises the step of detecting the presence or absence of one or more sequences selected from a group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof, indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is not pathogenic.

[0011] Advantageously, the method allows for direct identification of Ganoderma, and its pathogenicity from a sample, such as a soil sample. This translates into a shorter time for analyzing a sample, such as a soil sample, as the method eliminates the need for isolation of Ganoderma strains before analysis can be performed.

[0012] Disclosed is a method for detecting presence of a pathogenic Ganoderma species in a sample, comprising the steps of: (a) extracting DNA from the sample; (b) subjecting the DNA to sequencing to determine sequences of the extracted DNA; (c) comparing the DNA sequences determined from step (b) to a reference DNA sequence selected from a group consisting of SEQ ID NOs: 1 to 123 or a variant thereof; and (d) determining the absence or presence of one or more sequences selected from a group consisting of SEQ ID NOs: 1 to 123 or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 123 or a variant thereof indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 123 or a variant thereof indicates that the Ganoderma species is non-pathogenic.

[0012a] In another aspect, there is provided a method for detecting presence of pathogenic Ganoderma in a sample, comprising the steps of: (a) extracting DNA from the

sample; (b) subjecting the DNA to sequencing to determine the sequences of the extracted DNA; (c) comparing the DNA sequences determined from step (b) to a reference DNA sequence selected from a group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof; and (d) determining the absence or presence of one or more sequences selected from a group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is not pathogenic.

[0013] In another embodiment, there is provided a method for identifying a nucleic acid sequence containing a marker for Ganoderma pathogenesis comprising the steps of: (a) extracting DNA from a group of pathogenic and non-pathogenic Ganoderma strains; (b) preparing whole-genome sequencing libraries of each of the pathogenic and non-pathogenic Ganoderma strain using the extracted DNA of step (a); (c) sequencing the libraries from step (b) to form a plurality of sequencing reads; (d) assembling the sequencing reads from step (c) to generate a backbone sequence for the pathogenic Ganoderma strain and a backbone sequence for the non-pathogenic Ganoderma strain; (e) mapping the sequencing reads from step (c) from each strain to the backbone sequence of step (d) to perform comparative genomics to identify variant sequence(s); (f) designing and preparing primers from the variant sequence(s) from step (e); (g) applying the primers prepared from step (f) to DNA extracted from the pathogenic and non-pathogenic Ganoderma strains for DNA amplification and sequencing; and (h) classifying the variant sequence(s) into those associated with the

pathogenic Ganoderma strains and those associated with the non-pathogenic Ganoderma strains.

[0014] Disclosed is an oligonucleotide primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 124 to 369.

[0014a] In another aspect, there is provided an oligonucleotide primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 288-289, 178-179, 336-337, 198-199, 214-215, 234-235, 256-257, 312-313, 318-319, 344-345, 172-173, 250-251, 284-285, and 308-309.

[0015] Advantageously, the methods of the present disclosure are able to determine the presence of pathogenic Ganoderma in a sample, such as a soil sample, with confidence level of up to about 100%. For example, using one of the markers (SEQ ID NO: 83) isolated by the methods of the present disclosure, it is possible to determine the presence of pathogenic Ganoderma at about 100% confidence level.

[0016] Advantageously, the methods of the present disclosure are also highly sensitive in detecting DNA from pathogenic Ganoderma in a sample. For example, one of the markers identified by the present disclosure (SEQ ID NO: 25) is capable of detecting DNA from pathogenic Ganoderma in a soil sample that is present in an amount as low as 10^{-2} ng.

[0017] Disclosed is a kit for use in the methods defined herein, wherein the kit comprises: (a) one or more primer(s) selected from the group consisting of SEQ ID NOs: 124 to 369 for determining the presence or absence of one or more sequences selected from the group consisting of SEQ ID NOs: 1 to 123; (b) one or more container(s) suitable for sample collection; (c) one or more container(s) suitable for sample processing; (d) one or more reagents for sample processing; (e) one or more buffer(s); (f) one or more PCR reagent(s); and (g) instructions for use in accordance with the methods described herein.

[0017a] In another aspect, there is provided a kit for use in the methods defined herein, wherein the kit comprises: (a) one or more primer(s) selected from the group consisting of SEQ ID NOs: 288-289, 178-179, 336-337, 198-199, 214-215, 234-235, 256-257, 312-313, 318-319, 344-345, 172-173, 250-251, 284-285, and 308-309 for determining the presence or absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93; (b) one or more container(s) suitable for sample collection; (c) one or more container(s) suitable for sample processing; (d) one or more reagents for sample processing; (e) one or more buffer(s); (f) one or more PCR reagent(s); and (g) instructions for use in accordance with the methods described herein.

DEFINITION OF TERMS

[0018] The following words and terms used herein shall have the meaning indicated:

[0019] The term "molecular marker" or "marker", as used therein, refers to a sequence of genetic information that is associated with a location in the genome, a trait associated with the marker or a DNA-based feature, such as a repeat region or identifiable and conserved DNA region. A molecular marker may refer to the entirety of the sequence, or fragments of that sequence, or a region of flanking genetic region, that will allow for the identification of the molecular marker. Ideally, a molecular marker is polymorphic in nature so that it can be used to differentiate between the different features that they are related to. In the present disclosure, the feature related to the marker is pathogenicity of a Ganoderma isolate.

[0020] In one example, molecular markers may comprise but are not limited to single nucleotide polymorphisms (SNPs), simple sequence repeats (SSR), microsatellites, insertion-deletion of bases (INDELs), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism

CLAIMS

1. A method for determining the pathogenicity of Ganoderma species, wherein the method comprises the step of detecting the presence or absence of one or more sequences selected from a group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93 or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof, indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is not pathogenic.

2. The method of any one of the preceding claims, wherein the one or more sequences, or a variant thereof, comprises a single nucleotide polymorphism (SNP).

3. The method of claim 2, wherein the one or more sequences, or a variant thereof, comprises a SNP selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93.

4. The method of any one of the preceding claims, wherein the variant has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to one of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO:

95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93.

5. The method of any one of the preceding claims, wherein the detecting is conducted on a sample potentially containing or suspected of containing said Ganoderma species.

6. The method of the method of claim 5, wherein the sample is selected from the group consisting of soil, root, shoot, stem, bark, leaves, fruits, seed and an isolated microbial strain.

7. The method of any one of the preceding claims, wherein the detecting is conducted using a technique selected from the group consisting of sequencing, hybridization-based methods, enzyme-based methods, PCR-based methods, and post-amplification methods.

8. The method of claim 7, wherein the PCR is conducted using at least one set of primers selected from the group consisting of SEQ ID NOs: 288-289, 178-179, 336-337, 198-199, 214-215, 234-235, 256-257, 312-313, 318-319, 344-345, 172-173, 250-251, 284-285, and 308-309.

9. The method of any one of claims 5 to 8, comprising the steps of:

(a) extracting DNA from the sample;

(b) subjecting the DNA to sequencing to determine sequences of the extracted DNA;

(c) comparing the DNA sequences determined from step (b) to a reference DNA sequence selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof;

(d) determining the absence or presence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof.

10. The method of any one of the preceding claims, wherein the Ganoderma species is pathogenic in a plant selected from the group consisting of crops, wherein the crop is optionally selected from a group consisting of oil palm, *Mucuna bracteata*, coconut, betel nut, sugar palm, sago palm, rubber tree, and *Acacia mangium*; forest trees; and ornamental plants, optionally selected from *Chrysalidocarpus lutescens*.

11. The method of any one of the preceding claims, wherein the Ganoderma is selected from the group consisting of a *Ganoderma boninense*, *Ganoderma orbiforme*, *Ganoderma sp.*, *Ganoderma orbiforme*, *Ganoderma fornicatum*, *Ganoderma lucidum*, *Ganoderma weberianum*, *Ganoderma williamsianum*, *Ganoderma steyaertanum* and *Ganoderma applanatum*.

12. The method of claim 11, wherein the *Ganoderma sp.* is selected from the group consisting of *Ganoderma sp.* BL-6 18S (accession no. JN400509.1), *Ganoderma sp.* BL-9 18S (accession no. JN400510.1), *Ganoderma sp.* BL-94 18S (accession no. JN400511.1), *Ganoderma sp.* BP-16 18S (accession no. JN400513.1), *Ganoderma sp.* BRIUMSa (accession no. JN234427.1), *Ganoderma sp.* BRIUMSb (accession no. JN234428.1), *Ganoderma sp.* BRIUMSc (accession no. JN234429.1), *Ganoderma sp.* C16452 (accession no. EU239386.1), *Ganoderma sp.* MT-1 (accession no. AY220543.1), *Ganoderma sp.* G31 (accession no. KR093030.1), and *Ganoderma sp.* MEL 2382607 (accession no. KP012934.1)

13. The method of claim 11, wherein the Ganoderma is a *Ganoderma boninense, Ganoderma steyaertanum* or *Ganoderma orbiforme* species.

14. A method for detecting presence of pathogenic Ganoderma in a sample, comprising the steps of:

(a) extracting DNA from the sample;

(b) subjecting the DNA to sequencing to determine the sequences of the extracted DNA;

(c) comparing the DNA sequences determined from step (b) to a reference DNA sequence selected from a group consisting of SEQ ID NOs: SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID

NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof; and

(d) determining the absence or presence of one or more sequences selected from a group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof, wherein the presence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is pathogenic, and the absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO: 67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93, or a variant thereof indicates that the Ganoderma species is not pathogenic.

15. The method of claim 14, wherein the sample is selected from the group consisting of root, shoot, stem, bark, leaves, fruits, seed and an isolated microbial strain.

16. An oligonucleotide primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 288-289, 178-179, 336-337, 198-199, 214-215, 234-235, 256-257, 312-313, 318-319, 344-345, 172-173, 250-251, 284-285, and 308-309.

17. A kit for use in the methods of any one of claims 1 to 15, wherein the kit comprises:

(a) one or more primer(s) selected from the group consisting of SEQ ID NOs: 288-289, 178-179, 336-337, 198-199, 214-215, 234-235, 256-257, 312-313, 318-319, 344-345, 172-173, 250-251, 284-285, and 308-309 for determining the presence or absence of one or more sequences selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 28, SEQ ID NO: 107, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 56, SEQ ID NO:

67, SEQ ID NO: 95, SEQ ID NO: 98, SEQ ID NO: 111, SEQ ID NO: 25, SEQ ID NO: 64, SEQ ID NO: 81, and SEQ ID NO: 93;

(b) one or more container(s) suitable for sample collection;

(c) one or more container(s) suitable for sample processing;

(d) one or more reagents for sample processing;

(e) one or more buffer(s);

(f) one or more PCR reagent(s); and

(g) instructions for use in accordance with the methods any one of claims 1 to 15.