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(54) Title: METHODS FOR OBTAINING OIL PALM PLANTS THAT HAVE TOLERANCE TO *GANODERMA BONINENSE*

(57) Abstract: Methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, are disclosed. A treatment group and a control group of oil palm plants are cultivated together, roots of the treatment group, but not the control group, are contacted with *Ganodermo boninense*, and the groups are further cultivated together. Levels of transcripts of a cell-wall-related-protein gene, a transcription factor gene, a disease-resistance protein gene, and a jasmonic-acid-signalling gene are determined. Toleranceto *Ganoderma boninense* is predicted based on the levels of the transcripts being higher for treatment versus control. If the treatment group is predicted to have tolerance, then the treatment group is transplanted for cultivation. If not, then the treatment group is culled.

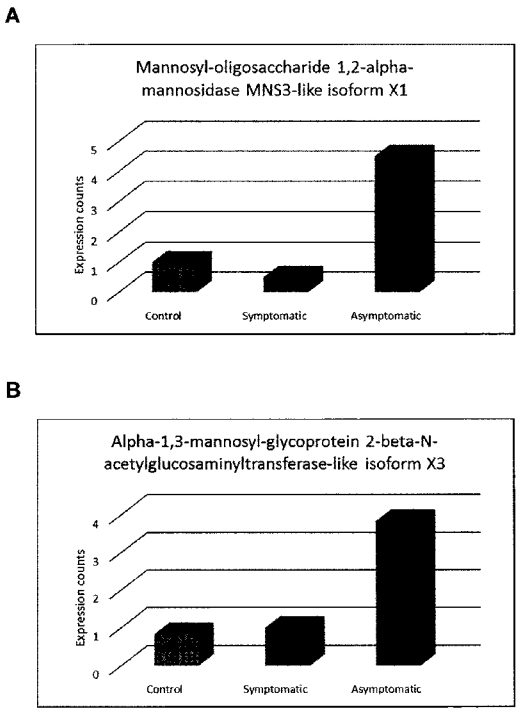

FIG. 1

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Methods for Obtaining Oil Palm Plants that Have Tolerance to *Ganoderma boninense*

Technical Field

This application relates to methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, and more particularly to methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, comprising (i) cultivating oil palm plants of a treatment group and oil palm plants of a control group together, the oil palm plants of the treatment group and the oil palm plants of the control group having been derived from a common lineage; (ii) contacting roots of the oil palm plants of the treatment group with *Ganoderma boninense*, while not contacting roots of the oil palm plants of the control group with *Ganoderma boninense*; (iii) further cultivating the oil palm plants of the treatment group and the oil palm plants of the control group together; (iv) determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, levels of transcripts of a cell-wall-related-protein gene, a transcription factor gene, a disease-resistance-protein gene, and a jasmonic-acid-signalling gene; (v) predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on the levels of the transcripts being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group; and (vi) if the oil palm plants of the treatment group are thereby predicted to have tolerance to *Ganoderma boninense*, then transplanting, for cultivation for agricultural use, oil palm plants remaining from the treatment group, whereas if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then culling the oil palm plants remaining from the treatment group.

Background Art

The African oil palm *Elaeis guineensis* Jacq. is an important oil-food crop. Oil palm plants are monoecious, i.e. single plants produce both male and female flowers, and are characterized by alternating series of male and female inflorescences. The male inflorescence is made up of numerous spikelets, and can bear well over 100,000 flowers. Oil palm is naturally cross-pollinated by insects and wind. The female inflorescence is a spadix which contains several thousands of flowers borne on thorny spikelets. A bunch carries 500 to 4,000 fruits. The oil palm fruit is a sessile drupe that is spherical to ovoid or elongated in shape and is composed of an exocarp, a mesocarp containing palm oil, and an endocarp surrounding a kernel.

Oil palm is important both because of its high yield and because of the high quality of its oil. Regarding yield, oil palm is the highest yielding oil-food crop, with a recent average yield of 3.67 tonnes per hectare per year and with best progenies known to produce about 10 tonnes per hectare per year. Oil palm is also the most efficient plant known for harnessing the

energy of sunlight for producing oil. Regarding quality, oil palm is cultivated for both palm oil, which is produced in the mesocarp, and palm kernel oil, which is produced in the kernel. Palm oil in particular is a balanced oil, having almost equal proportions of saturated fatty acids (≈ 55% including 45% of palmitic acid) and unsaturated fatty acids (≈ 45%), and it includes beta carotene. The palm kernel oil is more saturated than the mesocarp oil. Both are low in free fatty acids. The current combined output of palm oil and palm kernel oil is about 50 million tonnes per year, and demand is expected to increase substantially in the future with increasing global population and per capita consumption of oils and fats.

Basal stem rot is a disease of oil palm plants that is becoming an increasingly common problem among oil palm plantations in Malaysia and Indonesia and that threatens to limit productivity of the plantations. Basal stem rot is caused by a fungal pathogen, *Ganoderma boninense*, which infects basal stem and roots of oil palm plants and restricts flow of water and nutrients to upper portions of the oil palm plants. Infection is believed to be caused by contact of the pathogen with roots. Earliest visual symptoms include wilting of fronds and malnutrition. Diagnostic symptoms for confirmation of basal stem rot include detection of disease lesion (also termed dry rot) at the base of oil palm plants and the appearance of fruiting bodies. Basal stem rot can result in oil palm plants that have small canopies, are impaired with respect to fruit production, and are subject to being toppled by wind.

Basal stem rot threatens to reduce fresh fruit bunch yield in Malaysia and Indonesia, as shown in a recent case study at Johor estate (Roslan & Idris, 2012, Oil Palm Industry Economic Journal, 12(1), 24-30). Affected oil palm plants can die within six to twelves months after the development of symptoms, and up to 80% of plantings may die within the first half of the otherwise expected economic life of the oil palm plants. Recycled fields from coconuts planting and fields left with infected stumps have created a mass of inoculum of *Ganoderma boninense* in the soil. New plantings of oil palms in these fields will be subjected to infection, and thus these fields will have shorter cycles in the next generation plantings. Malaysia adopted a zero-burning policy in its land clearing activities for oil palm plantations as early as 1993, and lands with *Ganoderma boninense* infestation have been treated by other methods as alternatives to burning, such as removal of stumps, pulverizing of felled oil palm trunks, and harrowing and ploughing of fields (Mohd Noor, 2003, Oil Palm Industry Economic Journal, 3(1), 16-32). Unfortunately, the epidemiology of the disease is poorly understood, thus making early detection and chemical treatment harder. Although some methods of early detection have been reported, these methods at best will result in an earlier process for re-planting. Thus, the importance of identifying and propagating oil palms with resistance to infection by *Ganoderma boninense* has become a priority. Resistance of oil palm plants to infection by *Ganoderma boninense* may be achieved by crossing mainly germplasm or semi-germplasm material, but the resulting oil palm plants are of low interest due to low yield performance. Screening of

resistance materials has also reached bottle necks where introgression of these materials to current high oil producing lines needs more selection cycles and progeny testing.

Transcriptomic approaches have been undertaken by researchers who have been trying to unveil mechanisms underlying distinctions between resistant lines and susceptible lines of other plants of interest. Recent examples include studies in wheat (Xin et al., 2012, Genomics, Proteomics & Bioinformatics, 10, 94-106), chestnut (Barakat et al., 2012, BMC Plant Biology, 12, 38), soybean (Sá et al., 2012, Genetics and Molecular Biology, 35, 272-282), sorghum, (Yazawa et al., 2013, PloS One, 8, e62460) and *Arabidopsis* (Zhu et al., 2013, Gene, 512, 259-266). This method had been widely used to gather information of gene expression profiles, as well as to observe responses of plants during the process of infection. Transcriptome profiling has been effectively manifested by the improved technology of RNA-sequencing that uses deep sequencing as a platform. The improved technology of deep sequencing nowadays has enabled researchers to gather more information with a lower cost (Wang et al., 2009, Genetics 10, 57-63).

A recent study on oil palm plants examined transcriptional responses of oil palm roots treated with a *Ganoderma boninense* using a cDNA microarray approach (Tee et al., 2012, Tree Genetics & Genomes, DOI 10.1007/s11295-01200559-7). According to Tee, a total of 61 transcripts, from among 3,748 transcripts examined, were found to be significantly up- or down-regulated in oil palm roots infected with *Ganoderma boninense* at three weeks and six weeks post inoculation, compared to uninfected roots. The regulated transcripts relate to response to *Ganoderma boninense*, though, not to tolerance. Thus, it remains to be determined whether and to what extent methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, can be developed.

Accordingly, a need exists to improve oil palm through improved methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use.

Disclosure of Invention

A method for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, is disclosed. The method comprises a step of (i) cultivating oil palm plants of a treatment group and oil palm plants of a control group together, the oil palm plants of the treatment group and the oil palm plants of the control group having been derived from a common lineage. The method also comprises a step of (ii) contacting roots of the oil palm plants of the treatment group with *Ganoderma boninense*, while not contacting roots of the oil palm plants of the control group with *Ganoderma boninense*. The method also comprises a step of (iii) further cultivating the oil palm plants of the treatment group and the oil palm plants of the control group together. The method also comprises a step of (iv)

determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group: (1) a level of a transcript of a cell-wall-related-protein gene comprising one or more of: (a) a level of a transcript 1 of a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene, or (b) a level of a transcript 2 of an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene, (2) a level of a transcript of a transcription factor gene comprising one or more of: (a) a level of a transcript 3 of a probable WRKY transcription factor 40 gene, or (b) a level of a transcript 4 of a probable WRKY transcription factor 53 gene, (3) a level of a transcript of a disease-resistance-protein gene comprising one or more of: (a) a level of a transcript 5 of a dirigent protein 5-like gene, (b) a level of a transcript 6 of a G-type lectin S-receptor-like serine/threonine-protein kinase gene, (c) a level of a transcript 7 of a putative disease resistance protein RGA3 gene, or (d) a level of a transcript 8 of a polyamine oxidase gene, and (4) a level of a transcript of a jasmonic-acid-signalling gene comprising one or more of: (a) a level of a transcript 9 of a putative lipoxygenase 5 gene, or (b) a level of a transcript 10 of a 12-oxophytodienoate reductase 1-like gene. The method also comprises a step of (v) predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher, (2) at least one of the levels of the transcripts 3 or 4 being higher, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher, and (4) at least one of the levels of the transcripts 9 or 10 being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group. The method also comprises a step of (vi) if the oil palm plants of the treatment group are thereby predicted to have tolerance to *Ganoderma boninense*, then transplanting, for cultivation for agricultural use, oil palm plants remaining from the treatment group, whereas if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then culling the oil palm plants remaining from the treatment group.

Brief Description of Drawings

FIG. 1 shows expression counts of a bioinformatics analysis, indicating relative levels of transcripts, for cell-wall-related-protein genes (A) mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene of SEQ ID NO: 1, and (B) alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene of SEQ ID NO: 2, based on sampling roots of 12-month-old oil palm plants, at six months post inoculation with *Ganoderma boninense*, for control plants (n = 3), symptomatic plants (n = 3), and asymptomatic plants (n = 3).

FIG. 2 shows expression counts of a bioinformatics analysis, indicating relative levels of transcripts, for transcription factor genes (A) probable WRKY transcription factor 40 gene of SEQ ID NO: 3, and (B) probable WRKY transcription factor 53 gene of SEQ ID NO: 4, based

on sampling roots of 12-month-old oil palm plants, at six months post inoculation with *Ganoderma boninense*, for control plants (n = 3), symptomatic plants (n = 3), and asymptomatic plants (n = 3).

FIG. 3 shows expression counts of a bioinformatics analysis, indicating relative levels of transcripts, for disease-resistance-protein genes (A) dirigent protein 5-like gene of SEQ ID NO: 5, and (B) G-type lectin S-receptor-like serine/threonine-protein kinase gene of SEQ ID NO: 6, based on sampling roots of 12-month-old oil palm plants, at six months post inoculation with *Ganoderma boninense*, for control plants (n = 3), symptomatic plants (n = 3), and asymptomatic plants (n = 3).

FIG. 4 shows expression counts of a bioinformatics analysis, indicating relative levels of transcripts, for disease-resistance-protein genes (A) putative disease resistance protein RGA3 gene of SEQ ID NO: 7, and (B) polyamine oxidase gene of SEQ ID NO: 8, based on sampling roots of 12-month-old oil palm plants, at six months post inoculation with *Ganoderma boninense*, for control plants (n = 3), symptomatic plants (n = 3), and asymptomatic plants (n = 3).

FIG. 5 shows expression counts of a bioinformatics analysis, indicating relative levels of transcripts, for jasmonic-acid-signalling genes (A) putative lipoxygenase 5 gene of SEQ ID NO: 9, and (B) 12-oxophytodienoate reductase 1-like gene of SEQ ID NO: 10, based on sampling roots of 12-month-old oil palm plants, at six months post inoculation with *Ganoderma boninense*, for untreated control plants (n = 3), symptomatic plants (n = 3), and asymptomatic plants (n = 3).

FIG. 6 shows expression levels of qRT-PCR analyses, expressed as Target/Ref, indicating relative levels of transcripts, for cell-wall-related-protein genes (A) mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene of SEQ ID NO: 1, and (B) alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene of SEQ ID NO: 2, based on sampling roots of 20-month-old oil palm plants, at 14 months post inoculation with *Ganoderma boninense*, for three individual asymptomatic oil palm plants and two untreated control oil palm plants.

FIG. 7 shows expression levels of qRT-PCR analyses, expressed as Target/Ref, indicating relative levels of transcripts, for transcription factor genes (A) probable WRKY transcription factor 40 gene of SEQ ID NO: 3, and (B) probable WRKY transcription factor 53 gene of SEQ ID NO: 4, based on sampling roots of 20-month-old oil palm plants, at 14 months post inoculation with *Ganoderma boninense*, for three individual asymptomatic oil palm plants and two untreated control oil palm plants.

FIG. 8 shows expression levels of qRT-PCR analyses, expressed as Target/Ref, indicating relative levels of transcripts, for disease-resistance-protein genes (A) dirigent protein 5-like gene of SEQ ID NO: 5, and (B) G-type lectin S-receptor-like serine/threonine-protein

kinase gene of SEQ ID NO: 6, based on sampling roots of 20-month-old oil palm plants, at 14 months post inoculation with *Ganoderma boninense*, for three individual asymptomatic oil palm plants and two untreated control oil palm plants.

FIG. 9 shows expression levels of qRT-PCR analyses, expressed as Target/Ref, indicating relative levels of transcripts, for disease-resistance-protein genes (A) putative disease resistance protein RGA3 gene of SEQ ID NO: 7, and (B) polyamine oxidase gene of SEQ ID NO: 8, based on sampling roots of 20-month-old oil palm plants, at 14 months post inoculation with *Ganoderma boninense*, for three individual asymptomatic oil palm plants and two untreated control oil palm plants.

FIG. 10 shows expression levels of qRT-PCR analyses, expressed as Target/Ref, indicating relative levels of transcripts, for jasmonic-acid-signalling genes (A) putative lipoxygenase 5 gene of SEQ ID NO: 9, and (B) 12-oxophytodienoate reductase 1-like gene of SEQ ID NO: 10, based on sampling roots of 20-month-old oil palm plants, at 14 months post inoculation with *Ganoderma boninense*, for three individual asymptomatic oil palm plants and two untreated control oil palm plants.

Best Mode for Carrying Out the Invention

The application is drawn to methods for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use. The methods comprise steps of (i) cultivating oil palm plants of a treatment group and oil palm plants of a control group together, the oil palm plants of the treatment group and the oil palm plants of the control group having been derived from a common lineage; (ii) contacting roots of the oil palm plants of the treatment group with *Ganoderma boninense*, while not contacting roots of the oil palm plants of the control group with *Ganoderma boninense*; (iii) further cultivating the oil palm plants of the treatment group and the oil palm plants of the control group together; (iv) determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, (1) a level of a transcript of a cell-wall-related-protein gene, (2) a level of a transcript of a transcription factor gene, (3) a level of a transcript of a disease-resistance-protein gene, and (4) a level of a transcript of a jasmonic-acid-signalling gene; (v) predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on the levels of the transcripts being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group; and (vi) if the oil palm plants of the treatment group are thereby predicted to have tolerance to *Ganoderma boninense*, then transplanting, for cultivation for agricultural use, oil palm plants remaining from the treatment group, whereas if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then culling the oil palm plants remaining from the treatment group, as described in more detail below.

By treating 700 *dura* oil palm seedlings of the *Johor Labis* background with *Ganoderma boninense* PER71, cultivating the seedlings under nursery conditions, and identifying genes that are differentially expressed in those treated seedlings that have tolerance to *Ganoderma boninense*, in comparison to those treated seedlings that are susceptible to *Ganoderma boninense,* and to 90 control seedlings which were not inoculated but were otherwise cultivated under identical conditions, it has been determined that 122 candidate genes are upregulated in the treated seedlings that are tolerant to *Ganoderma boninense*, in comparison to the treated seedlings that are susceptible to *Ganoderma boninense* and to the control seedlings. Moreover, based on categorizing the candidate genes according to function, and validating a subset of ten of the candidate genes representing four functional categories in particular, it is proposed that oil palm plants that have tolerance to *Ganoderma boninense* can be obtained by measuring a level of a transcript of a cell-wall-related-protein gene, a level of a transcript of a transcription factor gene, a level of a transcript of a disease-resistance-protein gene, and a level of a transcript of a jasmonic-acid-signalling gene, and predicting tolerance to *Ganoderma boninense* of the treatment group based on the levels of the transcripts being higher in treated seedlings, in comparison to control seedlings.

The methods can be used to obtain oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use. Moreover, the corresponding genes may be inserted into other crop plants for which genetic modification can be carried out, towards modifying the other crop plants for resistance to fungi. In addition, potential markers may be cross-referenced with this list following identification of quantitative trait loci for tolerance and/or susceptibility to *Ganoderma boninense*. Further still, considering that fungal infections are detrimental to a range of tropical crops, identification of genes that contribute to tolerance of oil palm plants to *Ganoderma boninense* may reveal new pathways for tolerance to fungal infections, and potential bases for modifying plants to express such pathways, in tropical crops in general.

The singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

As noted above, a method for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, is disclosed.

The oil palm plants can be oil palm plants corresponding to an important oil-food crop. For example, the oil palm plants can correspond to African oil palm *Elaeis guineensis*.

Tolerance of oil palm plants to *Ganoderma boninense* can include, for example, resistance to initial infection, delay in the onset of symptoms of infection, delay in the progress of pathology of infection, decreased effects on yield of palm oil of infected oil palm plants, and/or delay in death of infected plants, relative to susceptible infected oil palm plants. As discussed above, *Ganoderma boninense* is a fungal pathogen that causes basal stem rot by

infecting basal stem and roots of oil palm plants and restricting flow of water and nutrients to upper portions of the oil palm plants. Infection is believed to be caused by contact of the pathogen with roots. Earliest visual symptoms include wilting of fronds and malnutrition. Diagnostic symptoms for confirmation of basal stem rot include detection of disease lesion (also termed dry rot) at the base of oil palm plants and the appearance of fruiting bodies. Basal stem rot can result in oil palm plants that have small canopies, are impaired with respect to fruit production, and are subject to being toppled by wind.

Tolerance of oil palm plants to *Ganoderma boninense* also can include, for example, survival through the course of an infection trial during an early stage of cultivation of the oil palm plants. The trial can include cultivation of the oil palm plants for a specific period following germination of the oil palm plants, e.g. about six months, infection of oil palm plants with a specific strain of *Ganoderma boninense*, e.g. *Ganoderma boninense* PER71, under standard conditions for infection, e.g. using rubber wood blocks (also termed RWBs) that have been fully colonized by *Ganoderma boninense*, and further cultivation of the oil palm plants for a further specific period, e.g. for at least about six months. For reference, *Ganoderma boninense* PER71 has been used as a standard strain of *Ganoderma boninense* by the Malaysian Palm Oil Board (also termed MPOB) and has been given to all oil palm companies. Cultures of *Ganoderma boninense* PER71 can be obtained from *Ganoderma* & Diseases Research of Oil Palm Unit Laboratory, MPOB.

Exemplary susceptible oil palm plants are oil palm plants of a population wherein if the oil palm plants are inoculated with *Ganoderma boninense* PER71 during cultivation under nursery conditions when the oil palm plants are six months old, then 50% or more of the population will have died due to infection by the *Ganoderma boninense* by the time that the oil palm plants are 20 months old.

Accordingly, in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit resistance to initial infection, e.g. based on being 10%, 20%, or 50% less likely to become infected, relative to susceptible oil palm plants. Also in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit delay in the onset of symptoms of infection, e.g. based on exhibiting a delay of 10%, 20%, or 50% in the onset of infection, relative to susceptible oil palm plants. Also in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit delay in the progress of pathology of infection, e.g. based on exhibiting 10%, 20%, or 50% less wilting of fronds, 10%, 20%, or 50% fewer signs of malnutrition, 10%, 20%, or 50% fewer disease lesions at the base of oil palm plants, and/or 10%, 20%, or 50% fewer fruiting bodies, relative to susceptible oil palm plants, within 3 months, 6 months, 12 months, and/or 24 months of infection. Also in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit

decreased effects on yield of palm oil of infected oil palm plants, e.g. yields of palm oil that are 10%, 20%, or 50% higher, relative to susceptible oil palm plants. Also in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit delay in death of infected plants, e.g. a life span that is 10%, 20%, or 50% longer, relative to susceptible oil palm plants. In each of these examples, the susceptible oil palm plants can be oil palm plants of a population wherein if the oil palm plants are inoculated with *Ganoderma boninense* PER71 during cultivation under nursery conditions when the oil palm plants are six months old, then 50% or more of the population will have died due to infection by the *Ganoderma boninense* by the time that the oil palm plants are 20 months old.

Moreover, in some examples the oil palm plants that have tolerance to *Ganoderma boninense* comprise oil palm plants that exhibit survival through the course of an infection trial during an early stage of cultivation of the oil palm plants, wherein the oil palm plants are cultivated for about six months following germination of the oil palm plants, the oil palm plants are inoculated with cultures of *Ganoderma boninense* PER71 using rubber wood blocks that have been fully colonized by *Ganoderma boninense* PER71, and the oil palm plants are further cultivated for at least about six months.

As noted, a method for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, is disclosed. Cultivation for agricultural use includes, for example, cultivation for mother palm selection and propagation, cultivation for introgressed mother palm selection and propagation, cultivation for pollen donor selection and propagation, and/or cultivation for production of palm oil.

The method comprises a step of (i) cultivating oil palm plants of a treatment group and oil palm plants of a control group together, the oil palm plants of the treatment group and the oil palm plants of the control group having been derived from a common lineage.

The oil palm plants of the treatment group can comprise oil palm plants that will be subjected to inoculation with *Ganoderma boninense*, e.g. wherein the oil palm plants are cultivated for about six months following their germination, the oil palm plants are inoculated with cultures of *Ganoderma boninense* PER71 using rubber wood blocks that have been fully colonized by *Ganoderma boninense* PER71, and the oil palm plants are further cultivated for at least about 6 to 18 more months. The oil palm plants of the treatment group can be in the form of seeds, seedlings, cell culture plants, zygotic embryo culture plants, or somatic tissue culture plants at the start of the cultivation.

In contrast, the oil palm plants of the control group can comprise oil palm plants that will not be subjected to inoculation with *Ganoderma boninense*, e.g. wherein the oil palm plants will not be inoculated with *Ganoderma boninense* but will otherwise be cultivated similarly or identically to the oil palm plants of the treatment group. Like the treatment group, the oil palm plants of the control group can be in the form of seeds, seedlings, cell culture

plants, zygotic embryo culture plants, or somatic tissue culture plants at the start of the cultivation.

Oil palm plants in the form of seeds, seedlings, cell culture plants, zygotic embryo culture plants, or somatic tissue culture plants at the start of the cultivation are in a form that is not yet mature, and thus that is not yet producing palm oil in amounts typical of commercial production, if at all. Accordingly, the method as applied to oil palm plants in such a form at the start of cultivation can be used to obtain oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, before the oil palm plants have matured sufficiently to allow direct measurement of palm oil production by the oil palm plants during commercial production.

As noted, step (i) comprises cultivating oil palm plants of a treatment group and oil palm plants of a control group together. This cultivation together can include, for example, germinating or otherwise initiating growth and development of the oil palm plants of the treatment group and the oil palm plants of the control group synchronously, cultivating the oil palm plants of the treatment group and the oil palm plants of the control group in a single location, such as an oil palm pre-nursery site, and/or subjecting the oil palm plants of the treatment group and the oil palm plants of the control group to similar or identical conditions with respect to light, water, and nutrients, among other factors.

Accordingly, in some examples step (i) is carried out from germination of the oil palm plants of the treatment group and the oil palm plants of the control group.

As also noted, in accordance with step (i), the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a common lineage. By common lineage, it is meant that the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a population of oil palm plants that are related to each other, e.g. based on the oil palm plants of the treatment group and the oil palm plants of the control group sharing at least one common parent based on sharing a mother palm, a father palm, or both.

The population of oil palm plants that are related to each other can comprise any suitable population of oil palm plants. The population can be specified in terms of fruit form and/or identity of the breeding material from which the population was generated.

In this regard, fruit form is a monogenic trait in oil palm that is important with respect to breeding and commercial production. Oil palms with either of two distinct fruit forms are generally used in breeding and seed production through crossing in order to generate palms for commercial production of palm oil, also termed commercial planting materials or agricultural production plants. The first fruit form is *dura* (genotype: sh+ sh+), which is characterized by a thick shell (also termed seed coat) corresponding to 28% to 35% of the fruit by weight, with no ring of black fibres around the kernel of the fruit. For *dura* fruits, the ratio of mesocarp to fruit

varies from 50% to 60%, with extractable oil content in proportion to bunch weight of 18% to 24%. The second fruit form is *pisifera* (genotype: sh- sh-), which is characterized by the absence of a shell, the vestiges of which are represented by a ring of fibres around a small kernel. Accordingly, for *pisifera* fruits, the ratio of mesocarp to fruit is 90% to 100%. The ratio of mesocarp oil to bunch is comparable to the *dura* at 16% to 28%. *Pisiferas* are however usually female sterile as the majority of bunches abort at an early stage of development.

Crossing *dura* and *pisifera* gives rise to palms with a third fruit form, the *tenera* (genotype: sh+ sh-). *Tenera* fruits have thin shells, typically corresponding to 8% to 10% of the fruit by weight, corresponding to a thickness of 0.5 to 4 mm, around which is a characteristic ring of black fibres. For *tenera* fruits, the ratio of mesocarp to fruit is comparatively high, typically in the range of 60% to 80%. Commercial *tenera* palms generally produce more fruit bunches than *duras*, although mean bunch weight is lower. The ratio of mesocarp oil to bunch is in the range of 20% to 30%, the highest of the three fruit forms, and thus *tenera* are typically used as commercial planting materials.

Identity of the breeding material can be based on the source and breeding history of the breeding material. *Dura* palm breeding populations used in Southeast Asia include Serdang Avenue, *Ulu Remis* (which incorporated some Serdang Avenue material, and which is also termed UR), *Banting dura* (also termed BD), *Johor Labis* (also termed JL), and Elmina estate, including Deli Dumpy, all of which are derived from Deli *dura*. *Pisifera* breeding populations used for seed production are generally grouped as Yangambi, AVROS, Binga, and URT. Other *dura* and *pisifera* populations are used in Africa and South America.

Oil palm plantation/breeding programs in Southeast Asia are using Deli *dura* origin, which originated from the four famous *dura* palms at Bogor in the year 1848. The Deli *dura* materials were subsequently distributed to several research stations across the region. Each station focused on different selection preferences over generations, leading to some differentiation between subpopulations, termed breeding populations of restricted origin (also termed BPRO). The important breeding populations of restricted origin derived from Deli *dura* are *Ulu Remis* and *Johor Labis*. The *Ulu Remis* origin was selected for high bunch number and high sex ratio (defined as ratio of females to total inflorescences) in Marihat Baris, Sumatra. Instead of bunch number, Socfindo in Sumatra had developed *Johor Labis* origin for bigger bunches (high bunch weight) and thinner shells.

Dura palms were commercially planted in Southeast Asia before the 1960's. The *Banting dura* was discovered in the commercial Deli *dura* planted in 1958 in Dusun Durian Estate. The material was selected for good bunch traits and number. *Banting dura* has become an important maternal source.

African *dura* materials are inferior to Deli *dura*. To increase oil yield, the main planting materials in Africa were *tenera* (*dura* x *pisifera*). This provided an opportunity to

discover a superior pollen source, i.e. AVROS *pisifera*. The material originated from the renowned Djongo palms that were planted in Eala Botanical Garden in Yangambi, Zaire, now the Democratic Republic of the Congo. The material was then further selected and produced BM119 at Kelanang Bharu Division of Dusun Durian Estate. The AVROS *pisifera* confers superiority in growth uniformity, general combining ability, precocity, and mesocarp oil yield in Deli x AVROS progeny (*tenera*). Thus, the introduction of Deli *dura* x BM119 AVROS *pisifera* in the region resulted in an increase in oil per hectare of 30% since the 1960's.

Oil palm breeding is primarily aimed at selecting for improved parental *dura* and *pisifera* breeding stock palms for production of superior *tenera* commercial planting materials. Such materials are largely in the form of seeds although the use of tissue culture for propagation of clones continues to be developed. Generally, parental *dura* breeding populations are generated by crossing among selected *dura* palms. Based on the monogenic inheritance of fruit form, 100% of the resulting palms will be *duras*. After several years of yield recording and confirmation of bunch and fruit characteristics, *duras* are selected for breeding based on phenotype. In contrast, *pisifera* palms are normally female sterile and thus breeding populations thereof must be generated by crossing among selected *teneras* or by crossing selected *teneras* with selected *pisiferas*. The *tenera* x *tenera* cross will generate 25% *duras*, 50% *teneras*, and 25% *pisiferas*. The *tenera* x *pisifera* cross will generate 50% *teneras* and 50% *pisiferas*. The yield potential of *pisiferas* is then determined indirectly by progeny testing with the elite *duras*, i.e. by crossing *duras* and *pisiferas* to generate *teneras*, and then determining yield phenotypes of the fruits of the *teneras* over time. From this, *pisiferas* with good general combining ability are selected based on the performance of their *tenera* progenies. Intercrossing among selected parents is also carried out with progenies being carried forward to the next breeding cycle.

Oil palm cultivation for commercial production of palm oil can be improved by use of the superior *tenera* commercial planting materials. Priority selection objectives include high oil yield per unit area in terms of high fresh fruit bunch yield (also termed FFB) and high oil to bunch ratio (also termed O/B), high early yield (precocity), and good oil qualities, among other traits. Progeny plants may be cultivated by conventional approaches, e.g. seedlings may be cultivated in polyethylene bags in pre-nursery and nursery settings, raised for about 18 months, and then transplanted for cultivation for agricultural use, with progeny that are known or predicted to exhibit high yields chosen for further cultivation, among other approaches.

As noted above, by treating 700 *dura* oil palm seedlings of the *Johor Labis* background with *Ganoderma boninense* PER71, cultivating the seedlings under nursery conditions, and identifying genes that are differentially expressed in those treated seedlings that have tolerance to *Ganoderma boninense*, in comparison to those treated seedlings that are susceptible to *Ganoderma boninense,* and to 90 control seedlings which were not inoculated but were otherwise cultivated under identical conditions, it has been determined that 122 candidate genes

are upregulated in the treated seedlings that are tolerant to *Ganoderma boninense*, in comparison to the treated seedlings that are susceptible to *Ganoderma boninense* and to the control seedlings. As also noted, cultivation for agricultural use includes, for example, cultivation for mother palm selection and propagation, cultivation for introgressed mother palm selection and propagation, cultivation for pollen donor selection and propagation, and/or cultivation for production of palm oil.

Accordingly, in some examples the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a *Johor Labis dura* x *Johor Labis dura* population, an *Ulu Remis dura* x *Ulu Remis dura* population, a *Banting dura* x *Banting dura* population, a *Johor Labis dura* x *Ulu Remis dura* population, a *Johor Labis dura* x *Banting dura* population, or an *Ulu Remis dura* x *Banting dura* population, and the agricultural use is (i) mother palm selection and propagation or (ii) introgressed mother palm selection and propagation.

Also in some examples the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from an AVROS *pisifera* x AVROS *tenera* population, or an AVROS *tenera* x AVROS *tenera* population, and the agricultural use is pollen donor selection and propagation.

Also in some examples, the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a Nigerian *dura* x AVROS *pisifera* population, a Deli *dura* x AVROS *pisifera* population, an *Ulu Remis dura* x AVROS *pisifera* population, or a *Banting dura* x AVROS *pisifera* population, and the agricultural use is production of palm oil.

Also in some examples, the common lineage can comprise identical parentage. Also in some examples, the identical parentage can comprise a *dura* mother palm plant and a *dura* father palm plant.

The method also comprises a step of (ii) contacting roots of the oil palm plants of the treatment group with *Ganoderma boninense*, while not contacting roots of the oil palm plants of the control group with *Ganoderma boninense*. As noted, the oil palm plants of the treatment group can comprise oil palm plants that will be subjected to inoculation with *Ganoderma boninense*, whereas the oil palm plants of the control group can comprise oil palm plants that will not be subjected to inoculation with *Ganoderma boninense*. Determination of which oil palm plants will be subjected to inoculation with *Ganoderma boninense* can be made at any time prior to the inoculation, e.g. prior to germination, during germination, within one month after germination, within three months after germination, and/or within six months after germination, among other times. Determination of which oil palm plants will be subjected to inoculation with *Ganoderma boninense* also can be made in accordance with standard principles of statistical analyses, e.g. based on random selection of samples and use of

sufficient sample sizes. Determination of which oil palm plants will be subjected to inoculation with *Ganoderma boninense* also can be made taking growth and health of oil palm plants into account, e.g. based on removing slow growing and/or unhealthy oil palm plants prior to inoculation.

The contacting of step (ii) can comprise inoculating the oil palm plants of the treatment group with cultures of *Ganoderma boninense*. The inoculating can be carried out, for example, by using rubber wood blocks that have been fully colonized by *Ganoderma boninense*, as follows. Rubber (*Hevea brasiliensis*) wood blocks can be custom-cut to 6 cm x 6 cm x 6 cm (216 cm^3). All rubber wood blocks can be washed and dried in an oven at 80 °C for overnight before autoclave at 121 °C for 1 hour. Each rubber wood block can be placed in a plastic bag with 120 ml of Malt Extract (ME) Broth. Bags can be sealed and autoclaved at 121 °C for 15 minutes and then left to solidify overnight. The 7 day old dikaryon inocula on a potato dextrose agar (also termed PDA) plate can be cut into 4 parts and a half of blocks agar can be inoculated onto the rubber wood block. The inoculated rubber wood blocks can be incubated at 25-28 °C at 60-70% relative humidity (also termed RH) for about 150 days during which time the *Ganoderma* mycelia will completely cover the wood blocks. Six months old seedlings in 6 inches x 9 inches (15 cm x 23 cm) polybags can be transplanted into 15 inches x 18 inches (38 cm x 46 cm) polybags filled with top soil which can be left settled for at least one week. Polybags should be watered to saturation the day before inoculation of the oil palm plants with *Ganoderma boninense*. During planting, the inoculated rubber wood block fully colonized by the pathogen can be placed in the middle of the bag, then the six-month-old seedlings with half of the soil removed from the bottom can be placed in closed contact with the inoculum block. Oil palm seedlings with rubber wood blocks without inoculum can be used as a control. The inoculating also can be carried out, for example, by variations, on this approach, e.g. with respect to time that the contacting is initiated, and/or by more specific embodiments, e.g. with respect to the specific strain of *Ganoderma boninense*.

For reference, a typical approach for cultivation of oil palm seedlings corresponds to cultivation of seedlings in 6 inches x 9 inches (15 cm x 23 cm) polybags including sieved top soil, in an oil palm pre-nursery site, in accordance with pre-nursery cultivation, from germination until the seedlings are about six months old and/or until the seedlings produce a bifid leaf. The typical approach then involves transplanting the six-month old seedlings from the 6 inches x 9 inches (15 cm x 23 cm) polybags to 15 inches x 18 inches (38 cm x 46 cm) polybags filled with top soil, followed by cultivation for another 12 months, in an oil palm nursery, in accordance with nursery cultivation, until the corresponding plants are about 18 months old and/or have about 15 green leaves. The typical approach then involves transplanting the 18-month old seedlings to an oil palm plantation for further cultivation.

Accordingly, for example, the contacting of step (ii) can be carried out at about the time of transplanting seedlings from pre-nursery cultivation to nursery cultivation, e.g. upon transplanting the seedlings from the 6 inches x 9 inches (15 cm x 23 cm) polybags to 15 inches x 18 inches (38 cm x 46 cm) polybags filled with top soil, e.g. when the seedlings are about six months old. Also for example, the contacting of step (ii) can be initiated when the oil palm plants of the treatment group are 4 to 8 months old, 5 to 7 months old, or about 6 months old, e.g. based on transplanting seedlings earlier or later than six months and/or based on initiating the contacting before or after the transplanting. Also for example, the contacting of step (ii) can comprise contacting the oil palm plants of the treatment group with an inoculum block, e.g. a rubber wood block that is colonized with *Ganoderma boninense*. Also for example, the *Ganoderma boninense* can comprise *Ganoderma boninense* PER71.

The method also comprises a step of (iii) further cultivating the oil palm plants of the treatment group and the oil palm plants of the control group together. The cultivation of step (iii) can be carried out as described above, e.g. by cultivating the oil palm plants of the treatment group and the oil palm plants of the control group in a single location, such as an oil palm nursery, and subjecting the oil palm plants of the treatment group and the oil palm plants of the control group to similar or identical conditions with respect to light, water, and nutrients, among other factors. Also, the cultivation of step (iii) can be carried out for a period of time suitable for the oil palm plants of the treatment group and the oil palm plants of the control group to mature sufficiently for transplantation from an oil palm nursery to another site for further cultivation, for example a palm grove, a small holding, or an oil palm plantation. For example, for plants for which the contacting of step (ii) was initiated when the oil palm plants of the treatment group were 6 months old, the cultivation of step (iii) can be carried out for 6 to 18 months, 9 to 15 months, 11 to 13 months, or about 12 months, and thus until the oil palm plants are 12 to 24 months old, 15 to 21 months old, 17 to 19 months old, or about 18 months old, respectively.

The method also comprises a step of (iv) determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, (1) a level of a transcript of a cell-wall-related-protein gene, (2) a level of a transcript of a transcription factor gene, (3) a level of a transcript of a disease-resistance-protein gene, and (4) a level of a transcript of a jasmonic-acid-signalling gene.

In accordance with step (iv), the level of the transcript of the cell-wall-related-protein gene comprises one or more of (a) a level of a transcript 1 of a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene, or (b) a level of a transcript 2 of an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene.

Mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene is predicted to encode a class I alpha-mannosidase. Class I alpha-mannosidases are essential for

early N-glycan processing, based on removing preferentially alpha-1,2-linked mannose residues from $Man_9GlcNAc_2$ to produce $Man_8GlcNAc_2$. Class I alpha-mannosidases are involved in root development and cell wall biosynthesis (Liebminger et al., 2009, The Plant Cell, 21(12), 3850-3867). In some examples, the transcript 1 of a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene is a transcript of mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene of SEQ ID NO: 1, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 1. In some examples, the transcript 1 includes an open reading frame of SEQ ID NO: 11, that codes for a protein of SEQ ID NO: 21.

Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene is predicted to encode a transferase that initiates complex N-linked carbohydrate formation. Such transfer is essential for the conversion of high-mannose to hybrid and complex N-glycans. Such transfer also is required for normal root growth and morphology (Wenderoth & von Schaewen, 2000, Plant physiology, 123, 1097-1108). N-glycans formation has been reported to be involved in an abundance of receptors and provides pattern recognition during plant and pathogen interactions (Häweker et al., 2010, The Journal of Biological Chemistry, 285, 4629-4636). In some examples, the transcript 2 of an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene is a transcript of alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene of SEQ ID NO: 2, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 2. In some examples, the transcript 2 includes an open reading frame of SEQ ID NO: 12, that codes for a protein of SEQ ID NO: 22.

Also in accordance with step (iv), the level of the transcript of the transcription factor gene comprises one or more of (a) a level of a transcript 3 of a probable WRKY transcription factor 40 gene, or (b) a level of a transcript 4 of a probable WRKY transcription factor 53 gene.

Probable WRKY transcription factor 40 gene is predicted to encode a transcription factor involved in disease resistance. Double mutants of WRKY18 and WRKY40 were susceptible to *Pseudomonas syringae* infection. Moreover, loss of function of WRKY40 was involved in jasmonic acid and salicylic acid signalling against *Golovinomyces orontii* but not *G. cichoracearum* and *G. cruciferarum* (Schön et al., 2013, Molecular Plant-Microbe Interactions: MPMI, 26, 758-767). Triple mutants of WRKY18, WRKY40, and PAD3 exhibited impaired camalexin biosynthesis, which is important in fungal defense (Schön et al., 2013). In some examples, the transcript 3 of a probable WRKY transcription factor 40 gene is a transcript of probable WRKY transcription factor 40 gene of SEQ ID NO: 3, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 3. In some examples, the transcript 3 includes an open reading frame of SEQ ID NO: 13, that codes for a protein of SEQ ID NO: 23.

Probable WRKY transcription factor 53 gene also is predicted to encode a transcription factor involved in disease resistance. WRKY53 was reported to be a positive regulator in

systemic acquired resistance against virulent bacteria *Pseudomonas syringae* in Arabidopsis (Wang et al., 2006, PLoS Pathog, 2, e123). Involvement of WRKY53 in senescence process and programmed cell death also has been reported (Zentgraf et al., 2010, European Journal of Cell Biology, 89, 133-137). In some examples, the transcript 4 of a probable WRKY transcription factor 53 gene is a transcript of probable WRKY transcription factor 53 gene of SEQ ID NO: 4, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 4. In some examples, the transcript 4 includes an open reading frame of SEQ ID NO: 14, that codes for a protein of SEQ ID NO: 24.

Also in accordance with step (iv), the level of the transcript of the disease-resistance-protein gene comprises one or more of (a) a level of a transcript 5 of a dirigent protein 5-like gene, (b) a level of a transcript 6 of a G-type lectin S-receptor-like serine/threonine-protein kinase gene, (c) a level of a transcript 7 of a putative disease resistance protein RGA3 gene, or (d) a level of a transcript 8 of a polyamine oxidase gene.

Dirigent protein 5-like gene is predicted to encode a dirigent protein. Dirigent proteins function to control the coupling of monolignols, as first discovered in *Forsythia intermedia* as the outcome of coupling of two molecules of *E*-coniferyl alcohol to give the lignan, (+)-pinoresinol (Davin et al., 1997, Plant Physiology, 123, 453-462). Lignans were reported to have anti-oxidant, antifungal, and antibacterial properties (Cespedes et al., 2005, Journal of Biosciences, 61, 35-43). Dirigent protein also was found to be induced in Arabidopsis by soil borne fungal *Verticillium longisporum* (Konig et al., 2014, The New Phytologist, 202, 823-837). In some examples, the transcript 5 of a dirigent protein 5-like gene is a transcript of dirigent protein 5-like gene of SEQ ID NO: 5, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 5. In some examples, the transcript 5 includes an open reading frame of SEQ ID NO: 15, that codes for a protein of SEQ ID NO: 25.

G-type lectin S-receptor-like serine/threonine-protein kinase gene is predicted to encode a lectin-like receptor kinase. Lectin-like receptor kinases are important in plant development and stress responses (Vaid et al., 2013, Molecular Plant, 6, 1405-1418). G-type lectin S-receptor-like serine/threonine-protein kinase were reported to be involved in defence mechanism after an elicitin (capsicein) from *Phytophthora* fungi were used to detect interactions using a pull-down method (Kim et al., 2010, Molecular Biology Reports, 37, 717-727). In some examples, the transcript 6 of a G-type lectin S-receptor-like serine/threonine-protein kinase gene is a transcript of G-type lectin S-receptor-like serine/threonine-protein kinase gene of SEQ ID NO: 6, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 6. In some examples, the transcript 6 includes an open reading frame of SEQ ID NO: 16, that codes for a protein of SEQ ID NO: 26.

Putative disease resistance protein RGA3 gene is predicted to encode a resistance gene analogue. Resistance gene analogues correspond to a large family of highly conserved genes

conferring resistance towards pathogens, as shown in soybean and apple (Perazzolli et al., 2014, PloS One 9, e83844; Kanazin et al., 1996, Proceedings of the National Academy of Sciences USA, 93, 11746-11750). Resistance gene analogues are able to be used as tools for cloning disease resistance genes and to study the evolution of interactions between host and pathogens (Sharma et al., 2009, J. Plant Biochem. Biotechnol., 18, 1-11). Disease resistance protein was reported to confer broad spectrum resistance in potato against potato late blight disease (Song et al., 2003, Proceedings of the National Academy of Sciences USA, 100, 9128-9133). In some examples, the transcript 7 of a putative disease resistance protein RGA3 gene is a transcript of putative disease resistance protein RGA3 gene of SEQ ID NO: 7, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 7. In some examples, the transcript 7 includes an open reading frame of SEQ ID NO: 17, that codes for a protein of SEQ ID NO: 27.

Polyamine oxidase gene is predicted to encode a polyamine oxidase. Polyamine oxidase is located predominantly at cell walls regulated in peroxisomes. The polyamine oxidase AtPAO4 was reported to be highly expressed in roots and to be important in maintaining homeostasis, i.e. back conversion of polyamines as conjugators in flavonoid and lignin synthesis pathways and release of hydrogen peroxide during programmed cell death (Jiménez-Bremont et al., 2014, Frontiers in Plant Science, 5, 95; Ahou et al., 2014, Journal of Experimental Botany, 65, 1585-1603). Polyamine oxidase also catabolizes spermine and spermidine, releasing hydrogen peroxide and inducing hypersensitive response via programmed cell death (Yoda et al., 2006, Plant Physiology, 142, 193-206). In some examples, the transcript 8 of a polyamine oxidase gene is a transcript of polyamine oxidase gene of SEQ ID NO: 8, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 8. In some examples, the transcript 8 includes an open reading frame of SEQ ID NO: 18, that codes for a protein of SEQ ID NO: 28.

Also in accordance with step (iv), the level of the transcript of the jasmonic-acid-signalling gene comprises one or more of (a) a level of a transcript 9 of a putative lipoxygenase 5 gene, or (b) a level of a transcript 10 of a 12-oxophytodienoate reductase 1-like gene.

Putative lipoxygenase 5 gene is predicted to encode a lipoxygenase containing a PLAT/LH2 domain. Lipoxygenases are non-heme, non-sulfur iron dioxygenases that act on lipid substrates containing one or more (Z,Z)-1,4-pentadiene moieties. In plants, the immediate products are involved in defense mechanisms against pathogens and may be precursors of metabolic regulators. The generally proposed function of PLAT/LH2 domains is to mediate interaction with lipids or membrane bound proteins. Lipoxygenase pathway was induced to produce antifungal phytooxylipin against *Botrytis cinerea* in tomato (Akram et al., 2008, BMC Plant Biol, 8, 113). 5-lipoxygenase activity also was increased in potato after exposure to fungal elicitor arachidonic acid (Bostock et al., 1992, Plant Physiology, 100, 1448-1456). In

some examples, the transcript 9 of a putative lipoxygenase 5 gene is a transcript of putative lipoxygenase 5 gene of SEQ ID NO: 9, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 9. In some examples, the transcript 9 includes an open reading frame of SEQ ID NO: 19, that codes for a protein of SEQ ID NO: 29.

12-oxophytodienoate reductase 1-like gene is predicted to encode a component of the jasmonic acid pathway. The jasmonic acid pathway is known to protect plants against necrotrophic fungus (Vijayan et al., 1998, Proceedings of the National Academy of Sciences USA, 95, 7209-7214). 12-Oxophytodienoate reductase (also termed OPR) is a flavin mononucleotide (FMN)-dependent oxidoreductase in plants that belongs to the family of Old Yellow Enzyme (OYE). 12-Oxophytodienoate reductase was initially characterized as an enzyme involved in the biosynthesis of the plant hormone jasmonic acid. OPR1 has been shown to be expressed in Arabidopsis cell cultures treated with fungal elicitors (Chivasa et al., 2006, Journal of Experimental Botany, 57, 1553-1562). In some examples, the transcript 10 of a 12-oxophytodienoate reductase 1-like gene is a transcript of 12-oxophytodienoate reductase 1-like gene of SEQ ID NO: 10, i.e. the transcript comprises the polynucleotide sequence of SEQ ID NO: 10. In some examples, the transcript 10 includes an open reading frame of SEQ ID NO: 20, that codes for a protein of SEQ ID NO: 30.

Step (iv) can be carried out when the oil palm plants of the treatment group and the oil palm plants of the control group have been cultivated for a sufficient time for differences to be apparent with respect to (1) the level of the transcript of the cell-wall-related-protein gene, (2) the level of the transcript of the transcription factor gene, (3) the level of the transcript of the disease-resistance-protein gene, and (4) the level of the transcript of the jasmonic-acid-signalling gene, for the oil palm plants of the treatment group in comparison to the oil palm plants of the control group. Such differences may become apparent before, during, or after the period of time suitable for the oil palm plants of the treatment group and the oil palm plants of the control group to mature sufficiently for transplantation from an oil palm nursery to another site for further cultivation, for example a palm grove, a small holding, or an oil palm plantation.

Accordingly, in some examples, step (iv) can be carried out when the oil palm plants of the treatment group and the oil palm plants of the control group are 12 to 24 months old, e.g. 15 to 21 months, 17 to 19 months, or about 18 months.

The level of one or more of the transcripts 1-10 may be determined in samples of the oil palm plants of the treatment group and samples of the oil palm plants of the control group that comprise an organ, tissue, cell, or other part of the corresponding oil palm plants that includes sufficient RNA to allow for determination of the level of one or more of the transcripts 1-10.

For example, the sample can comprise an organ, tissue, cell, or other part of the oil palm plants of the treatment group that has come in direct contact with *Ganoderma boninense*

by inoculation, e.g. roots of the oil palm plants of the treatment group. Also for example, the sample can comprise a corresponding organ, tissue, cell, or other part of the oil palm plants of the control group, e.g. roots of the oil palm plants of the control group. In accordance with these examples, the level of one or more of the transcripts 1-10 may differ for samples of the oil palm plants of the treatment group in comparison to samples of the oil palm plants of the control group based on direct interaction between *Ganoderma boninense* and an organ, tissue, cell, or other part of the oil palm plants of the treatment group, but not of the oil palm plants of the control group.

Also for example, the sample can comprise an organ, tissue, cell, or other part of the oil palm plants of the treatment group that has not come in direct contact with *Ganoderma boninense* by inoculation, e.g. leaves of the oil palm plants of the treatment group. Also for example, the sample can comprise a corresponding organ, tissue, cell, or other part of the oil palm plants of the control group, e.g. leaves of the oil palm plants of the control group. In accordance with these examples, the level of one or more of the transcripts 1-10 may differ for samples of the oil palm plants of the treatment group in comparison to samples of the oil palm plants of the control group based on transduction of a signal from an organ, tissue, cell, or other part of the oil palm plants of the treatment group that has come in direct contact with *Ganoderma* boninense, to another organ, tissue, cell, or other part that has not come in direct contact, with no such direct contact occurring for the oil palm plants of the control group, and thus no such transduction of a signal occurring for those oil palm plants.

The level of one or more of the transcripts 1-10 also may be determined in samples of the oil palm plants of the treatment group and samples of the oil palm plants of the control group based on destructive sampling, e.g. by a method that involves removal of an organ, tissue, cell, or other part of the oil palm plants of the treatment group and the oil palm plants of the control group.

Accordingly, in some examples the determining of step (iv) comprises determining the level of one or more of the transcripts 1-10 individually in roots of the subset of the oil palm plants of the treatment group, in comparison to roots of the oil palm plants of the control group. Also, in some examples step (iv) comprises determining the level of one or more of the transcripts 1-10 individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, by destructive sampling.

As noted, the method comprises the step of (iv) determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, (1) a level of a transcript of a cell-wall-related-protein gene, (2) a level of a transcript of a transcription factor gene, (3) a level of a transcript of a disease-resistance-protein gene, and (4) a level of a transcript of a jasmonic-acid-signalling gene. Accordingly, step (iv) includes determining the levels of the transcripts in a subset of the oil palm plants of the treatment

group, i.e. in at least one, but fewer than all, of the oil palm plants of the treatment group, and more particularly determining the levels of the transcripts with respect to individual oil palm plants of the subset. Moreover, step (iv) includes determining the levels of the transcripts in the oil palm plants of the control group, and more particularly determining the levels of the transcripts with respect individual oil palm plants of the control group.

By determining the levels of the transcripts with respect to individual oil palm plants of the subset of the treatment group, as opposed for example to the entire treatment group, it remains possible to continue cultivation of oil palm plants of the treatment group that have not been subjected to destructive sampling, i.e. oil palm plants remaining from the treatment group, for possible transplantation later. Moreover, by determining the levels of the transcripts with respect to individual oil palm plants of both the subset of the oil palm plants of the treatment group and the oil palm plants of the control group, it is possible to determine the levels of the transcripts relative to expression of another transcript that serves as an internal control, e.g. a transcript that is expressed constitutively in oil palm plants, to control for variations in yields of RNA that may occur between different preparations of RNA and/or different oil palm plants.

Accordingly, in some examples the subset of oil palm plants of the treatment group that are subjected to the determining of step (iv) corresponds to 5% to 30% of the oil palm plants of the treatment group, e.g. 7% to 20%, 9% to 15%, or about 10% of the oil palm plants of the treatment group. Also in some examples step (iv) further comprises determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group, the levels of the transcripts relative to expression of another transcript that serves as an internal control.

In accordance with step (iv), (1) the level of a transcript of a cell-wall-related-protein gene, (2) the level of a transcript of a transcription factor gene, (3) the level of a transcript of a disease-resistance-protein gene, and (4) the level of a transcript of a jasmonic-acid-signalling gene can be determined by various methods, including for example quantitative reverse transcription polymerase chain reaction (also termed qRT-PCR), RNA-seq, hybridization, or microarray.

Regarding qRT-PCR in particular, reverse transcription of RNA obtained from samples of the oil palm plants of the treatment group and the oil palm plants of the control group can be carried out to obtain complementary DNA (also termed cDNA) by standard methods, which are known in the art. For example, reverse transcriptions can be performed by using SensiFAST™ cDNA Synthesis Kit (Bioline, UK). Also for example, samples of 2 μg of RNA from each biological replicate can be used for cDNA conversion. Also for example, the samples can be diluted 5 times prior to use.

Following reverse transcription, the cDNA can be amplified by PCR by standard methods, which also are known in the art. For example, PCR mixtures of 20 μL, consisting of

1X SyBr Green Master I, 400 nM of forward and reverse primers with 2 µL of 5-fold diluted cDNA, can be set up in ice. Also for example, the qPCR can be performed on Roche LightCycler 480 with the settings as follow: (1) preincubation (95 °C, hold time of 5 min, ramp rate of 4.4 °C/sec), (2) amplification (95 °C, hold time of 10 sec, ramp rate of 4.4 °C/sec, then 60 °C, hold time of 10 sec, ramp rate of 2.2 °C/sec, then 72 °C, single acquisition, hold time of 10 sec, ramp rate of 4.4 °C/sec, single mode acquisition), (3) melting curve (95 °C, hold time of 5 sec, ramp rate of 4.4 °C/sec, then 65 °C, hold time of 1 min, ramp rate of 2.2 °C/sec, then 97 °C, continuous acquisition, ramp rate of 0.11 °C/sec, continuous acquisition mode), and (4) cooling (40 °C, hold time of 30 sec, ramp rate of 2.2 °C/sec). Also for example, suitable primers for qPCR of transcripts 1-10 include the following: (1) for transcript 1, a forward primer of SEQ ID NO: 31 and a reverse primer of SEQ ID NO: 32, (2) for transcript 2, a forward primer of SEQ ID NO: 33 and a reverse primer of SEQ ID NO: 34, (3) for transcript 3, a forward primer of SEQ ID NO: 35 and a reverse primer of SEQ ID NO: 36, (4) for transcript 4, a forward primer of SEQ ID NO: 37 and a reverse primer of SEQ ID NO: 38, (5) for transcript 5, a forward primer of SEQ ID NO: 39 and a reverse primer of SEQ ID NO: 40, (6) for transcript 6, a forward primer of SEQ ID NO: 41 and a reverse primer of SEQ ID NO: 42, (7) for transcript 7, a forward primer of SEQ ID NO: 43 and a reverse primer of SEQ ID NO: 44, (8) for transcript 8, a forward primer of SEQ ID NO: 45 and a reverse primer of SEQ ID NO: 46, (9) for transcript 9, a forward primer of SEQ ID NO: 47 and a reverse primer of SEQ ID NO: 48, and (10) for transcript 10, a forward primer of SEQ ID NO: 49 and a reverse primer of SEQ ID NO: 50.

Accordingly, in some examples the determining of step (iv) comprises determining the level of one or more of the transcripts 1-10 by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Also, in some of these examples the qRT-PCR comprises determining the level of one or more of the transcripts 1-10 by use of (1) a forward primer of SEQ ID NO: 31 and a reverse primer of SEQ ID NO: 32, (2) a forward primer of SEQ ID NO: 33 and a reverse primer of SEQ ID NO: 34, (3) a forward primer of SEQ ID NO: 35 and a reverse primer of SEQ ID NO: 36, (4) a forward primer of SEQ ID NO: 37 and a reverse primer of SEQ ID NO: 38, (5) a forward primer of SEQ ID NO: 39 and a reverse primer of SEQ ID NO: 40, (6) a forward primer of SEQ ID NO: 41 and a reverse primer of SEQ ID NO: 42, (7) a forward primer of SEQ ID NO: 43 and a reverse primer of SEQ ID NO: 44, (8) a forward primer of SEQ ID NO: 45 and a reverse primer of SEQ ID NO: 46, (9) a forward primer of SEQ ID NO: 47 and a reverse primer of SEQ ID NO: 48, or (10) a forward primer of SEQ ID NO: 49 and a reverse primer of SEQ ID NO: 50, respectively.

The method also comprises a step of (v) predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher, (2) at least one of the levels of the transcripts 3 or 4 being

higher, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher, and (4) at least one of the levels of the transcripts 9 or 10 being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the one or more oil palm plants of the control group. As noted above, based on categorizing 122 candidate genes according to function, and validating a subset of ten of the candidate genes representing four functional categories in particular, it is proposed that oil palm plants that have tolerance to *Ganoderma boninense* can be obtained by measuring a level of a transcript of a cell-wall-related-protein gene, a level of a transcript of a transcription factor gene, a level of a transcript of a disease-resistance-protein gene, and a level of a transcript of a jasmonic-acid-signalling gene, and predicting tolerance to *Ganoderma boninense* of the treatment group based the levels of the transcripts being higher in treated seedlings, in comparison to control seedlings. As also noted, the level of the transcript of the cell-wall-related-protein gene comprises one or more of (a) a level of a transcript 1 of a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene, or (b) a level of a transcript 2 of an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene. Moreover, the level of the transcript of the transcription factor gene comprises one or more of (a) a level of a transcript 3 of a probable WRKY transcription factor 40 gene, or (b) a level of a transcript 4 of a probable WRKY transcription factor 53 gene. In addition, the level of the transcript of the disease-resistance-protein gene comprises one or more of (a) a level of a transcript 5 of a dirigent protein 5-like gene, (b) a level of a transcript 6 of a G-type lectin S-receptor-like serine/threonine-protein kinase gene, (c) a level of a transcript 7 of a putative disease resistance protein RGA3 gene, or (d) a level of a transcript 8 of a polyamine oxidase gene. Furthermore, the level of the transcript of the jasmonic-acid-signalling gene comprises one or more of (a) a level of a transcript 9 of a putative lipoxygenase 5 gene, or (b) a level of a transcript 10 of a 12-oxophytodienoate reductase 1-like gene. Thus, in accordance with step (v), the treatment group is predicted to have tolerance to *Ganoderma boninense* based on (1) at least one of the levels of the cell-wall-related-protein gene transcripts 1 or 2 being higher, (2) at least one of the levels of the transcription factor gene transcripts 3 or 4 being higher, (3) at least one of the levels of the disease-resistance-protein gene transcripts 5, 6, 7, or 8 being higher, and (4) at least one of the levels of the jasmonic-acid-signalling gene transcripts 9 or 10 being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group.

In accordance with step (v), the predicting of tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group is based on the levels of the transcripts being higher individually in the subset the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group. Similarly as for step (iv), by predicting tolerance based on the levels of the transcripts being higher individually in the subset of the oil palm plants of the

treatment group, in comparison to the oil palm plants of the control group, it is possible to control for variations in the levels of the transcripts that may occur in different oil palm plants. Moreover, the predicting of tolerance can be based on (1) at least one of the levels of the transcripts 1 or 2 being higher in one or more plants of the subset of the oil palm plants of the treatment group, (2) at least one of the levels of the transcripts 3 or 4 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, and (4) at least one of the levels of the transcripts 9 or 10 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group. By using this approach, it is possible to predict tolerance based on properties of the oil palm plants of the treatment group considered as a population, e.g. based on statistical properties, and thus to continue cultivation of oil palm plants remaining from the treatment group, i.e. oil palm plants of the treatment group that have not been subjected to destructive sampling, for possible transplantation later, while still controlling for variations in the levels of the transcripts that may occur in different oil palm plants.

Accordingly, like for step (iv), in some examples step (v) further comprises comparing the levels of the transcripts, individually in a subset of the oil palm plants of the treatment group, in comparison to oil palm plants of the control group, wherein the levels of the transcripts have been determined relative to expression of another transcript that serves as an internal control.

Also in some examples the predicting of step (v) comprises predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher in one or more plants of the subset of the oil palm plants of the treatment group, (2) at least one of the levels of the transcripts 3 or 4 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, and/or (4) at least one of the levels of the transcripts 9 or 10 being higher in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group.

Also in some examples the predicting of step (v) comprises predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher by at least 5%, at least 10%, or at least 20%, (2) at least one of the levels of the transcripts 3 or 4 being higher by at least 5%, at least 10%, or at least 20%, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher by at least 5%, at least 10%, or at least 20%, and/or (4) at least one of the levels of the transcripts 9 or 10

being higher by at least 5%, at least 10%, or at least 20%, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group.

Also in some examples the predicting of step (v) comprises predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher by at least 5%, at least 10%, or at least 20% in one or more plants of the subset of the oil palm plants of the treatment group, (2) at least one of the levels of the transcripts 3 or 4 being higher by at least 5%, at least 10%, or at least 20% in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher by at least 5%, at least 10%, or at least 20% in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, and/or (4) at least one of the levels of the transcripts 9 or 10 being higher by at least 5%, at least 10%, or at least 20% in the same or a different one or more plants of the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group.

The method also comprises a step of (vi) if the oil palm plants of the treatment group are thereby predicted to have tolerance to *Ganoderma boninense*, then transplanting, for cultivation for agricultural use, oil palm plants remaining from the treatment group, whereas if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then culling the oil palm plants remaining from the treatment group.

As noted above, oil palm seedlings are typically cultivated in a nursery for about 12 months, until the seedlings are about 18 months old and/or have about 15 green leaves, followed by transplanting the 18-month old seedlings to an oil palm plantation for further cultivation. The transplantation can be from the nursery to another site for further cultivation, for example a palm grove, a small holding, or an oil palm plantation. Following transplantation, and further cultivation, in the absence of disease the seedling should begin producing oil palm fruit within about two to three years, and continue for 25 or more years.

As also noted above, affected oil palm plants can die within six to twelves months after the development of symptoms, and up to 80% of plantings may die within the first half of the otherwise expected economic life of the oil palm plants. Moreover, recycled fields from coconuts planting and fields left with infected stumps have created a mass of inoculum of *Ganoderma boninense* in the soil, and new plantings of oil palms in these fields will be subjected to infection, and thus these fields will have shorter cycles in the next generation plantings.

In accordance with step (vi), if the oil palm plants of the treatment group are predicted to have tolerance to *Ganoderma boninense*, then oil palm plants remaining from the treatment group are transplanted, for cultivation for agricultural use. Because such oil palm plants remaining from the treatment group have already been exposed to *Ganoderma boninense*, and

have been predicted to be tolerant thereto, the oil palm plants remaining from the treatment group can be expected to be tolerant to *Ganoderma boninense* that may be present at the site for further cultivation.

In contrast, if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then the oil palm plants remaining from the treatment group are culled, e.g. the plants are set aside, not cultivated further, and/or destroyed, and in any case are not transplanted for cultivation, for agricultural use. Because such oil palm plants remaining from the treatment group have not been predicted to have tolerance to *Ganoderma boninense*, such culling of the oil palm plants remaining from the treatment group can be expected to decrease the frequency of transplantation of oil palm plants that will ultimately die from basal stem rot disease and to avoid a corresponding waste of resources associated with agricultural use of such oil palm plants.

This method is expected to be particularly advantageous with respect to transplantation of oil palm plants to an oil palm plantation in an area in which basal stem rot disease, as caused by *Ganoderma boninense*, is endemic. This is because cultivation of oil palm plants that have tolerance to *Ganoderma boninense* in such an area should result in longer life span and greater productivity of the oil palm plants in comparison to cultivation, in the same area, of oil palm plants that do not have tolerance to *Ganoderma boninense*, thus at least partially restoring the area as effective and useful for such cultivation.

Accordingly, in some examples step (iii) is carried out at an oil-palm nursery, and the transplanting of step (vi) comprises transplanting the oil palm plants remaining from the treatment group to an oil-palm plantation. Also, in some of these examples the oil-palm plantation is an oil palm plantation in an area in which basal stem rot disease, as caused by *Ganoderma boninense*, is endemic.

Moreover, in some examples all of the oil palm plants remaining from the treatment group are transplanted, for cultivation for agricultural use. Also in some examples at least one, but fewer than all, of the oil palm plants remaining from the treatment group are transplanted, for cultivation for agricultural use.

The method for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, can be carried out with respect to a plurality of treatment groups, for example based on treatment groups being derived from crosses of different parents and/or different crosses of the same parents. In such cases, each treatment group may have been derived from a different lineage than at least one other treatment group of the plurality. By carrying out the method with respect to a plurality of treatment groups, it is expected that new populations of oil palm plants that have tolerance to *Ganoderma boninense* will be identified over time.

Accordingly, in some examples, the method further comprises carrying out steps (i) to (vi) with respect to a plurality of treatment groups, each treatment group of the plurality having been derived from a different lineage than at least one other treatment group of the plurality.

The following examples are for purposes of illustration and are not intended to limit the scope of the claims.

Examples

Example 1 – Identification 122 Candidate Genes

A total of 122 candidate genes were found to be differentially expressed in tolerant (also termed asymptomatic) oil palm seedlings in comparison to susceptible (also termed symptomatic) oil palm seedlings and untreated control oil palm seedlings. Root samples were taken from 12-month old oil palm seedlings six months after challenge of the seedlings with *Ganoderma boninense*. RNA was extracted from the root samples and sent for RNA sequencing via Illumina platform. Transcriptome analysis showed that a group of 122 genes were expressed more highly in tolerant seedlings compared to control seedlings and susceptible seedlings. The identified genes mainly included genes that regulate cellular functions, genes encoding disease resistance proteins, and genes encoding transcription factors. The genes potentially can be used for purposes of genetic modification to produce oil palm plants that are tolerant to fungus. The genes can also be used to validate putative tolerant oil palm seedlings subjected to *Ganoderma boninense*, if the signal with respect to differential expression is systemically transduced to the other parts of the palm.

Experimental Scope & Data

Crossing program

The Dura palm selected was from treatment 40, with palm number 40. This Dura palm was from the *Johor Labis* genetic background. Crossing of the Dura palm were carried out for this experiment. BM 1372/40 comes from Progeny Trial 59 in Dusun Durian Estate, planted in May 1979, as indicated in TABLE 1. In 4 years of census, which was conducted annually in 2007, 2008, 2009, and 2011, the palm showed no sign of symptoms of infection by *Ganoderma boninense*.

TABLE 1: Background of the crossing palm.

TRT	FNO	Pedigree	FS	MS
40	BM 1372	BM 207/40 x BM 208/13	JL	JL

The germinated seeds were planted in 10 inches x 12 inches (25 cm x 30 cm) black polythene bag filled with unsterilized top soil. The seedlings were watered regularly and

fertilized following standard practices. All seedlings were maintained until six months for pathogenicity testing.

Sources of Ganoderma Culture

Cultures of *Ganoderma boninense* PER71 were obtained from *Ganoderma* & Diseases Research of Oil Palm Unit Laboratory, MPOB. This strain has been used as a standard strain of *Ganoderma boninense* by MPOB and has been given to all oil palm companies. The aim was to standardize the strain for screening and study of the pathogenicity. The culture was maintained at Crop Protection Unit, Sime Darby Research Centre, Banting, Selangor.

Preparation of Ganoderma Inoculum using Rubber Wood Blocks

Rubber wood blocks were obtained from suppliers, who custom-cut them to 6 cm x 6 cm x 6 cm (216 cm^3). All rubber wood blocks were washed and dried in an oven at 80°C overnight before being autoclaved at 121 °C for 1 hour. Each rubber wood block was placed in a plastic bag with 120 ml of Malt Extract (ME) Broth added into each bag. Bags were sealed and autoclaved at 121 °C for 15 minutes and then left to solidify overnight. The 7-day-old dikaryon inocula on the potato dextrose agar plate were cut into 4 parts and half of blocks of agar were inoculated onto the rubber wood block. The inoculated rubber wood blocks were incubated at 25-28 °C at 60-70% relative humidity for about 150 days, during which time the *Ganoderma* mycelia completely covered the rubber wood blocks.

Infection Trial

Oil palm seedlings aged 6 months were infected using a sitting technique, as previously described (Abdullah F et al., 2003, Research Bulletin Science Putra, 11, 31–33). The seedlings were transplanted into a 15 inches x 18 inches (38 cm x 46 cm) black polythene bag filled with unsterilized top soil. During planting, the inoculated rubber wood blocks, fully colonized by the pathogen, were placed in the middle of the bag, then the six-month-old seedlings were placed in closed contact with the inoculum block. Oil palm seedlings without inoculum were used as a control. Extreme care was taken to minimize injury during inoculation and planting. A concrete slab was placed underneath each polybag to prevent the geotropism growth of roots into the soil. A total of 700 seedlings were infected with *Ganoderma*, with 90 untreated seedlings as control. All seedlings were maintained and kept under artificial shade at Crop Protection Unit Nursery, Sime Darby Research Centre, Banting, Selangor. Upon 6 month-post-infection, seedlings were grouped into 3 different groups and harvested for further analysis.

Plant source

The roots from 12-month-old seedlings with 6 months post inoculation with *Ganoderma boninense* strain PER71 were harvested and cleaned with running tap water to remove the soil debris. The roots were later dapped dry by tissue paper before being cut to smaller pieces and frozen in liquid nitrogen. Samples were sent for sequencing as indicated in TABLE 2.

TABLE 2: Samples sent for RNA Sequencing

Treatment	**Palm ID**
Control	746, 747, 759
Tolerant	297, 90, 373
Susceptible DSI (Class 3) with DS (internal) (Class 2)	219, 245, 245

Disease Severity Index and Disease Severity (internal) Classification

Disease Severity Index (also termed DSI) and Disease Incidence (internal) is the most widely use scoring system for *Ganoderma boninense* infection trials. DSI and DS (internal) were calculated based on a formula as previously described (Abdullah F et al., 2003; Ilias et al., 2000, *Trichoderma* and its efficacy as a bio-control agent of basal stem rot of oil palm (*Elaeis guineensis* Jacq.), [Ph.D. Thesis.] Universiti Putra Malaysia, Selangor, Malaysia; Sapak et al., 2008, International Journal of Agriculture & Biology 10, 127-132). DSI = (number of seedlings in the rating x rating number/disease class value)/(total number of seedlings assessed x highest rating or disease class value). For DSI calculation, the seedlings were scored for disease class value based on the signs or symptoms of infection delineated in TABLE 3. DS (internal) = (number of seedlings in the rating x rating number) x 100/(total number of seedlings assessed x highest rating). For DS (internal), the seedlings were scored in accordance with TABLE 4. In this study, the palms have been classified as stage 3 with DSI although there is no formation of basidioma on the stem during sampling time.

TABLE 3: Disease Severity Index

Class				
0	**1**	**2**	**3**	**4**
Healthy (green leaves, no signs of infection)	Presence of white mycelium, with or without chlorosis/ necrosis leaves	Presence of basidioma initials on the stem base with chlorosis/ necrosis leaves (1-3 leaves)	Formation of basidioma on the stem base with chlorosis/ necrosis leaves (>3 leaves)	Presence of fruiting body with 50% necrosis leaves; plants dried or dead

TABLE 4: Disease Severity (internal)

Class

0	1	2	3	4
Healthy (no internal rot)	20% rotting of tissues	20-50% rotting of tissues	>50% rotting of tissues	>90% rotting of tissues

Methodology

RNA Extraction: Total RNAs were extracted from the three biological replicates of the root specimens using a method as previously described (Asemota & Shah, 2004, African Journal of Biotechnology, 3(11), 595-598) with some modifications. Total RNA was extracted by using a CTAB method as previously described (Asemota & Shah, 2004) with modifications from roots of three biological replicates from each control, tolerant, and susceptible group. RNA samples were then treated by DNase I (Epicentre) and heat inactivated before another round of ethanol precipitation. RNA was quantified by Nanodrop ND-1000 and the quality was checked by the readings of 260/280 and 260/230 which ranges from 1.8-2.0. The integrity of the RNA was also assessed from the sharpness of the 28s and 18s ribosomal RNA on agarose (1%) gels.

Sequencing Library Preparation: cDNA sequencing libraries from nine samples were prepared using ScriptSeq™ v2 RNA-Seq Library Preparation Kit (Epicentre, Illumina). RNA samples were treated with terminator 5'-phosphate dependent exonuclease (Epicentre, Illumina) to deplete ribosomal RNA according to manufacturer's recommendation.

Sequencing: A total of nine cDNA libraries were constructed to generate 242 million paired end reads. Sequencing was performed by using Illumina Hi-Seq 2500 platform to generate total transcriptome from roots samples.

Bioinformatics analysis: De novo assembly of raw counts were performed by using Trinity software (Broad Institute, Hebrew University of Jerusalem) and unigenes were generated by CAP3 (Huang & Madan, 1999, Genome Research 9, 868-877). NOISeq analysis was used to determine differential expression between nine samples (Tarazona et al., 2011, Genome research 21, 2213-2223). RPKM (normalized count) for each of the samples were first calculated, and M value, which is the log base 2-fold change and D value, the absolute difference of the comparison, were later determined. With this, the noise distributions were generated for the background, M-D, using the replicates. The probability values were calculated by comparing the M-D of the gene versus the noise distribution and were set at >0.75. Annotations were performed by using BLAST against UniProt database at www.uniprot.org and internal database for oil palm transcriptome.

Results

Results of differential expression of ten candidate genes, from among the 122 candidate genes that were tested, are provided in FIG. 1, FIG. 2, FIG. 3, FIG. 4, and FIG. 5. As can be seen, levels of transcripts of mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like

isoform X1 gene, alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene, probable WRKY transcription factor 40 gene, probable WRKY transcription factor 53 gene, dirigent protein 5-like gene, G-type lectin S-receptor-like serine/threonine-protein kinase gene, putative disease resistance protein RGA3 gene, polyamine oxidase gene, putative lipoxygenase 5 gene, and 12-oxophytodienoate reductase 1-like gene were higher in oil palm plants that had been treated with *Ganoderma boninense* and that were asymptomatic, i.e. having tolerance to *Ganoderma boninense*, six months after treatment, than in oil palm plants that had been treated with *Ganoderma boninense* and that were symptomatic, i.e. not having tolerance to *Ganoderma boninense*, six months after treatment, as well as in oil palm plants that had not been treated with *Ganoderma boninense*, i.e. control.

Example 2 – Validation of Ten Candidate Genes

Results Validation by Quantitative Reverse Transcription Polymerase Chain Reaction

Ten candidate genes were selected from the 122 candidate genes according to four categories: (1) cell-wall-related-protein genes, (2) transcription factor genes, (3) disease-resistance-protein genes, and (4) jasmonic-acid-signalling genes. Oil palm root samples were taken from 20-month old tolerant oil palms (inoculated with *Ganoderma boninense* at six months old) and control oil palms (non-inoculated). No susceptible seedlings were taken as all susceptible samples had succumbed to disease infection. RNA was extracted as described above and subjected to quantitative reverse transcriptase polymerase chain reaction (also termed qRT-PCR) analysis.

Methodology

cDNA Generation: Reverse transcriptions were performed by using SensiFAST™ cDNA Synthesis Kit (Bioline, UK). Samples of 2 µg of RNA from each biological replicate were used for cDNA conversion. The samples were diluted five-fold prior to use.

Primers Design and Internal Standard Curves Generation: Primer sets for the ten genes were designed on Advanced Primer 3 web-based software. Internal standard curves were generated with different dilution with a mixture of cDNA pooled samples. The standard curves must have the efficiency of 1.85-2.05 with the slope value of -3.4 to -3.8. The PCR efficiency can be calculated using the formula $E=10^{-1/slope}$. Analyses of relative expression were performed by using Efficiency Method (E-method). Cp value for the target gene and reference gene were determined by Second Derivative Maximum method. The reference gene used was the OBERON 4-like gene.

Quantitative PCR and Analysis: A PCR mixture of 20 µL consisted of 1X SyBr Green Master I, 400 nM of forward and reverse primers with 2 µL of 5-fold diluted cDNA. The qPCR was performed on the Roche LightCycler 480 with the settings shown on TABLE 5.

TABLE 5: qPCR settings

Target (°C)	Acquisition	Hold time	Ramp Rate (°C/sec)	Acquisition Mode
1) Preincubation				
95	-	5 minute	4.4	-
2) Amplification				
95	-	10 seconds	4.4	-
60	-	10 seconds	2.2	-
72	Single	10 seconds	4.4	Single
3) Melting Curve				
95	-	5 seconds	4.4	-
65	-	1 minute	2.2	-
97	Continuous	-	0.11	Continuous
4) Cooling				
40	-	30 seconds	2.2	-

Results

Results of validations of the ten candidate genes are provided in FIG. 6, FIG. 7, FIG. 8, FIG. 9, and FIG. 10. As can be seen, levels of transcripts of mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene, alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene, probable WRKY transcription factor 40 gene, probable WRKY transcription factor 53 gene, dirigent protein 5-like gene, G-type lectin S-receptor-like serine/threonine-protein kinase gene, putative disease resistance protein RGA3 gene, polyamine oxidase gene, putative lipoxygenase 5 gene, and 12-oxophytodienoate reductase 1-like gene were higher in one or more of three oil palm plants that had been treated with *Ganoderma boninense* and that were asymptomatic, i.e. having tolerance to *Ganoderma boninense*, 14 months after treatment, than in either of two oil palm plants that had not been treated with *Ganoderma boninense*, i.e. control.

Example 3 – Standard Operating Procedure

Objective

The objective of the standard operating procedure (also termed SOP) is to cultivate the oil palm seedlings and subject them to *Ganoderma boninense* strain PER71 prior to screening for putative tolerant oil palms. The putative tolerant seedlings will be randomly selected from about 10% of the balance to be checked for transcripts via qRT-PCR method to confirm up-

regulation of genes involved in conferring tolerance. All nursery practices are in accordance with standard practices for cultivation of oil palm seedlings.

Methodology

Pre-nursery

Polybag filling: Only sieved top soil should be used for polybags filling. Prior to infection trial, polybags of 10 inches x 12 inches (25 cm x 30 cm) should be used to keep the seedlings until 6 months old.

Seeds sowing: Polybags should be watered to saturation the day before the planting of seeds. Seeds should be placed with the shoot facing upwards 20 mm from above the soil.

Watering: If overhead sprinkler system is not available in the nursery, manual watering is advisable. Each seedling should receive at least 0.5 litre of water per day and up to 2 litres per day by the time the seedlings are 11 months old.

Weeding: Weeding process should be carried out manually at monthly interval. The soil surface of the polybags should be lightly forked to prevent pan forming.

Culling: Abnormal seedlings should be culled at two intervals; 3 months and prior to infection trial at 6 months old. Different types of seedlings abnormalities shall be determined by breeding unit staffs with reference to standard practices for cultivation of oil palm seedlings. 30% more than required seedlings are to be sowed for infection trials to carry out the culling procedure.

Fertilizing and manuring: Seedlings of 5 to 8 weeks old should be sprayed weekly with foliar fertiliser (11:8:6) - Bayfolan at the rate of 15 mL in 15 litres of water for 100 seedlings. Seedlings of 9 to 12 weeks old should be sprayed weekly with foliar fertiliser (11:8:6) e.g. Grofas at the rate of 15 mL in 15 litres of water for 25 seedlings. Basal organic fertiliser application from 6 to 12 weeks on top of foliar spray should be continued with basal organic fertiliser including nitrogen, phosphorus, potassium, and magnesium (14:13:9:2.5), e.g. Compound 25, as indicated in the TABLE 6.

TABLE 6: Application of basal organic fertiliser.

Weeks after planting	**Fertiliser**	**Quantity (g) for Coastal soil**
6	Compound 25	2
8	Compound 25	2
10	Compound 25	3
12	Compound 25	5
14	Compound 25	5
16	Compound 25	5
18	Compound 25	10

20	Compound 25	10
22	Compound 25	10
26	Compound 25	20
30	Compound 25	20
34	Compound 25	20
38	Compound 25	20
42	Compound 25	25
46	Compound 25	25
50	Compound 25	25
54	Compound 25	25
58	Compound 25	25

Infection trial

Sources of *Ganoderma* culture: *G. boninense* strain PER71 were obtained from *Ganoderma* & Diseases Research of Oil Palm Unit Laboratory, MPOB. This is a standard strain that is used by MPOB and provided to all oil palm companies. The aim was to standardize the strain for screening and study of the pathogenicity between the companies.

Preparation of *Ganoderma* inoculum: Rubber wood blocks are custom-cut to 6 cm x 6 cm x 6 cm (216 cm^3). The rubber wood blocks are washed and dried in an oven at 80°C for overnight before autoclaving at 121 °C for 1 hour. Each rubber wood block will be placed in a plastic bag with 120 ml of Malt Extract (ME) Broth. Bags are sealed and autoclaved at 121 °C for 15 minutes and then left to solidify overnight. The 7 day old dikaryon inocula on the potato dextrose agar plate are cut into 4 parts and a half of blocks agar will be inoculated onto the rubber wood blocks. The inoculated rubber wood blocks are incubated at 25-28 °C at 60-70% relative humidity for about 150 days during which time the *Ganoderma* mycelia completely covered the rubber wood blocks.

Infection trial: Six months old seedlings in 10 inches x 12 inches (25 cm x 30 cm) polybags are required to be transplanted into 15 inches x 18 inches (38 cm x 46 cm) polybags filled with top soil which are left settled for at least one week. Polybags should be watered to saturation the day before infection trial. During planting, the inoculated rubber wood block fully colonized by the pathogen, will be placed in the middle of the bag, then the six-month-old seedlings with half of the soil removed from the bottom are placed in closed contact with the inoculum block. Oil palm seedlings with rubber wood blocks without inoculum are used as a control.

Shading: Infection trial will be carried out in a shaded nursery with 50 % light cut off until 9 months post infection to acclimatize for another 3 months before the seedlings to be planted in the field after the infection trial.

Lab validation

Sampling: 10% of the putative tolerant seedlings will be sampled destructively to check the internal disease severity and to be tested with quantitative reverse transcriptase polymerase chain reaction method.

RNA extraction: Total RNAs were extracted from the biological replicates of the root specimens using a method as previously described (Asemota & Shah, 2004) with some modifications. Steps were as follows:

a) Grind the root tissue in liquid nitrogen with pre-treated RNAse free mortar and pestle and homogenize well before keeping in -80 °C freezer.
b) Transfer the powder form tissue into RNase free 50 ml tube (2 g/tube).
c) Add 2% (w/v) PVPP powder to fresh sample according to CTAB buffer volume (10 mL).
d) Add extraction buffer into the tube and mix well with vortex (5 mL/g tissue).
e) Add equal volume of Phenol: Chloroform: Isoamyl alcohol (P:C:I) (pH 4.3 for RNA purification).
f) Mix well by vortex for 30 seconds to 1 minute.
g) Spin at 10k rpm for 15 minutes at 4 °C.
h) Transfer aqueous layer to new tube and add equal volume of chloroform: isoamyl alcohol (24:1). Spin at 10k rpm for 15 mins at 4 °C.
i) After spun, transfer the aqueous layer to new tube and add 1/3 V of 8 M LiCl (final concentration 2 M).
j) Precipitate overnight at 4 °C.
k) Spin at 10k rpm for 20 minutes at 4 °C to pellet the RNA.
l) Discard the supernatant.
m) Re-suspend the pellet in 500 µL RNase free water and transfer into several 1.5 mL tubes.
n) Add 1/3 volume of 8 M LiCl and incubate at 4 °C for 3 hours.
o) Spin at 12 k rpm for 20 minutes at 4 °C.
p) Discard supernatant and re-suspend in 400 µL RNase-free water.
q) Add 1/10 volume of 3M sodium acetate and 2.5 volume of absolute ethanol.
r) Incubate for 3 hours at -80 °C.
s) Spin at 12 k rpm for 20 minutes at 4 °C.
t) Discard supernatant and wash with 500 µL of 70% (v/v) ethanol.

u) Spin 12 k rpm for 5 minutes and discard supernatant.

v) Air dry the RNA pellet and re-suspend in 50 or 100 µL RNase-free water.

cDNA generation: Reverse transcriptions are performed by using SensiFAST™ cDNA Synthesis Kit (Bioline, UK). Samples of 2 µg of RNA from each biological replicate are suggested for cDNA conversion according to manufacturer's guideline. The samples are to be diluted 5 times prior to use.

Quantitative PCR: PCR mixture of 20 µL consist of 1X SyBr Green Master I, 400 nM of forward and reverse primers with 2 µL of 5-fold diluted cDNA to be set up in ice. The qPCR was performed on the Roche LightCycler 480 with the settings shown in TABLE 7. The forward and reverse primers are provided in TABLE 8.

TABLE 7: qPCR settings.

Target (°C)	Acquisition	Hold time	Ramp Rate (°C/sec)	Acquisition Mode
1) Preincubation				
95	-	5 minute	4.4	-
2) Amplification				
95	-	10 seconds	4.4	-
60	-	10 seconds	2.2	-
72	Single	10 seconds	4.4	Single
3) Melting Curve				
95	-	5 seconds	4.4	-
65	-	1 minute	2.2	-
97	Continuous	-	0.11	Continuous
4) Cooling				
40	-	30 seconds	2.2	-

TABLE 8: Forward and reverse primers to be used in qPCR.

	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
Cell wall related proteins		

A: Mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1	tcggaaatctgtacccaagg (SEQ ID NO: 31)	gggactgaagccaccattag (SEQ ID NO: 32)
B: Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3	gcagcacttggattttacacc (SEQ ID NO: 33)	tcttgcgatcttgtagtatgcaat (SEQ ID NO: 34)
Transcription factors		
C: Probable WRKY transcription factor 40	aacctgcagagccagctaat (SEQ ID NO: 35)	gaccgacccctatctgag (SEQ ID NO: 36)
D: Probable WRKY transcription factor 53	ggacgatggctatagctgga (SEQ ID NO: 37)	tggatgtttggctccaagt (SEQ ID NO: 38)
Disease resistance protein		
E: Dirigent protein 5-like	atccgccaagtcttgttcc (SEQ ID NO: 39)	gtgagcaaggcctatttgga (SEQ ID NO: 40)
F: G-type lectin S-receptor-like serine/threonine-protein kinase	tgcttacaagtgatgcgaatg (SEQ ID NO: 41)	ttctccttccaaagaatgcag (SEQ ID NO: 42)
G: Putative disease resistance protein RGA3	tttaggcaaagagcatttacacc (SEQ ID NO: 43)	cgccaatttcaatcagctct (SEQ ID NO: 44)
H: Polyamine oxidase	aagctgggatcaggagcat (SEQ ID NO: 45)	gatagtatccttgcaccataagtcc (SEQ ID NO: 46)
Jasmonic acid signalling		
J: Putative lipoxygenase 5	agcgttgacggtgaggaa (SEQ ID NO: 47)	agctggcttgcaatcttctc (SEQ ID NO: 48)
K: 12-oxophytodienoate reductase 1-like	aaagatatccccggtatttgg (SEQ ID NO: 49)	cagcattcactattggtttcca (SEQ ID NO: 50)

Analysis: Analysis of the expression results is carried out relative to the uninfected control seedlings.

Industrial Applicability

The methods disclosed herein are useful for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, and thus for improving commercial production of palm oil.

Claims

1. A method for obtaining oil palm plants that have tolerance to *Ganoderma boninense*, for cultivation for agricultural use, the method comprising steps of:

(i) cultivating oil palm plants of a treatment group and oil palm plants of a control group together, the oil palm plants of the treatment group and the oil palm plants of the control group having been derived from a common lineage;

(ii) contacting roots of the oil palm plants of the treatment group with *Ganoderma boninense*, while not contacting roots of the oil palm plants of the control group with *Ganoderma boninense*;

(iii) further cultivating the oil palm plants of the treatment group and the oil palm plants of the control group together;

(iv) determining, individually in a subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group:

(1) a level of a transcript of a cell-wall-related-protein gene comprising one or more of:

(a) a level of a transcript 1 of a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene, or

(b) a level of a transcript 2 of an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene,

(2) a level of a transcript of a transcription factor gene comprising one or more of:

(a) a level of a transcript 3 of a probable WRKY transcription factor 40 gene, or

(b) a level of a transcript 4 of a probable WRKY transcription factor 53 gene,

(3) a level of a transcript of a disease-resistance-protein gene comprising one or more of:

(a) a level of a transcript 5 of a dirigent protein 5-like gene,

(b) a level of a transcript 6 of a G-type lectin S-receptor-like serine/threonine-protein kinase gene,

(c) a level of a transcript 7 of a putative disease resistance protein RGA3 gene, or

(d) a level of a transcript 8 of a polyamine oxidase gene, and

(4) a level of a transcript of a jasmonic-acid-signalling gene comprising one or more of:

(a) a level of a transcript 9 of a putative lipoxygenase 5 gene, or

(b) a level of a transcript 10 of a 12-oxophytodienoate reductase 1-like gene;

(v) predicting tolerance to *Ganoderma boninense* of the oil palm plants of the treatment group based on (1) at least one of the levels of the transcripts 1 or 2 being higher, (2) at least one of the levels of the transcripts 3 or 4 being higher, (3) at least one of the levels of the transcripts 5, 6, 7, or 8 being higher, and (4) at least one of the levels of the transcripts 9 or 10

being higher, individually in the subset of the oil palm plants of the treatment group, in comparison to the oil palm plants of the control group; and

(vi) if the oil palm plants of the treatment group are thereby predicted to have tolerance to *Ganoderma boninense*, then transplanting, for cultivation for agricultural use, oil palm plants remaining from the treatment group, whereas if the oil palm plants of the treatment group are predicted not to have tolerance to *Ganoderma boninense*, then culling the oil palm plants remaining from the treatment group.

2. The method of claim 1, wherein the transcripts 1-10 are transcripts of (1) a mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1 gene of SEQ ID NO: 1, (2) an alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3 gene of SEQ ID NO: 2, (3) a probable WRKY transcription factor 40 gene of SEQ ID NO: 3, (4) a probable WRKY transcription factor 53 gene of SEQ ID NO: 4, (5) a dirigent protein 5-like gene of SEQ ID NO: 5, (6) a G-type lectin S-receptor-like serine/threonine-protein kinase gene of SEQ ID NO: 6, (7) a putative disease resistance protein RGA3 gene of SEQ ID NO: 7, (8) a polyamine oxidase gene of SEQ ID NO: 8, (9) a putative lipoxygenase 5 gene of SEQ ID NO: 9, and (10) a 12-oxophytodienoate reductase 1-like gene of SEQ ID NO: 10, respectively.

3. The method of claim 1 or 2, wherein step (i) is carried out from germination of the oil palm plants of the treatment group and the oil palm plants of the control group.

4. The method of claims 1, 2, or 3, wherein the contacting of step (ii) is initiated when the oil palm plants of the treatment group are 4 to 8 months old.

5. The method of any of claims 1-4, wherein the contacting of step (ii) comprises contacting the oil palm plants of the treatment group with an inoculum block that is colonized with *Ganoderma boninense*.

6. The method of any one of claims 1-5, wherein the *Ganoderma boninense* comprises *Ganoderma boninense* PER71.

7. The method of any one of claims 1-6, wherein step (iv) is carried out when the oil palm plants of the treatment group and the oil palm plants of the control group are 12 to 24 months old.

8. The method of any one of claims 1-7, wherein the determining of step (iv) comprises determining the level of one or more of the transcripts 1-10 individually in roots of the subset

of the oil palm plants of the treatment group, in comparison to roots of the oil palm plants of the control group.

9. The method of any one of claims 1-8, wherein the determining of step (iv) comprises determining the level of one or more of the transcripts 1-10 by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

10. The method of claim 9, wherein the qRT-PCR comprises determining the level of one or more of the transcripts 1-10 by use of (1) a forward primer of SEQ ID NO: 31 and a reverse primer of SEQ ID NO: 32, (2) a forward primer of SEQ ID NO: 33 and a reverse primer of SEQ ID NO: 34, (3) a forward primer of SEQ ID NO: 35 and a reverse primer of SEQ ID NO: 36, (4) a forward primer of SEQ ID NO: 37 and a reverse primer of SEQ ID NO: 38, (5) a forward primer of SEQ ID NO: 39 and a reverse primer of SEQ ID NO: 40, (6) a forward primer of SEQ ID NO: 41 and a reverse primer of SEQ ID NO: 42, (7) a forward primer of SEQ ID NO: 43 and a reverse primer of SEQ ID NO: 44, (8) a forward primer of SEQ ID NO: 45 and a reverse primer of SEQ ID NO: 46, (9) a forward primer of SEQ ID NO: 47 and a reverse primer of SEQ ID NO: 48, or (10) a forward primer of SEQ ID NO: 49 and a reverse primer of SEQ ID NO: 50, respectively.

11. The method of any one of claims 1-10, wherein the determining of step (iv) comprises determining the levels of all of the transcripts 1-10 individually in roots of the subset of the oil palm plants of the treatment group, in comparison to roots of the oil palm plants of the control group.

12. The method of any one of claims 1-11, wherein the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a *Johor Labis dura* x *Johor Labis dura* population, an *Ulu Remis dura* x *Ulu Remis dura* population, a *Banting dura* x *Banting dura* population, a *Johor Labis dura* x *Ulu Remis dura* population, a *Johor Labis dura* x *Banting dura* population, or an *Ulu Remis dura* x *Banting dura* population, and the agricultural use is (i) mother palm selection and propagation or (ii) introgressed mother palm selection and propagation.

13. The method of any one of claims 1-11, wherein the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from an AVROS *pisifera* x AVROS *tenera* population, or an AVROS *tenera* x AVROS *tenera* population, and the agricultural use is pollen donor selection and propagation.

14. The method of any one of claims 1-11, wherein the oil palm plants of the treatment group and the oil palm plants of the control group have been derived from a Nigerian *dura* x AVROS *pisifera* population, a Deli *dura* x AVROS *pisifera* population, an *Ulu Remis dura* x AVROS *pisifera* population, or a *Banting dura* x AVROS *pisifera* population, and the agricultural use is production of palm oil.

15. The method of any one of claims 1-14, wherein step (iii) is carried out at an oil-palm nursery, and the transplanting of step (vi) comprises transplanting the oil palm plants remaining from the treatment group to an oil-palm plantation.

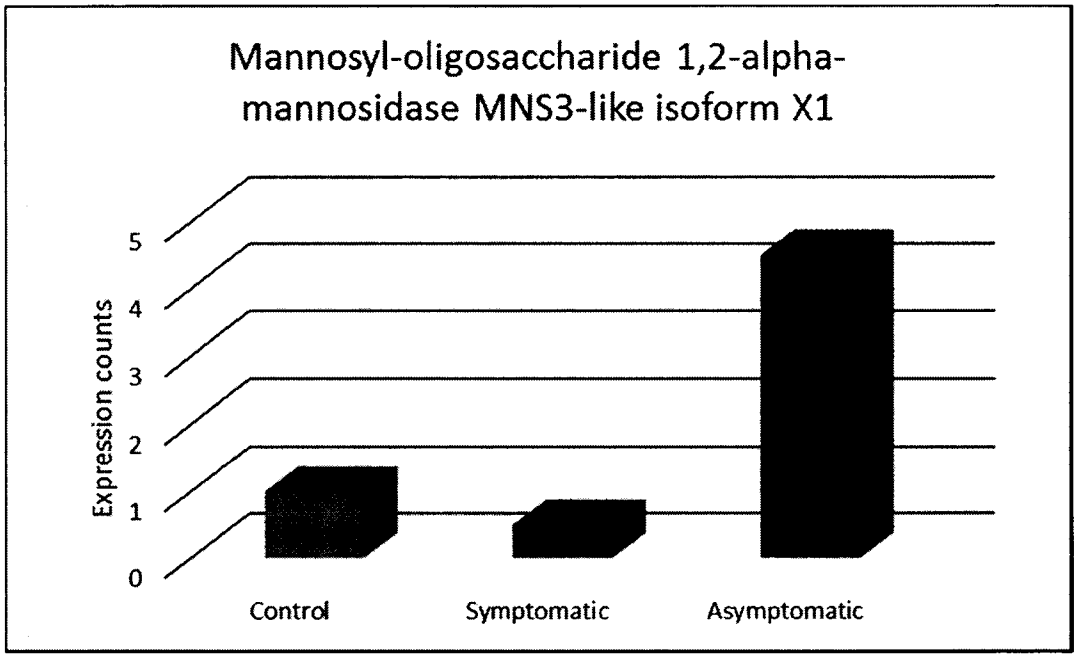
16. The method of claim 15, wherein the oil-palm plantation is an oil palm plantation in an area in which basal stem rot disease, as caused by *Ganoderma boninense*, is endemic.

17. The method of any one of claims 1-16, in which the common lineage comprises identical parentage.

18. The method of claim 17, in which the identical parentage comprises a *dura* mother palm plant and a *dura* father palm plant.

19. The method of any one of claims 1-18, further comprising carrying out steps (i) to (vi) with respect to oil palm plants of a plurality of treatment groups, each treatment group of the plurality having been derived from a different lineage than at least one other treatment group of the plurality.

A


Mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1
Expression counts
0
1
2
3
4
5
Control
Symptomatic
Asymptomatic

B

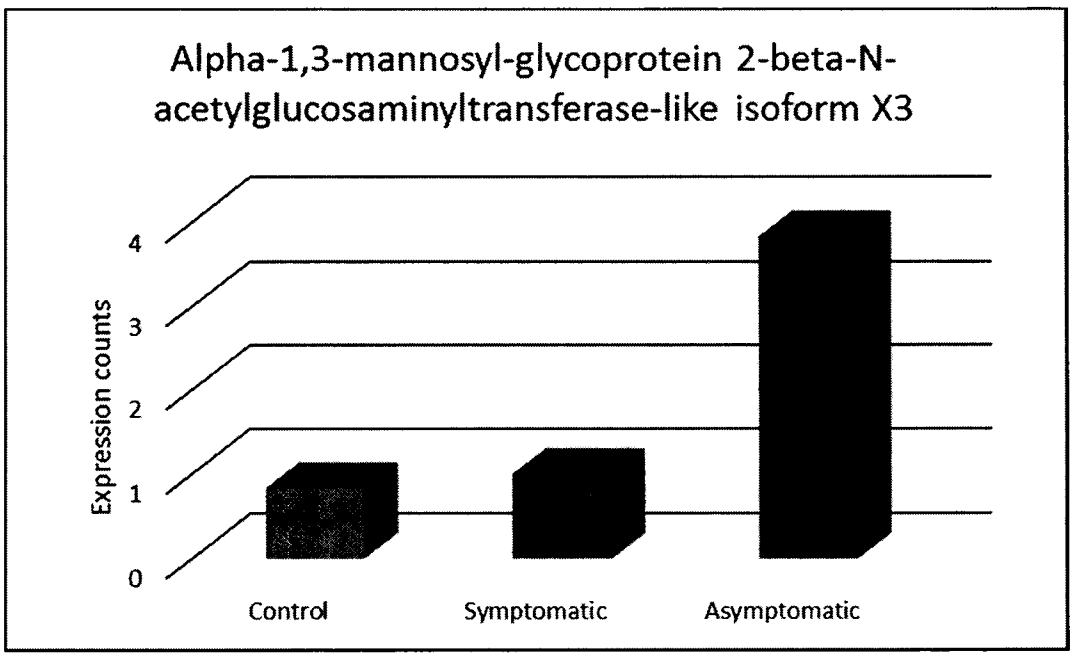
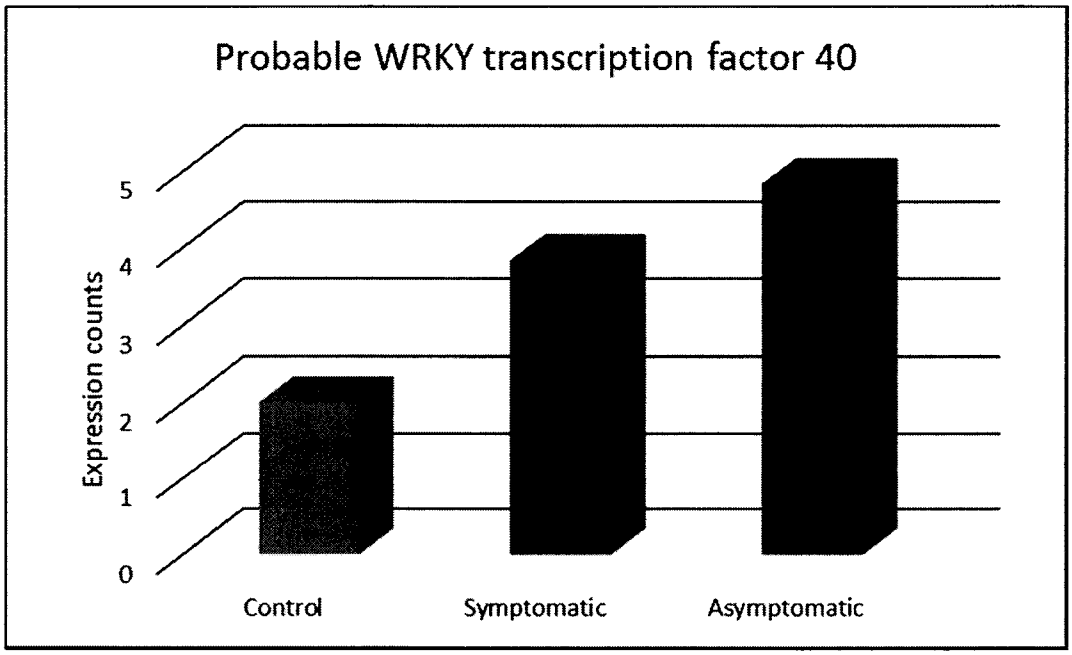

Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3
Expression counts
0
1
2
3
4
Control
Symptomatic
Asymptomatic

FIG. 1

A


Probable WRKY transcription factor 40
Expression counts
0
1
2
3
4
5
Control
Symptomatic
Asymptomatic

B

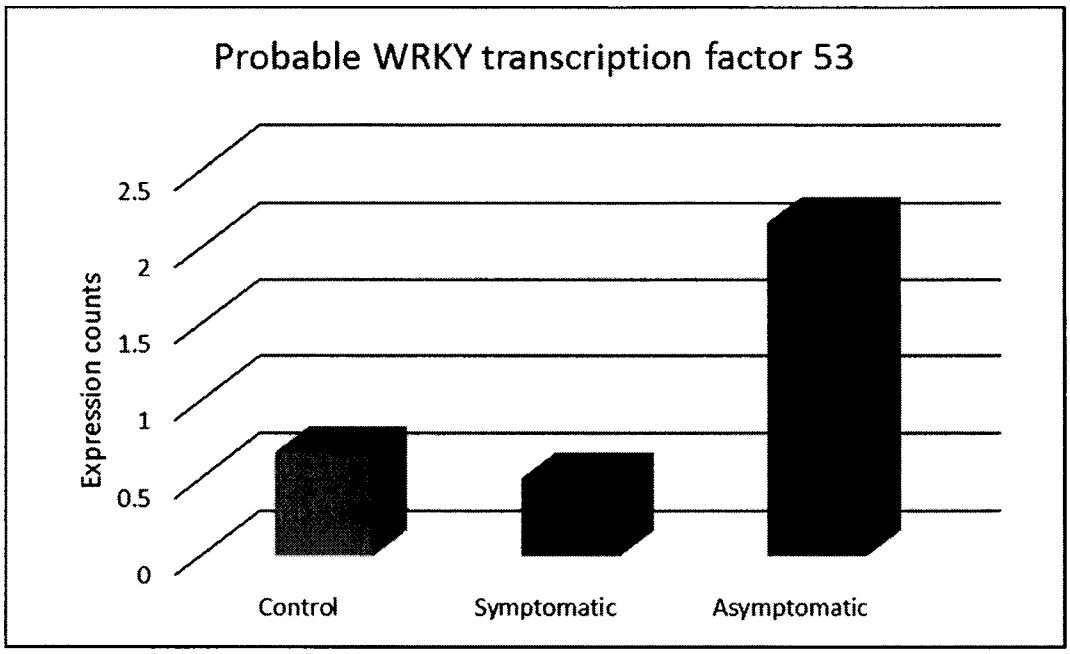
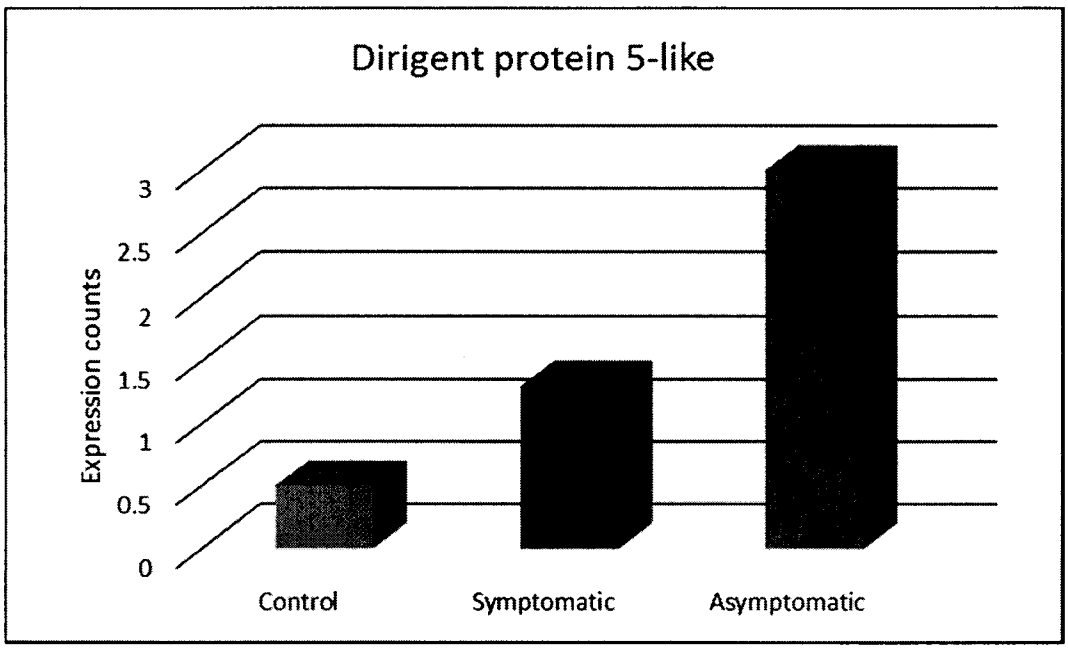

Probable WRKY transcription factor 53
Expression counts
0
0.5
1
1.5
2
2.5
Control
Symptomatic
Asymptomatic

FIG. 2

A


Dirigent protein 5-like
Expression counts
3
2.5
2
1.5
1
0.5
0
Control
Symptomatic
Asymptomatic

B

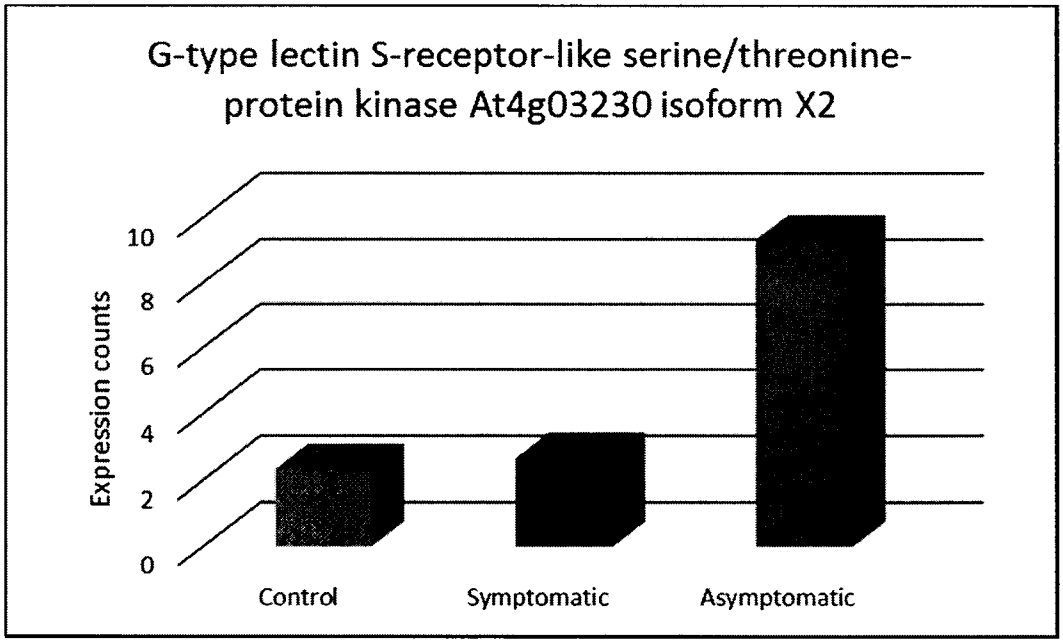
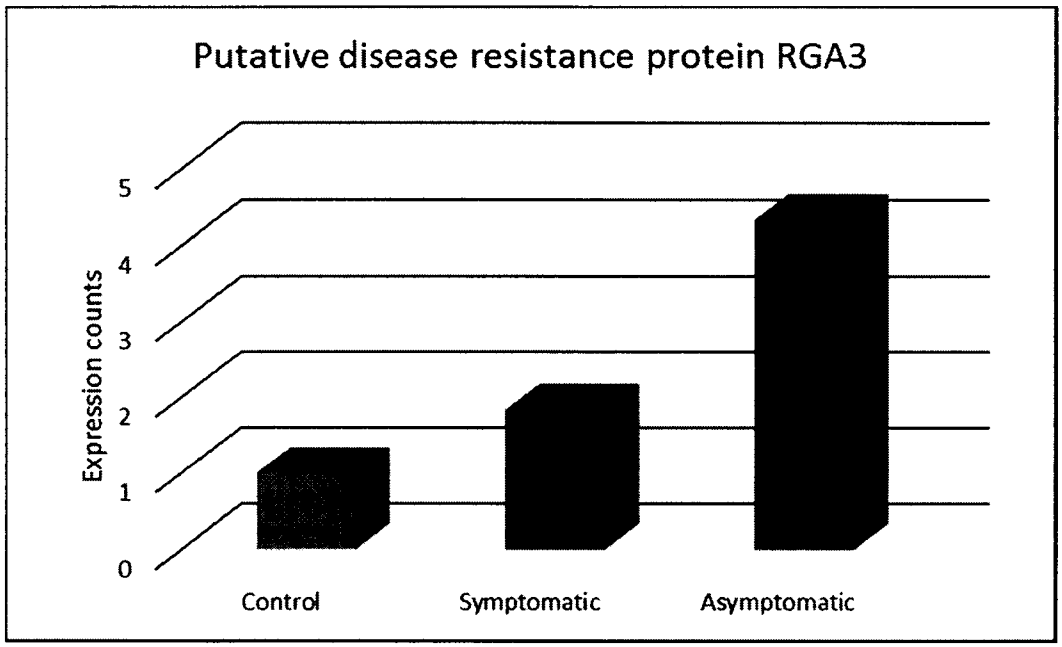

G-type lectin S-receptor-like serine/threonine-protein kinase At4g03230 isoform X2
Expression counts
10
8
6
4
2
0
Control
Symptomatic
Asymptomatic

FIG. 3

A


Putative disease resistance protein RGA3
Expression counts
0
1
2
3
4
5
Control
Symptomatic
Asymptomatic

B

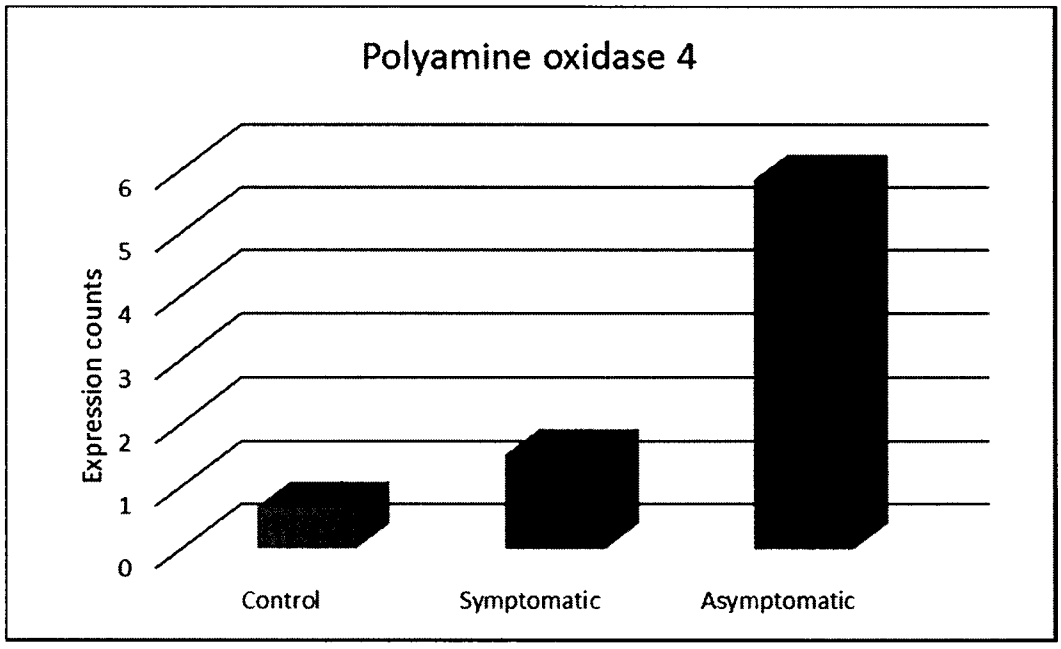
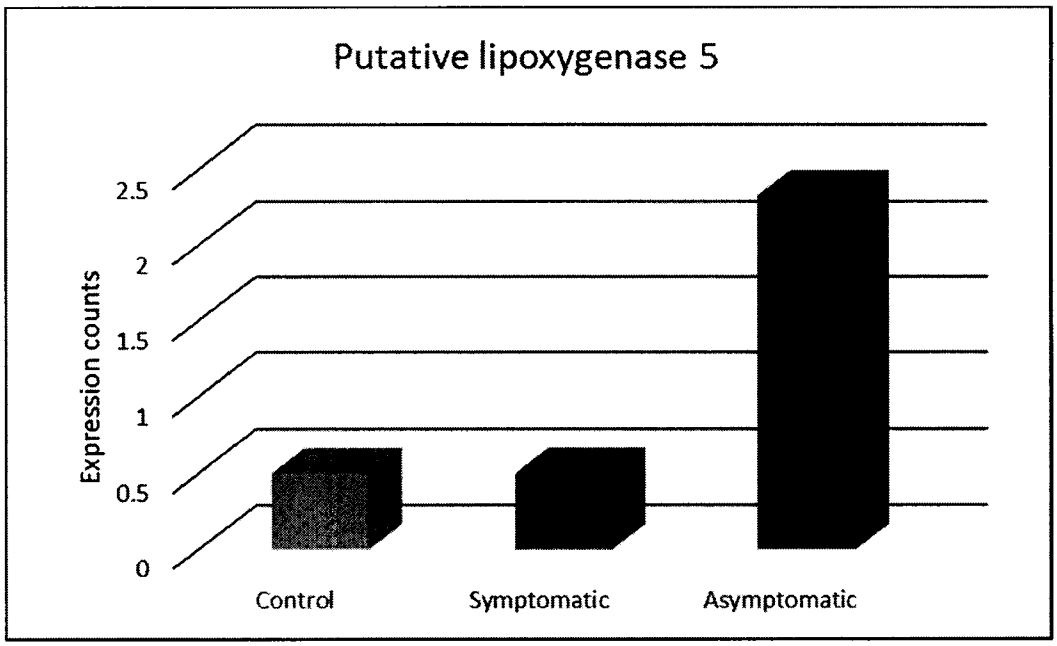

Polyamine oxidase 4
Expression counts
0
1
2
3
4
5
6
Control
Symptomatic
Asymptomatic

FIG. 4

A


Putative lipoxygenase 5
Expression counts
2.5
2
1.5
1
0.5
0
Control
Symptomatic
Asymptomatic

B

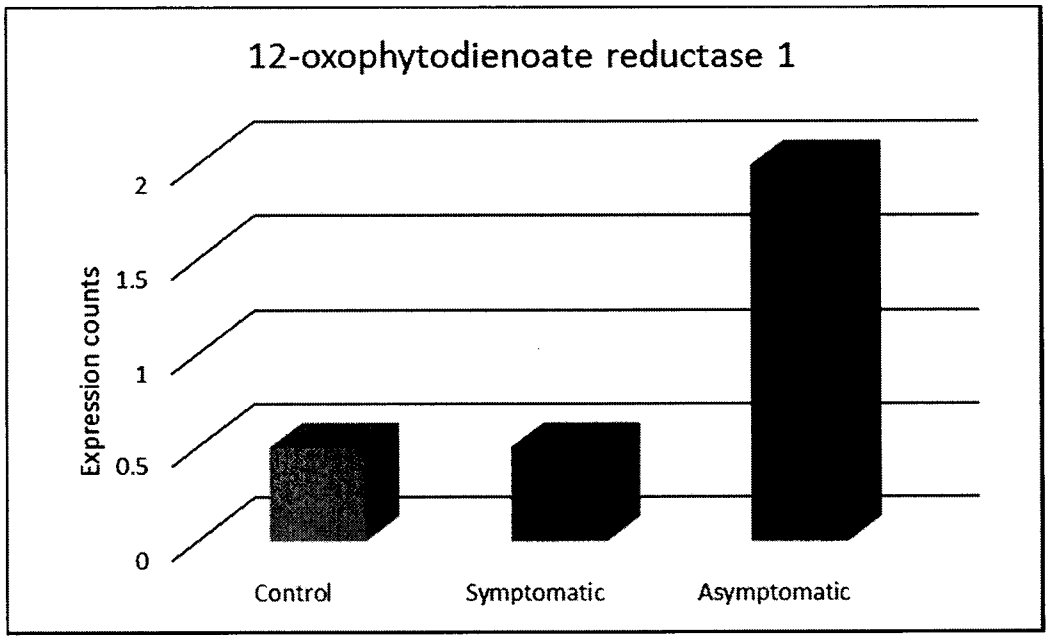
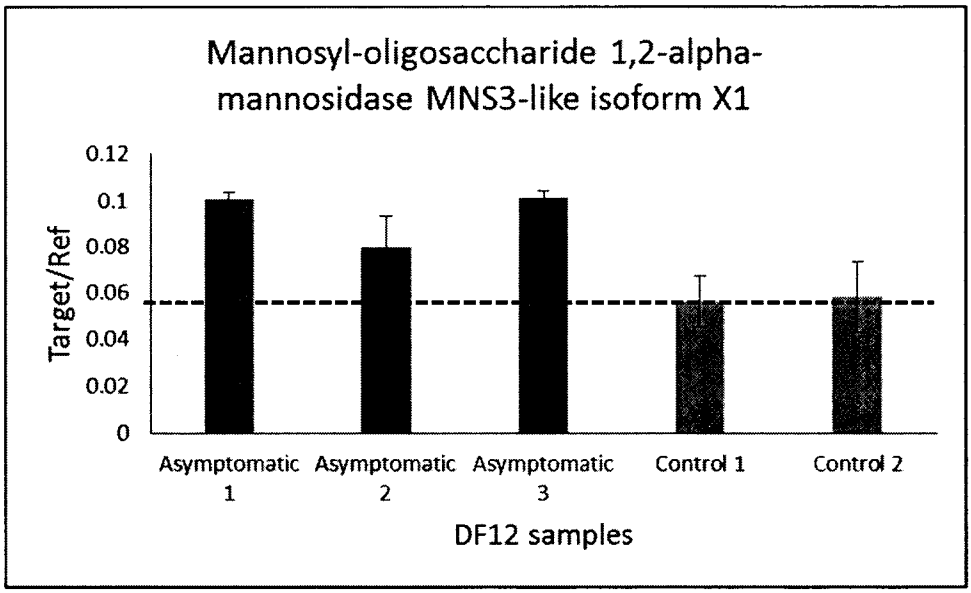

12-oxophytodienoate reductase 1
Expression counts
2
1.5
1
0.5
0
Control
Symptomatic
Asymptomatic

FIG. 5

A


Mannosyl-oligosaccharide 1,2-alpha-mannosidase MNS3-like isoform X1
Target/Ref
0.12
0.1
0.08
0.06
0.04
0.02
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

B

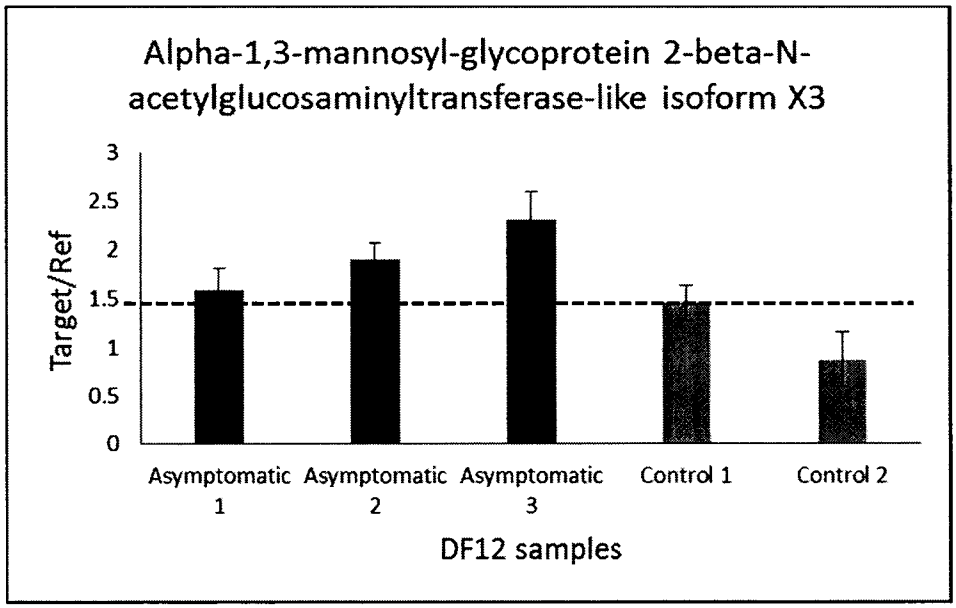
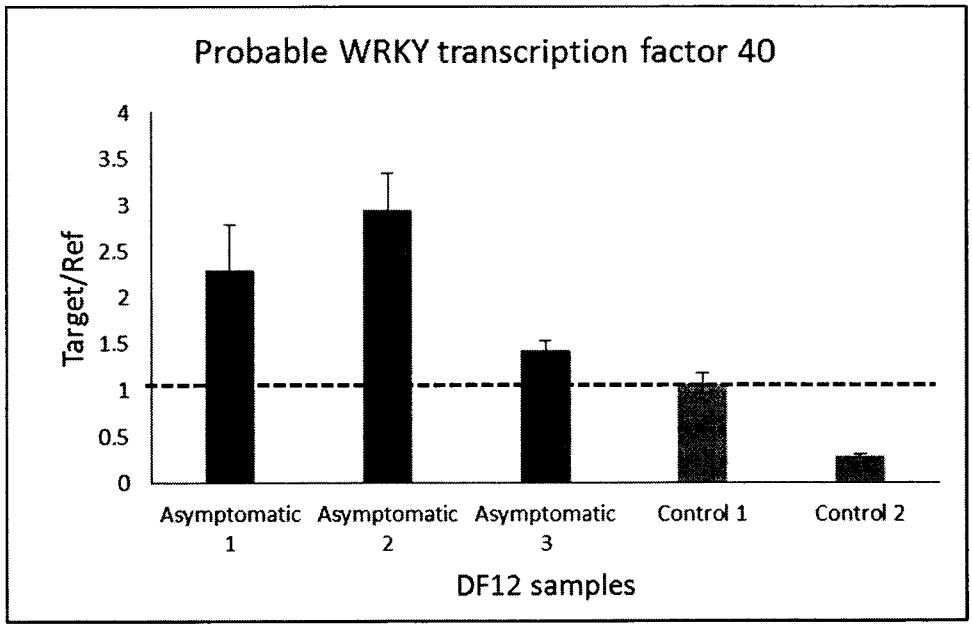

Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like isoform X3
Target/Ref
3
2.5
2
1.5
1
0.5
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

FIG. 6

A


Probable WRKY transcription factor 40
Target/Ref
4
3.5
3
2.5
2
1.5
1
0.5
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

B

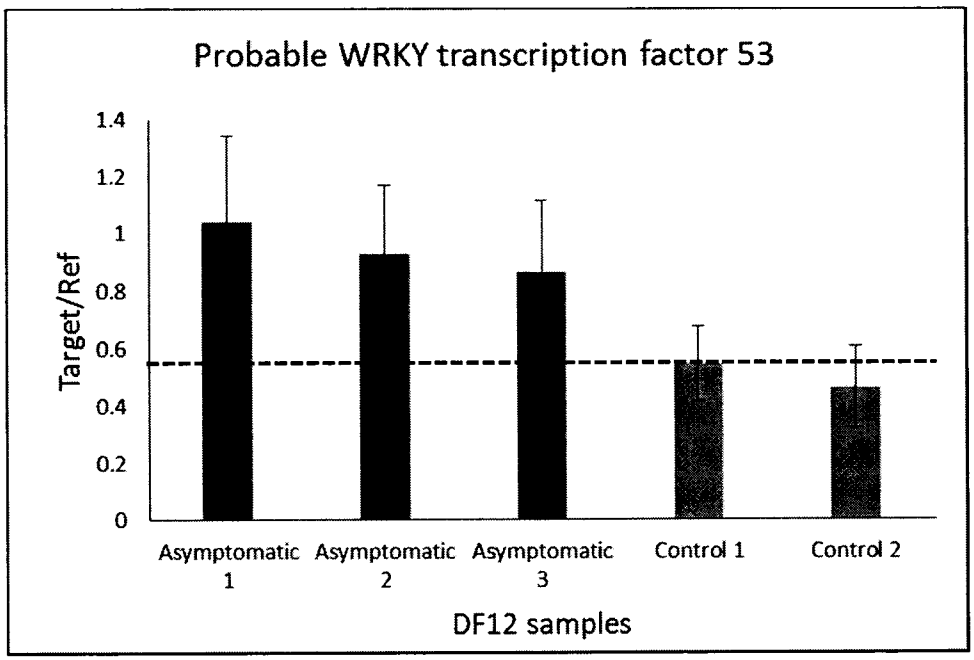
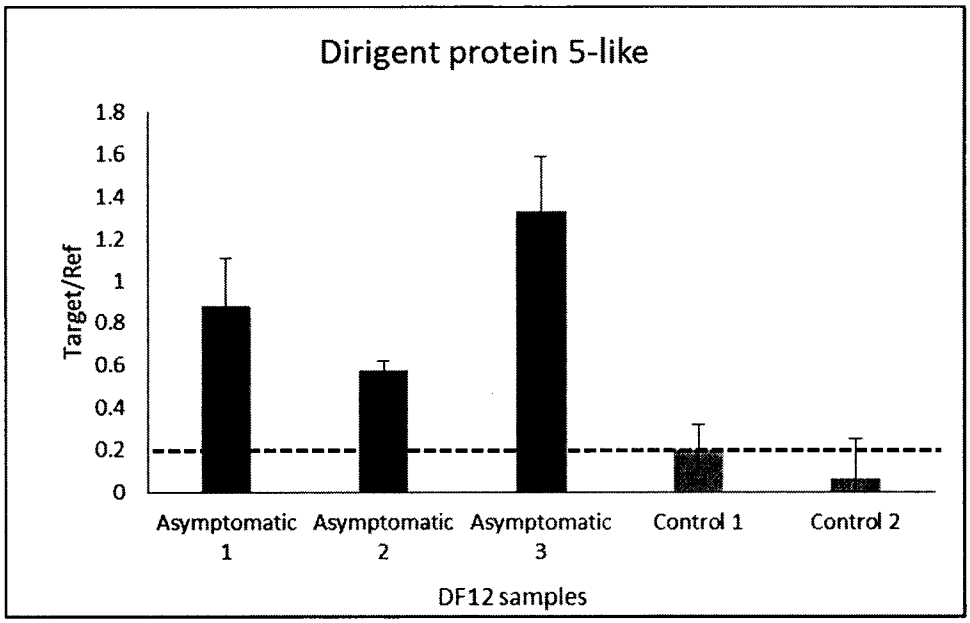

Probable WRKY transcription factor 53
Target/Ref
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

FIG. 7

A


Dirigent protein 5-like
Target/Ref
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

B

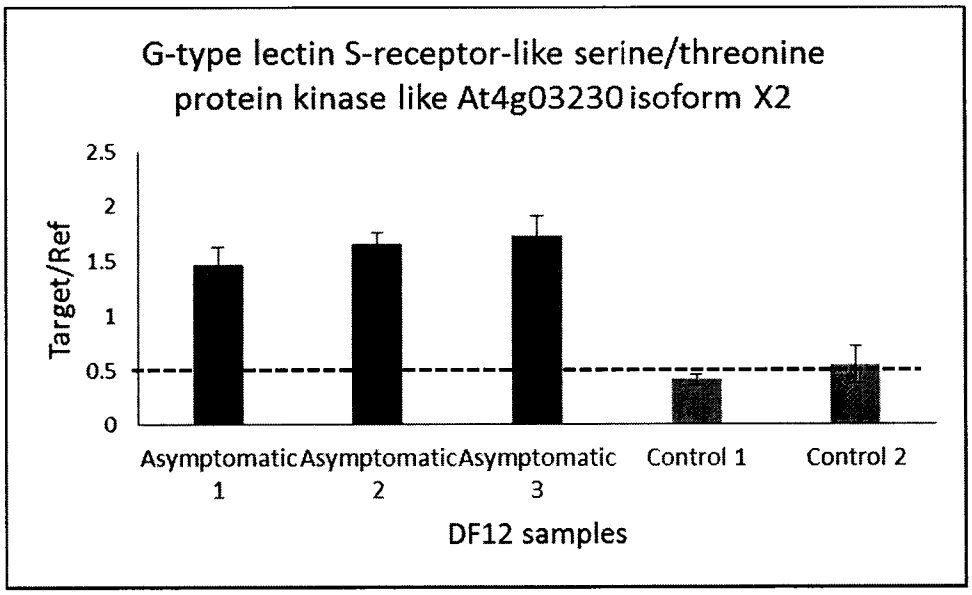
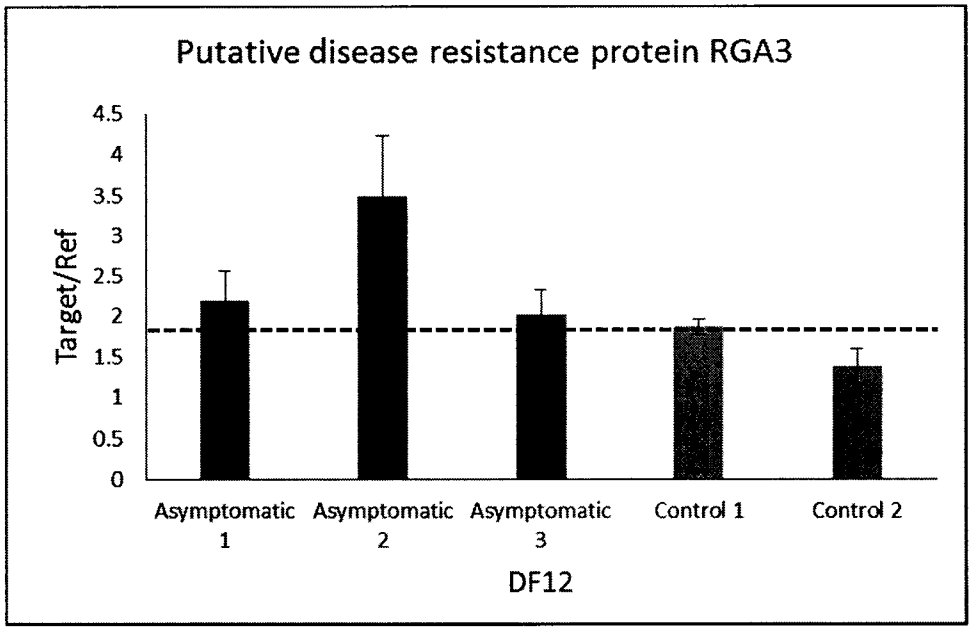

G-type lectin S-receptor-like serine/threonine protein kinase like At4g03230 isoform X2
Target/Ref
2.5
2
1.5
1
0.5
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

FIG. 8

A


Putative disease resistance protein RGA3
Target/Ref
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12

B

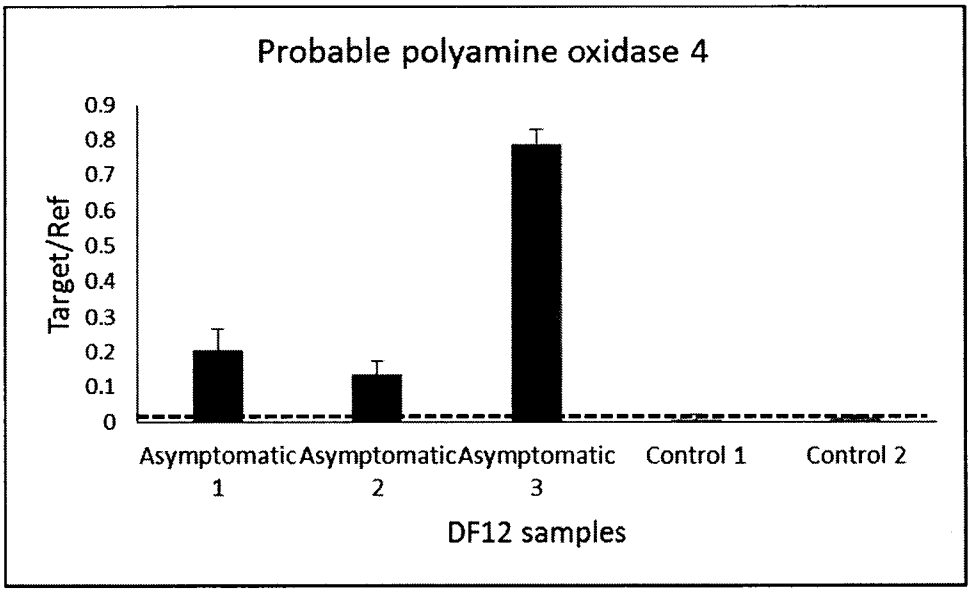
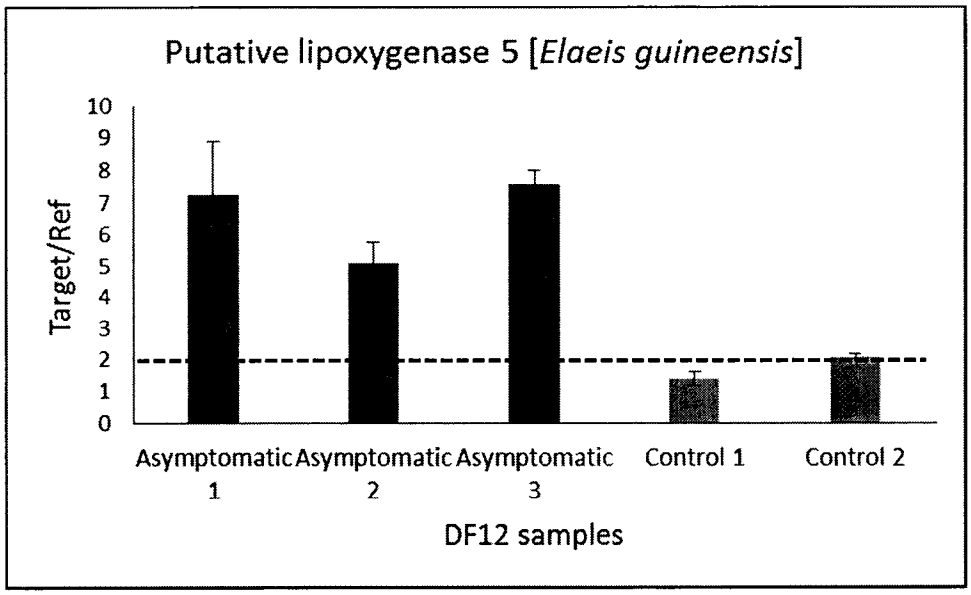

Probable polyamine oxidase 4
Target/Ref
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

FIG. 9

A


Putative lipoxygenase 5 [*Elaeis guineensis*]
Target/Ref
10
9
8
7
6
5
4
3
2
1
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

B

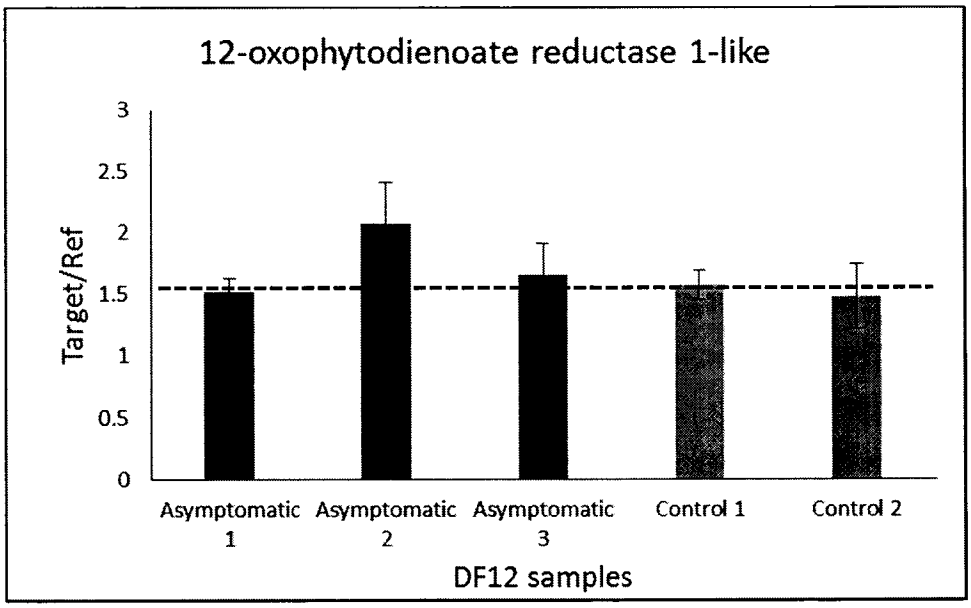

12-oxophytodienoate reductase 1-like
Target/Ref
3
2.5
2
1.5
1
0.5
0
Asymptomatic 1
Asymptomatic 2
Asymptomatic 3
Control 1
Control 2
DF12 samples

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/MY2017/000003

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/04 A01H5/10 C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/084714 A1 (UNIV PUTRA MALAYSIA [MY]) 5 June 2014 (2014-06-05) page 3, lines 1-21	1-19
A	WO 2015/170961 A1 (MALAYSIAN PALM OIL BOARD [MY]) 12 November 2015 (2015-11-12) page 5, lines 1-15	1-19

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
6 June 2017	21/06/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bilang, Jürg

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAHEBI MAHBOD ET AL: "Towards immunity of oil palm against Ganodermafungus infection", ACTA PHYSIOLOGIAE PLANTARUM, PWN-POLISH SCIENTIFIC PUBLISHERS, WARSZAW, PL, vol. 37, no. 10, 195, 4 September 2015 (2015-09-04), pages 1-16, XP035575823, ISSN: 0137-5881, DOI: 10.1007/S11738-015-1939-Z [retrieved on 2015-09-04] page 2, right-hand column, paragraph 3 page 8, left-hand column, paragraph 2 - page 9, left-hand column, paragraph 2	1-19
A	HO C-L ET AL: "Molecular defense response of oil palm to Ganoderma infection", PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 114, 12 November 2014 (2014-11-12), pages 160-169, XP029603896, ISSN: 0031-9422, DOI: 10.1016/J.PHYTOCHEM.2014.10.016 page 164, paragraph 4. Gene expression ...	1-19
A	SUE-SEAN TEE ET AL: "Transcriptome of oil palm (Elaeis guineensis Jacq.) roots treated with Ganoderma boninense", TREE GENETICS & GENOMES, vol. 9, no. 2, 1 April 2013 (2013-04-01), pages 377-386, XP055378653, Berlin/Heidelberg ISSN: 1614-2942, DOI: 10.1007/s11295-012-0559-7 the whole document	1-19
A	MARIA EUGENIA LISEI DE SÁ ET AL: "Transcriptome analysis of resistant soybean roots infected by Meloidogyne javanica", GENETICS AND MOLECULAR BIOLOGY, vol. 35, no. 1, 1 June 2012 (2012-06-01), pages 272-282, XP055378752, BR ISSN: 1415-4757, DOI: 10.1590/S1415-47572012000200008 the whole document	1-19

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	XIAO QIANG LIU ET AL: "OsWRKY03, a rice transcriptional activator that functions in defense signaling pathway upstream of OsNPR1", CELL RESEARCH - XIBAO YANJIU, vol. 15, no. 8, 1 August 2005 (2005-08-01) , pages 593-603, XP055378783, GB, CN ISSN: 1001-0602, DOI: 10.1038/sj.cr.7290329 abstract	1-19
A,P	SARGOLZAEI M ET AL: "Characterization of novel type I ribosome-inactivating proteins isolated from oil palm (Elaeis guineensis) inoculated with Ganoderma boninense, the causal agent of basal stem rot", PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, vol. 94, 19 April 2016 (2016-04-19), pages 53-61, XP029631891, ISSN: 0885-5765, DOI: 10.1016/J.PMPP.2016.04.002 abstract	1-19
A	HAMA-ALI EMAD OMER ET AL: "Association between basal stem rot disease and simple sequence repeat markers in oil palm, Elaeis guineensis Jacq.", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 202, no. 2, 9 October 2014 (2014-10-09), pages 199-206, XP035451305, ISSN: 0014-2336, DOI: 10.1007/S10681-014-1270-6 [retrieved on 2014-10-09] the whole document	1-19

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/MY2017/000003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014084714 A1	05-06-2014	NONE	
WO 2015170961 A1	12-11-2015	NONE	