(12) STANDARD PATENT (11) Application No. AU 2022422213 B2
(19) AUSTRALIAN PATENT OFFICE

(54) Title
A METHOD FOR SCREENING AND BREEDING PLANTS WHICH ARE RESISTANT TO PLANT PATHOGENS

(51) International Patent Classification(s)
A01H 1/04 (2006.01) ***A01H 5/00*** (2018.01)
A01H 1/00 (2006.01) ***G01N 33/00*** (2006.01)
A01H 3/00 (2006.01)

(21) Application No: **2022422213** (22) Date of Filing: **2022.12.23**

(87) WIPO No: **WO23/115155**

(30) Priority Data

(31) Number	(32) Date	(33) Country
2021904250	**2021.12.23**	**AU**

(43) Publication Date: **2023.06.29**
(44) Accepted Journal Date: **2024.06.13**

(71) Applicant(s)
THE CROWN IN RIGHT OF THE STATE OF NEW SOUTH WALES ACTING THROUGH REGIONAL NSW

(72) Inventor(s)
MILGATE, Andrew William;OVENDEN, Benjamin Walter;YANG, Nannan

(74) Agent / Attorney
FB Rice Pty Ltd, L 23 44 Market St, Sydney, NSW, 2000, AU

(56) Related Art
WO 2023/115155 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 June 2023 (29.06.2023)

(10) International Publication Number
WO 2023/115155 A1

(51) International Patent Classification:
A01H 1/04 (2006.01) *A01H 5/00* (2018.01)
A01H 1/00 (2006.01) *G01N 33/00* (2006.01)
A01H 3/00 (2006.01)

(21) International Application Number:
PCT/AU2022/051581

(22) International Filing Date:
23 December 2022 (23.12.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2021904250 23 December 2021 (23.12.2021) AU

(71) Applicant: THE CROWN IN RIGHT OF THE STATE OF NEW SOUTH WALES ACTING THROUGH REGIONAL NSW [AU/AU]; Department of Primary Industries, 105 Prince Street, Orange, New South Wales 2800 (AU).

(72) Inventors: MILGATE, Andrew William; c/o The Crown in right of the State of New South Wales acting through Regional NSW, Department of Primary Industries, 105 Prince Street, Orange, New South Wales 2800 (AU). OVENDEN, Benjamin Walter; c/o The Crown in right of the State of New South Wales acting through Regional NSW, Department of Primary Industries, 105 Prince Street, Orange, New South Wales 2800 (AU). YANG, Nannan; c/o The Crown in right of the State of New South Wales acting through Regional NSW, Department of Primary Industries, 105 Prince Street, Orange, New South Wales 2800 (AU).

(74) Agent: FB RICE; Level 23, 44 Market Street, Sydney, New South Wales 2000 (AU).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV,

(54) **Title:** A METHOD FOR SCREENING AND BREEDING PLANTS WHICH ARE RESISTANT TO PLANT PATHOGENS

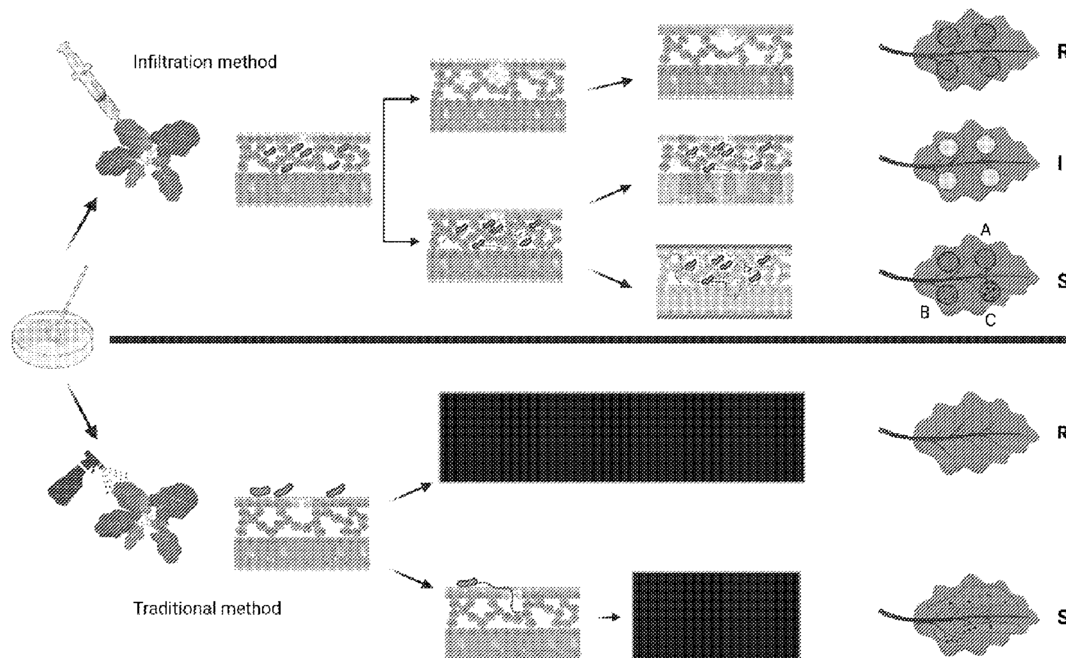

Figure 1

(57) **Abstract:** The present disclosure relates to a phenotype-based method for screening and identifying plants which exhibit resistance phenotypes to plant pathogens, and the use of same in plant breeding.

[Continued on next page]

GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

" A method for screening and breeding plants which are resistant to plant pathogens"

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims priority from Australian Provisional Patent Application No 2021904250 filed on 23 December 2021, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

10 The present disclosure relates to a phenotype-based method for screening and identifying plants which exhibit resistance phenotypes to plant pathogens, and the use of same in plant breeding.

BACKGROUND

15 Plants possess a powerful system to defend themselves against potential threats by pathogenic organisms. For agriculturally important plants, however, current measures to combat such pathogens have proved conservative and, thus, not sufficiently effective. Some recent efforts have focused on the identification of novel host-resistance factors to assist in controlling plant diseases naturally through the identification of resistant germplasm, the isolation and 20 characterization of resistance genes, and the molecular breeding of resistant cultivars. Unfortunately, however, resistance genes to fungal and bacterial pathogens in agricultural crops are rare. It also takes considerable breeding effort to introduce such genes into a single cultivar.

Modern agricultural practices provide suitable environmental conditions for infectious plant pathogens to evolve and regularly overcome host resistance. Continuous search for new 25 resistant plants carrying novel disease resistance genes, therefore, is a key component of breeding activities around the globe. Individual resistance genes, unfortunately, may not provide lasting protection to crops. For example, the wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*) pathogen has a well-documented history of evolving new strains against single wheat resistance genes. In Australia the yellow rust strain 134 E16 A+ entered the country in 2002 and quickly 30 evolved acquiring resistance to the genes Yr17 and Yr10 by 2006 (Wellings 2007). Combinations of resistance genes could increase the evolutionary barriers a pathogen must overcome to cause significant disease.

The infection of plants by fungal pathogens is a complex process that can be divided into multiple steps. Traditional disease phenotyping methodologies used to identifying plants 35 exhibiting a disease resistant phenotype were designed to mimic a natural infection process where the pathogen must overcome external barriers to enter the plant. The rationale behind this approach is to minimise phenotyping false positives (type I error) in controlled environment

studies, which ware often relied on to predict field performance of the identified resistance traits. Breeders must be sure that the phenotypes they select would translate into genetic gain and improved cultivars, generally with the lowest cost phenotyping approach for maximum breeding program efficiency. The key deficiency with traditional phenotyping methods is that they do not provide any indication of how and when the plant disease resistance is activated in response to the pathogen other than a binary view of the disease outcome.

Traditional phenotyping techniques have been widely used to discover and clone over 314 resistance genes in multiple crop species to date, such as in rice (50 genes), tomatoes (43 genes), wheat (27 genes) and barley (26 genes) (Kourelis and van der Hoorn 2018). However, these methods provide no insight into differentiation of plant resistance mechanisms or defence actions of the host. Such information can only be obtained once a functional analysis of the gene is completed. The mechanisms for gene action has only been suggested in approximately 41% of the cloned resistance genes (Kourelis and van der Hoorn 2018). Investigating the functional role of a single plant resistance gene currently takes approximately 10 years to conclude and is very resource intensive, preventing breeders from selecting optimal resistance combinations to achieve crop protection.

As the available methodologies to identify disease resistance treat the total infection process as a single event, it limits the ability of breeders and researchers to identify the activation of plant resistance at different stages of infection. There is currently no rapid phenotyping method available to identify plant resistance at different stages of infection by fungal pathogens.

SUMMARY

Classical phenotyping methods are designed to mimic natural infection processes and cannot distinguish when a pathogen is recognized by the plant or how it then acts to prevent or slow infection and disease. The inventors have invented a phenotyping method which allows plant reactions against plant pathogens (such as fungal pathogens) to be categorized into different classes of defence. As the pathogenic inoculum gets applied in two separate steps to plant leaves by surface spraying and infiltration of spores and/or mycelium fragments, it results in dissection of the infection process, thereby allowing detection of when defence responses are triggered to protect the plant. The new method described in this disclosure, as mentioned above, dissects the infection process into two main phases, pre and post entry of the pathogen past the leaf external layers. Plants can trigger defence responses to a pathogen at different points along the infection pathway. The recognition of the presence of the pathogen by the host is the first step in this process and when this occurs the plant triggers a defence response. This recognition step can occur at the earliest point of a spore landing on a leaf or at any point of the following stages of infection and disease development. The inventors have taken a novel approach by combining two different inoculation techniques which allows the identification and separation of when defence is triggered by the plant. The phenotyping method represents a large step forward in developing

crop varieties with multiple layers of defence against pathogens, such as fungi, particularly for species of plant where there is little or no knowledge of the genetic background which confers disease resistance or susceptibility.

Accordingly, in one example, the present disclosure provides a method of identifying a plant which exhibits a resistance phenotype to a plant pathogen, the method comprising:

(a) performing a first screening method comprising:

(i) contacting the surface of a first plant or part thereof, or first group of plants or parts thereof, with an inoculum of the plant pathogen;

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the plant pathogen to infect the plant(s) or plant part(s); and

(iii) determining one or more responses of the plant(s) or plant part(s) to the pathogen at a plurality of time points during step (ii);

(b) performing a second screening method comprising:

(i) infiltrating a second plant or part thereof, or second group of plants of parts thereof, with an inoculum of the plant pathogen, wherein the second plant(s) or plant part(s) are of the same species and/or cultivar as the first plant(s);

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the plant pathogen to infect the plant(s) or plant part(s) and

(iii) determining one or more responses of the plant(s) or plant part(s) to the pathogen at a plurality of time points during step (ii); and

(c) determining whether or not the plant species or cultivar exhibits a resistance phenotype to the plant pathogen based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second screening methods.

In one example, the method comprises determining whether or not the plant species or cultivar exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof, based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second methods. For example, the method may comprise determining that the plant species or cultivar exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, but not after entry of the plant pathogen into the plant or part thereof, based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second methods. For example, the method may comprise determining that the plant species or cultivar exhibits a resistance phenotype to the plant pathogen after entry of the plant pathogen into the plant or part thereof, but not prior to and/or during entry of the pathogen into the plant or part thereof, based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second methods. In some examples, the method may comprise determining that the plant species or cultivar exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, as

well as after entry of the plant pathogen into the plant or part thereof, based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second methods.

In one example, the first screening method comprises contacting the surface of the first plant(s) or plant part(s) with the inoculum by spraying, swiping and/or swabbing the inoculum onto the surface of the plant(s) or plant part(s), and/or dipping or soaking the plant(s) or plant part(s) in the inoculum. For example, the first screening method may comprise contacting the surface of the first plant(s) or plant part(s) with the inoculum by spraying the plant(s) or plant part(s) with the inoculum. For example, the first screening method may comprise contacting the surface of the first plant(s) or plant part(s) with the inoculum by swiping and/or swabbing the surface of the plant(s) or plant part(s) with the inoculum. For example, the first screening method may comprise contacting the surface of the first plant(s) or plant part(s) with the inoculum by dipping, immersing and/or soaking the plant(s) or plant part(s) in the inoculum. Any one or more of the fore-mentioned methods may be used for contacting the surface of the first plant(s) or plant part(s) with the inoculum.

In one example, the second screening method comprises infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe infiltration, syringe pressure infiltration and/or vacuum infiltration. For example, the second screening method may comprise infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe infiltration. For example, the second screening method may comprise infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe pressure infiltration. For example, the second screening method may comprise infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by vacuum infiltration.

The one or more responses of the plant(s) or plant part(s) to the pathogen may comprise one or more phenotypic responses to the pathogen. For example, the method may comprise classifying the plant(s) or plant part(s) into response phenotypes comprising:

(i) a Class 1 response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the second screening method;

(ii) a Class 2 response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method and the second screening method;

(iii) a Class 3 response phenotype characterised by chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and an absence of chlorosis, necrosis and pathogen fruiting bodies form on the plant(s) or plant part(s) following performance of the second screening method;

(iv) a Class 4 response phenotype characterised by colonization of the plant(s) or plant part(s) by the pathogen, but infection is halted with minimal chlorosis, following performance of the first screening method and/or the second screening method;

(v) a Class 5 response phenotype characterised by chlorosis, necrosis and formation of pathogenic fruiting structures with evidence of host resistance, wherein host resistance is characterised by a reduction in number of lesion and/or lesion area compared to a plant or plant part in which there is no evidence of host resistance; or

(vi) a Class 6 response phenotype characterised by chlorosis, necrosis and formation of pathogen fruiting structures with no evidence of host resistance.

A plant or plant part which exhibits a Class 1 response phenotype elicits a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue, but not post entry.

A plant or plant part which exhibits a Class 2 response phenotype elicits a defence response to the pathogen prior to, during and post entry of the pathogen into the plant tissue.

A plant or plant part which exhibits a Class 3 response phenotype elicits a defence response to the pathogen post entry of the pathogen into the plant tissue, but not prior to or during entry of the pathogen into the plant tissue.

A plant or plant part which exhibits a Class 4 response phenotype does not elicit a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue, but does elicit a defence response to the pathogen post entry of the pathogen into the plant tissue and colonisation of the plant by the pathogen still occurs.

A plant or plant part which exhibits a Class 5 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, but shows evidence of resistance to the pathogen represented by a reduced number of lesion and/or reduced lesion area compared to a plant or plant part in which there is no evidence of host resistance (*e.g.,* compared to a plant or plant part which exhibits a Class 6 response phenotype).

A plant or plant part which exhibits a Class 6 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, and shows no evidence of resistance to the pathogen.

Selecting one or more plants or plant parts classified as being resistant to the plant pathogen based on a defence response to the pathogen at one or more time points in the infection cycle.

In each of the foregoing examples, the method may comprise selecting one or more plants or plant parts classified as being resistant to the plant pathogen based on a defence response to the pathogen prior to or during entry of the plant pathogen to the plant tissue. Alternatively, the method may comprise selecting one or more plants or plant parts classified as being resistant to the plant pathogen based on a defence response to the pathogen post entry of the plant pathogen to the plant tissue. However, in other examples, the method may comprise

selecting one or more plants or plant parts classified as being resistant to the plant pathogen based on a defence response to the pathogen prior to, during and post entry of the plant pathogen to the plant tissue.

In some examples, the one or more responses of the plant(s) or plant part(s) to the pathogen comprises a change of expression in one or more immune response genes. Accordingly, in some examples, the method may further comprise determining an expression profile of one or more immune response genes in the plant(s) or plant part(s) prior to and following inoculation with the pathogen to determine a change of expression in one or more immune response genes.

In each of the foregoing examples, the plant pathogen may be a fungal pathogen or an oomycete pathogen.

In one example, the plant pathogen is a fungal pathogen. For example, the fungal pathogen may be selected from the group consisting of *Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp., *Blumeria graminis*, *Mycosphaerella graminicola* (*Zymoseptoria tritici*), *Colletotrichum* spp., *Melampsora lini*, *Phyrenophora tritici-repentis, Leptosphaeria maculans, Phakopsora pachyrhizi* (Asian Soybean Rust), *Phakopsora meibomiae* (New world Soybean Rust), *Puccinia striiformis f. sp. tritici, Puccinia recondite, Magnaporthe grisea, Cercospora zeae-maydis, Cercospora Zeina, Septoria lycopersici, Rhynchosporium commune, Phyrenophora teres-maculata, Phyrenophora teres-teres, Alternaria solani, Alternaria alternada, Septoria apiicola, Septoria glycines, and Thekospora minima.* For example, the fungal pathogen may be *Mycosphaerella graminicola* (*Zymoseptoria tritici*). For example, the fungal pathogen may be *Phyrenophora tritici-repentis*. For example, the fungal pathogen may be *Puccinia striiformis f. sp. tritici.* For example, the fungal pathogen may be *Rhynchosporium commune.* For example, the fungal pathogen may be *Septoria apiicola.* For example, the fungal pathogen may be *Thekospora minima.* For example, the fungal pathogen may be *Leptosphaeria maculans.* For example, the fungal pathogen may be *Septoria lycopersici.*

In one example, plant pathogen is a oomycete pathogen. For example, the oomycete pathogen may be selected from the group consisting of *Phytophthora infestans, Hyaloperonospora arabidopsidis, Phytophthora ramorum, Phytophthora sojae, Phytophthora capsici, Plasmopara viticola, Phytophthora parasitica,* and *Albugo candida.*

The plants or plant parts which are screened in the method of the disclosure may be of any species or variety. For example, the plant may be a gymnosperm, a monocot or a dicot.

In one example, the plant is selected from the group consisting of a fruiting plant, a leguminous plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant, a medicinal plant and a noxious plant or weed.

In one example, the plant is a cereal plant. For example, the cereal plant may be selected from the group consisting of wheat, durum, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa and buckwheat. In one example, the cereal plant is wheat.

In one example, the plant is wheat and the plant pathogen is *Mycosphaerella graminicola* (*Zymoseptoria tritici*), *Pyrenophora tritici-repentis* or *Puccinia striiformis f. sp. tritici.* In one example, the plant is barley and the plant pathogen is *Rhyncosporium commune*. In one example, the plant is canola and the plant pathogen is *Leptosphaeria maculans*. In one example, the plant is blueberry and the plant pathogen is *Thekospora minima*. In one example, the plant is tomato and the plant pathogen is *Septoria lycopersici.* In one example, the plant is celery or celeriac and the plant pathogen is *Septoria apiicola.*

In each of the foregoing examples, the method of identifying a plant which exhibits a resistance phenotype or a susceptibility phenotype to a plant pathogen may further comprise identifying one or more molecular markers associated with resistance to the plant pathogen and/or identifying one or more molecular markers associated with susceptibility to the plant pathogen, comprising:

(i) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(ii) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a susceptibility phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(iii) comparing the sequence data at (i) and (ii) and identifying one or more molecular markers which are associated with:

a. a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof,
b. a resistance phenotype after entry of the plant pathogen into the plant or part thereof,
c. a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof, and/or
d. a susceptibility phenotype after entry of the plant pathogen into the plant or part thereof.

A method of identifying one or more molecular markers associated with resistance of a plant to a plant pathogen and/or identifying one or more molecular markers associated with susceptibility of a plant to a plant pathogen, said method comprising:

(a) performing the screening method described herein to identify one or more plants which exhibit a resistance phenotype to a plant pathogen and one or more plants which exhibit a susceptibility phenotype to the plant pathogen;

(b) identifying one or more molecular markers associated with resistance to the plant pathogen and/or identifying one or more molecular markers associated with susceptibility to the plant pathogen, comprising:

(i) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(ii) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a susceptibility phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(iii) comparing the sequence data at (i) and (ii) and identifying one or more molecular markers which are associated with:

1) a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof,
2) a resistance phenotype after entry of the plant pathogen into the plant or part thereof,
3) a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof, and/or
4) a susceptibility phenotype after entry of the plant pathogen into the plant or part thereof.

In each of the foregoing examples, the polynucleotide sequence data may be DNA sequence data and/or RNA sequence data. In one example, the polynucleotide sequence data is DNA sequence data. In one example, the polynucleotide sequence data is RNA sequence data.

In one example, the one or more molecular markers identified are associated with a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof. For example, the one or more molecular markers identified may be associated with a resistance phenotype prior to entry of the pathogen into the plant or part thereof. Alternatively, or in addition, the one or more molecular markers identified may be associated with a resistance phenotype during entry of the pathogen into the plant or part thereof.

In another example, the one or more molecular markers identified are associated with a resistance phenotype after entry of the pathogen into the plant or part thereof.

In one example, the one or more molecular markers identified are associated with a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof. For example, the one or more molecular markers identified may be associated with a susceptibility phenotype prior to entry of the pathogen into the plant or part thereof. Alternatively, or in addition, the one or more molecular markers identified may be associated with a susceptibility phenotype during entry of the pathogen into the plant or part thereof.

In another example, the one or more molecular markers identified are associated with a susceptibility phenotype after entry of the pathogen into the plant or part thereof.

The one or more molecular markers may be selected from the group consisting of a single nucleotide polymorphism (SNP) marker, an amplified fragment length polymorphism (AFLP) marker, a DNA amplification fingerprinting (DAF) marker, a random amplified polymorphic DNA (RAPD) marker, microsatellite markers (e.g. simple sequence repeats (SSRs)), insertion mutation markers, sequence-characterized amplified region (SCAR) markers, cleaved amplified polymorphic sequence (CAPS) markers, kompetitive allele specific PCR (KASP) markers and any combinations thereof.

In one example, comparing the sequence data at (iii) comprises performing one or more sequence alignments to identify the one or more molecular markers.

In some examples, the method comprises generating the polynucleotide sequence for the plant of the species or cultivar by performing one or more assays selected from a nucleic acid isolation assay, a nucleic acid purification assay, a digestion assay, an assay for enrichment of molecular markers, a nucleic acid amplification assay, nucleic acid sequencing and combinations thereof.

The present disclosure also provides a method of breeding a plant which is resistant to a plant pathogen, comprising crossing or selfing a plant identified as being resistant to the plant pathogen using the method described herein. The method may comprise one or more selection steps to select for progeny which exhibit resistance to the plant pathogen.

In one example, the method of breeding a plant which is resistant to a plant pathogen comprises selecting the plant to be crossed or selfed based on the presence of one or more molecular markers associated with resistance to the plant pathogen and/or the absence of one or more molecular markers associated with susceptibility to the plant pathogen, wherein the one or more molecular markers are identified using the method described herein. For example, the plant to be crossed or selfed is selected based on the presence of one or more molecular markers associated with resistance to the plant pathogen. For example, the plant to be crossed or selfed is selected based on the absence of one or more molecular markers associated with susceptibility to the plant pathogen.

The present disclosure also provides a method of breeding a plant which is resistant to a plant pathogen, comprising:

(i) obtaining a plant identified as being resistant to the plant pathogen using the method described herein; and

(ii) introducing one or more foreign nucleic acids to the plant and/or modifying or editing one or more polynucleotides within the plant's genome.

In some examples, the method of breeding the plant comprising performing the method of identifying a plant which exhibits a resistance phenotype to the plant pathogen as described herein.

The plants which are bred in the method of the disclosure may be of any species or variety. For example, the plant may be a gymnosperm, a monocot or a dicot.

In one example, the plant which is bred is selected from the group consisting of a fruiting plant, a leguminous plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant and a noxious plant or weed.

In one example, the plant which is bred is a cereal plant. For example, the cereal plant may be selected from the group consisting of wheat, durum, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa and buckwheat. In one example, the cereal plant is wheat.

The present disclosure also provides a plant or plant part which has been bred by performing the method of breeding a plant as described herein. In one example, the plant part is a plant propagation material. For example, the plant propagation material may be a seed, a plant cutting, callus or scion.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustrating the Defend system, whereby two inoculation techniques (topical spray and infiltration) are used to identify plant genotypes with different defence trigger mechanisms. The upper panel represents the infiltration method, whereby spores are forced into the leaf. The lower panel represent a traditional topical spray in which spores are applied to the leaf surface. R = resistant phenotype, I = intermediate phenotype, S = susceptible phenotype. The susceptible reaction of the infiltration method can be assessed for presence of fungal fruiting structures A = necrosis with no fungal fruiting structure, B = moderate density of fungal fruiting structure, C = High density of fungal fruiting structures.

Figure 2 shows comparative results obtained using the first screening method (i.e., spray method) and the second screening method (i.e., infiltration method) for three varieties of wheat, 'Lorikeet' , 'Currawong' and 'Summit'. On the left of each paired leaves, it shows the result from the spray method, on the right of each pair, it shows the result from the infiltration method.

Figure 3 shows asexual fruiting body of called pycnidium (pl. pycnidia) which appear as tiny black spots on the surface of infected leaves.

Figure 4 illustrates the difference in phenotype following performance of the spray method (left hand side) and the infiltration method (right hand side) for the celery varieties 'Jive' (A), 'Stringless Organic' (B), 'Tall Utah' (C), 'Amsterdam' (D), 'Peppermint Stick' (E), 'Balena' (F), 'Giant Pascal' (G) and 'Laboni' (H) with *S. apiicola* spores.

5 Figure 5 illustrates the difference in phenotype following performance of the spray and infiltration methods for wheat cultivars 'Lancer', 'McVey' and 'Tammarian Rock' with *Pst* spores.

Figure 6 illustrates the exemplary 'Defend' classes identified in tomato varieties following challenge with *S. lycopersici*.

10 Figure 7 illustrates the difference in phenotype following performance of the spray and infiltration methods for Blueberry cultivars 'Peach Sorbent' and 'Pink Icing' with *T. minima spores*. Red circles indicate the sites where the infiltration was conducted. The Defend Class was categorized according to Table 3.

Figure 8 illustrates the difference in phenotype following performance of the infiltration 15 and wound methods for canola varieties 'Westar', 'Egra' and 'ATR-Beacon' with *L. maculans.*

Figure 9 illustrates the difference in phenotype following performance of the spray method (left hand side) and the infiltration method (right hand side) for the barley varieties 'Briar' (A), 'Atlas 46' (B), 'Franklin' (C), and 'ICARDA4' (D), with *R. commune* spores.

Figure 10 illustrates the difference in phenotype following performance of the spray and 20 infiltration methods for wheat cultivars 'ISIS', 'Oasis' and 'Spear' with the isolate YLS WAI240 of *P. tritici-repentis*. The Defend Class is categorized according to Table 3.

DETAILED DESCRIPTION

General Techniques and Definitions

25 Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, plant pathology, plant molecular pathology, plant molecular genetics, plant breeding, cell culture, protein chemistry, wine production and biochemistry).

Unless otherwise indicated, the recombinant DNA, recombinant protein, cell culture, and 30 immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical 35 Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors)

Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", is understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements. The indefinite article “a” or “an” thus usually means “at least one”.

The term “and/or”, *e.g.,* “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein, the term about, unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, more preferably +/- 1% of the designated value.

All publications, patents and patent applications, including any drawings and appendices, herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

<u>Phenotypic screening method</u>

The present disclosure relates to a method of identifying a plant which exhibits a resistance phenotype to a plant pathogen, comprising the steps of:

(a) performing a first screening method comprising:

(i) contacting the surface of a first plant or part thereof, or first group of plants or parts thereof, with an inoculum of the plant pathogen;

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the plant pathogen to infect the plant(s) or plant part(s); and

(iii) determining one or more responses of the plant(s) or plant part(s) to the pathogen at a plurality of time points during step (ii);

(b) performing a second screening method comprising:

(i) infiltrating a second plant or part thereof, or second group of plants of parts thereof, with an inoculum of the plant pathogen, wherein the second plant(s) or plant part(s) are of the same species and/or cultivar as the first plant(s);

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the plant pathogen to infect the plant(s) or plant part(s) and

(iii) determining one or more responses of the plant(s) or plant part(s) to the pathogen at a plurality of time points during step (ii); and

(c) determining whether or not the plant species or cultivar exhibits a resistance phenotype to the plant pathogen based on the one or more responses of the plants(s) or plant part(s) to the pathogen in the first and second screening methods.

As used herein, a "cultivar" or a "variety" refers to a group of similar plants that belong to the same species and that, by structural features and performance, may be distinguished from other varieties within the same species. Two essential characteristics of a variety are identity and reproducibility. Identity is necessary so that the variety may be recognized and distinguished from other varieties within the crop species. The distinguishing features may be morphological characteristics, molecular markers, colour markings, physiological functions, disease reaction, or performance. Most agricultural varieties are pure for the characteristic or for those characteristics that identify the variety; per se. Reproducibility is needed in order that the characteristic(s) by which the variety is identified will be reproduced in the progeny. For the purpose of this disclosure, the terms "cultivar" and "variety" are used interchangeably to refer to a group of plants within a species that share certain constant characters which separate them from the typical form and from other possible varieties within that species. While possessing at least the distinctive trait, a "variety" of the invention also may be characterized by a substantial amount of overall variation between individuals within the variety, based primarily on the Mendelian segregation of traits among the progeny of succeeding generations. On the other hand, "cultivar" or "variety" also can denote a clone, since some plant cultivars may individually be reproduced asexually, via stem cuttings, and all of the clones would be essentially identical genetically.

Techniques for applying an inoculum comprising a pathogen (*e.g.,* pathogenic spores) to the surface of a plant or a plant part (*e.g.,* foliage, stems, roots or seeds etc) in accordance with the first screening method are known in the art (Russell (1978) Plant breeding for pest and disease resistance. Butterworth, London Boston;; Eyal *et al* (1987) *The Septoria diseases of wheat: Concepts and methods of disease management*. CIMMYT, Mexico; and McIntosh et al., (1995) *Wheat rusts: An atlas of resistance genes*. CSIRO). One exemplary method for applying an inoculum to the surface of a plant is by spraying the inoculum using any conventional means for spraying liquids, such as spray nozzles or spinning-disk atomizers or hand held spray bottles. In other examples, inoculum may be applied to the surface of a plant or plant part by swiping or swabbing the inoculum onto the surface of the plant or plant part, or dipping, soaking or otherwise immersing the plant or plant part in the inoculum.

In accordance with the second screening method, inoculum comprising a pathogen (*e.g.,* pathogenic spores or mycelial fragments) is infiltrated into the plants tissue (*e.g.,* leaf, stem or fruit), or into the intercellular air spaces of the plant tissue (*e.g.,* leaf, stem of fruit) or the sub-stromal cavity, with the help of pressure using a syringe or other device known in the art (Chincinska 2021). Several factors might influence the successful entry of spores or other

inoculum (*e.g.,* mycelial fragments) into the sub-stromal cavity and intracellular spaces without causing physical damage or eliciting a hypertensive response from the plant tissue. These factors include stomata density, leaf age, time of day, cell turgor, and spore concentration. A skilled person would be capable of performing infiltration of inoculum into plant tissue using methods known in the art. However, in one example, the second screening method comprise infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe infiltration. In one example, the second screening method comprises infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe pressure infiltration. In one example, the second screening method comprises infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by vacuum infiltration.

As used herein, the term "inoculum" refers to a preparation suitable for use in inoculation of plant tissue with a plant pathogen of interest. In accordance with example in which the plant pathogen of interest is capable of sporulation, the inoculum may comprise spores derived from the plant pathogen. However, it will be appreciated that an “inoculum” may comprise any part of the pathogen that can initiate infection. Thus, in fungi, an inoculum may comprise spores, sclerotia (i.e., a compact mass of mycelium), or fragments of mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, an inoculum refers to whole individuals of bacteria, mollicutes, protozoa, viruses, and viroids, respectively. An inoculum may comprise a single individual of a pathogen, *e.g.,* one spore or one multicellular sclerotium, or may comprise a plurality of individual pathogens, such as millions of individuals of a pathogen, *e.g.,* bacteria carried in a drop of water (Agrios GN (2005) Fifth Edition Plant Pathology, Fifth edn. Elsevier, London).

The inoculum may be provided in any form which is convenient for the method of application. However, in one example, the inoculum is in the form of a liquid. For example, the inoculum may comprise spores of the plant pathogen and a diluent, carrier and/or excipient. Suitable carriers include but not limited to beta glucan, sugar, Tween 20, gelatin or agar. In some examples, the inoculum may comprise spores of the plant pathogen in a suitable storage and/or growth media. In some examples, the inoculum may comprise spores of the plant pathogen provided in the form of a dry powder suspended in an oil. In other examples, the inoculum may comprise spores of the plant pathogen suspended in an oil-in-water emulsion.

A skilled person would be able to determine the appropriate concentration of spores in the inoculum based on the plant pathogen of interest and plant to be infected. For example, the inoculum may comprise spores at a concentration of at least 10^2 spores/mL, or at a concentration of at least 10^3 spores/mL, or at a concentration of at least 10^4 spores/mL, or at a concentration of at least 10^5 spores/mL, or at a concentration of at least 10^6 spores/mL, or at a concentration of at least 10^7 spores/mL. In one example, the composition comprises spores at a concentration of at least $1x10^4$ spores/mL. In one example, the composition comprises spores at a concentration of at least $1x10^5$ spores/mL. In one example, the composition comprises spores at a concentration

of at least $1x10^6$ spores/mL. In another example, the composition comprises spores at a concentration of $1x10^7$ spores/mL or more.

As used herein, the term “response” shall be understood to mean any change in a plant or plant part following exposure to a stimuli, such as an environmental stimuli or a plant pathogen of the disclosure. The response of the plant or plant part may manifest as observable changes in one or more traits of the plant or plant part (*e.g.,* a change in a plant phenotype) and/or may occur at a molecular or cellular level without resulting in any observable change in the plant appearance or plant phenotype.

A “phenotypic response”, “response phenotype” or similar shall therefore be understood to mean one or more changes in the phenotype of the plant or plant part in response to the plant pathogen. A skilled person will appreciate that the nature and extent of a phenotypic response of a plant or plant part to a pathogen will depend on a range of factors, including the type of plant pathogen, the species of plant and its stage of development, the part of the plant affected by the plant pathogen, susceptibility or resistance of the plant to the plant pathogen, and any plant defences to the pathogen. However, some exemplary phenotypic responses of plants to plant pathogens may include, but are not limited to, no change in phenotype (resistance to pathogen), chlorosis, necrosis of plant tissue, leaf curl, leave wrinkling or a change in rate of growth of the plant or plant part (*e.g.,* stunting), fungal fruiting structures forming (*e.g.,* acervulus, apothecium, ascocarp, ascoma, basidiocarp, coremium, pycnidium, perithecium, sporcarp, spordochium, etc.), hypersensitive response, melanism or programmed cell death.

The response phenotype of the plant or plant part may be indicative of a plant’s susceptibility to the plant pathogen or the plant’s ability to resist the pathogen at different stages of the pathogen’s lifecycle or stage of infection. The response phenotype may also be a reflection of a plant’s inherent defences against the particular plant pathogen. In this regard, plants have developed a wide variety of constitutive and inducible defences in order to protect themselves from damage (including from pathogens). Constitutive (continuous or structural) defences include many preformed barriers such as cell walls, waxy epidermal cuticles, and bark. These substances not only protect the plant from invasion, they also give the plant strength and rigidity. In addition to preformed barriers, virtually all living plant cells have the ability to detect invading pathogens and respond with inducible defences including the production of toxic chemicals, pathogen-degrading enzymes, and deliberate cell suicide. Plants often wait until pathogens are detected before producing toxic chemicals or defence-related proteins because of the high energy costs and nutrient requirements associated with their production and maintenance.

Plants perceive pathogen invasion via interactions between pattern recognition receptors on the cell surface and conserved molecular signature molecules known as pathogen/microbe-associated molecular patterns (PAMPs/MAMPs). Following pathogen recognition, a series of defence responses is induced, collectively known as PAMP-triggered

immunity (PTI). Over time, however, specific pathogens have acquired the ability to suppress PTI in plants. These pathogens secrete various PTI-interfering effectors in the host plants. However, if the host plant acquires the ability to recognize these effectors via R (resistance) proteins, effector-triggered immunity (ETI) is induced, which involves stronger and longer-lasting responses than PTI. Early defence responses common to PTI and ETI include an increase in cytosolic Ca^{2+} concentration, production of reactive oxygen species (ROS), activation of the mitogen-activated protein kinases (MAPKs), expression of various defence-related genes, and increased biosynthesis of phytoalexins and defence hormones, such as salicylic acid (SA) and jasmonic acid (JA).

The method may comprise classifying the plant(s) or plant part(s) based on their phenotypic response to the plant pathogen following inoculation using the first and second screening methods. These classifications (Classes 1-6) are described below.

Class 1 response phenotype: A plant or plant part which exhibits a Class 1 response phenotype elicits a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue, but not post entry. For example, a Class 1 response phenotype may be characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the second screening method.

Class 2 response phenotype: A plant or plant part which exhibits a Class 2 response phenotype elicits a defence response to the pathogen prior to, during and post entry of the pathogen into the plant tissue. For example, a Class 2 response phenotype may be characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method and the second screening method.

Class 3 response phenotype: A plant or plant part which exhibits a Class 3 response phenotype elicits a defence response to the pathogen post entry of the pathogen into the plant tissue, but not prior to or during entry of the pathogen into the plant tissue. For example, a Class 3 response phenotype may be characterised by chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and an absence of chlorosis, necrosis and pathogen fruiting bodies form on the plant(s) or plant part(s) following performance of the second screening method.

Class 4 response phenotype: A plant or plant part which exhibits a Class 4 response phenotype does not elicit a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue, but does elicit a defence response to the pathogen post entry of the pathogen into the plant tissue and colonisation of the plant by the pathogen still occurs. For example, a Class 4 response phenotype may be characterised by colonization of the plant(s) or plant part(s) by the pathogen, but infection is halted with minimal chlorosis, following performance of the first screening method and/or the second screening method.

Class 5 response phenotype: A plant or plant part which exhibits a Class 5 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, but shows evidence of resistance to the pathogen represented by a reduced number of lesion and/or reduced lesion area compared to a plant or plant part in which there is no evidence of host resistance (*e.g.,* compared to a plant or plant part which exhibits a Class 6 response phenotype). For example, a Class 5 response phenotype may be characterised by chlorosis, necrosis and formation of pathogenic fruiting structures with evidence of host resistance, wherein host resistance is characterised by a reduction in number of lesion and/or lesion area compared to a plant or plant part in which there is no evidence of host resistance

Class 6 response phenotype: A plant or plant part which exhibits a Class 6 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, and shows no evidence of resistance to the pathogen. For example, a Class 6 response phenotype may be characterised by chlorosis, necrosis and formation of pathogen fruiting structures with no evidence of host resistance.

Plants screened using the phenotyping method of the disclosure may categorised into one of Classes 1-6 based on the ability (or inability) of the plant to trigger a defence response to a pathogen at different points along the infection pathway, as determined by the plant's response phenotype at different points along the infection pathway. Based on these classifications, it may be possible to identify varieties and/or specific plants capable of mounting a defence response to the plant pathogen at a desired time point within the infection lifecycle. Alternatively, it may be possible to identify varieties and/or specific plants which mount a poor defence response or no defence response to the plant pathogen at one or more desired time points within the infection lifecycle if that were desirable.

In some examples, a plant may be categorised as exhibiting a resistance phenotype at one or more time points along the infection pathway. As used herein, the term "resistance phenotype" refers to a phenotype which is indicative of a level of resistance of a plant to the plant pathogen. The resistance phenotype may be characterised in terms of morphological features of the plant or plant part and/or in term of functional characteristics. For example, a resistance phenotype may be one in which there are few or no disease symptoms (*e.g.,* chlorosis or necrosis) exhibited by the plant following exposure to a pathogen. The skilled person would appreciate that a resistance phenotype will differ according to the plant species and plant pathogen, for example.

The term "resistance" or "resistant" refers to the ability of a plant to mount a defence response to the plant pathogen prior to, during or post entry of the pathogen into the plant tissue, such that the plant develops no disease symptoms or few disease symptoms following exposure of the plant to the plant pathogen. In other examples, "resistance" may be determined relative to a plant that is susceptible to the pathogen. For example, a plant that exhibits resistance may exhibit fewer disease symptoms than a plant that is susceptible to the pathogen and which does not exhibit disease resistance. "Resistance" includes complete resistance to the disease and also

varying degrees of resistance manifested as decreased or weakening disease symptoms, longer survival, recovery or weakening of other disease parameters.

Conversely, the term "susceptible", "susceptible phenotype" or similar as used herein, shall be understood to mean a plant that does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, and shows no evidence of resistance to the pathogen. For example, a plant which is determined to be susceptible to a plant pathogen following performance of the method of the disclosure will exhibit a Class 6 response phenotype.

One or more plants exhibiting a desired response phenotype may then be selected for downstream breeding activities and/or genetic characterisation based on the response phenotype classification attributed to the plant. For example, the method may comprise selecting a plant or part thereof which is capable of eliciting a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 1 or Class 2 response phenotype). For example, the method may comprise selecting a plant or part thereof which is capable of eliciting a defence response to the pathogen post entry of the pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 2, Class 3 or Class 4 response phenotype). For example, the method may comprise selecting a plant or part thereof which is capable of eliciting a defence response to the pathogen prior to, during and post entry of the pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 2 response phenotype). For example, the method may comprise selecting a plant or part thereof which does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue but which still shows evidence of resistance to the pathogen (*e.g.,* a plant exhibiting a Class 5 response phenotype). For example, the method may comprise selecting a plant or part thereof which is susceptible to the plant pathogen (*e.g.,* a plant exhibiting a Class 6 response phenotype). Genetic characterisation studies may be performed to identify genes and/or gene variants involved in the plant-pathogen interaction pathway which confer the response phenotype of interest.

In some examples, the one or more responses of the plant(s) or plant part(s) to the pathogen may comprise a change of expression in one or more immune response genes. According to examples in which one or more immune response genes of a plant are known, the method may further comprise determining an expression profile of one or more immune response genes in the plant(s) or plant part(s) prior to and following inoculation with the pathogen to determine a change of expression in one or more immune response genes. As used herein, an "immune response gene" (also referred to as a "resistance gene") shall be understood to mean a nucleic acid encoding a protein that is directly or indirectly involved in the detection of the pathogen or in the induction of a signal transduction pathway leading to a plant defence response against the plant pathogen following contact of the plant with that particular pathogen. Examples of immune response genes include genes involved in PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI).

As described herein, the first and second screening methods comprise determining one or more responses of the plant(s) or plant part(s) to the plant pathogen at a plurality of time points, including pre-inoculation and at least 5 days (*e.g.,* at least 5, or at least 10, or at least 15, or at least 20, or at least 25, or at least 30 days, or at least 35 days, or at least 40 days, or at least 45 days, or at least 50 days, or at least 55 days, or at least 60 days or more) post inoculation. A skilled person would appreciate that the time differs for species of pathogen and plant. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 5 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 10 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 15 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 20 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 25 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 30 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 35 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 40 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 45 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 50 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 55 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for at least about day 60 days post inoculation. In some examples, the methods comprise monitoring the plants and determining one or more responses of the plant(s) or plant part(s) to the plant pathogen for more than 60 days post inoculation.

A "pathogen" of plant refers to an organism which can infect a plant, or which can cause a disease in a plant. Pathogens which can infect a plant, or which can cause a disease in a plant, include but not limited to fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, and nematodes. However, in the context of the method of the disclosure,

a "plant pathogen" shall be understood to be a fungal pathogen or fungus-like pathogen (such as, for example, Chromista) belonging to the group comprising Plasmodiophoramycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota and Deuteromycetes (Fungi imperfecti). Plant pathogens include, by way of example, but not by limitation, those detailed in
5 Tables 1 and 2, and the diseases which are associated with them.

Table 1. Diseases caused by biotrophic and/or heminecrotrophic phytopathogenic fungi

Disease	**Pathogen**
Leaf rust	*Puccinia recondita*
Yellow rust	*Puccinia striiformis*
Powdery mildew	*Erysiphe graminis / Blumeria graminis*
Rust (common corn)	*Puccinia sorghi*
Rust (Southern corn)	*Puccinia polysora*
Tobacco leaf spot	*Cercospora nicotianae*
Rust (soybean)	*Phakopsora pachyrhizi, P. meibomiae*
Rust (tropical corn)	*Physopella pallescens, P. zeae = Angiopsora zeae*

Table 2: Diseases caused by necrotrophic and/or hemibiotrophic fungi and Oomycetes

Disease	**Pathogen**
Glume blotch	*Septoria (Stagonospora) nodorum*
Leaf blotch	*Septoria tritici*
Ear fusarioses	*Fusarium spp.*
Late blight	*Phytophthora infestans*
Anthrocnose leaf blight Anthracnose stalk rot	*Colletotrichum graminicola (teleomorph: Glomerella graminicola Politis); Glomerella tucumanensis (anamorph: Glomerella falcatum Went)*
Curvularia leaf spot	*Curvularia clavata, C. eragrostidis, = C. maculans (teleomorph: Cochliobolus eragrostidis), Curvularia inaequalis, C. intermedia (teleomorph: Cochliobolus intermedius), Curvularia lunata (teleomorph: Cochliobolus lunatus), Curvularia pallescens (teleomorph: Cochliobolus pallescens), Curvularia senegalensis, C. tuberculata (teleomorph: Cochliobolus tuberculatus)*
Didymella leaf spot	*Didymella exitalis*
Diplodia leaf spot or streak	*Stenocarpella macrospora = Diplodialeaf macrospora*

Disease	**Pathogen**
Brown stripe downy mildew	*Sclerophthora rayssiae var. zeae*
Crazy top downy mildew	*Sclerophthora macrospora = Sclerospora macrospora*
Green ear downy mildew (graminicola downy mildew)	*Sclerospora graminicola*
Leaf spots, minor	*Alternaria alternata, Ascochyta maydis, A. tritici, A. zeicola, Bipolaris victoriae = Helminthosporium victoriae (teleomorph: Cochliobolus victoriae), C. sativus (anamorph: Bipolaris sorokiniana = H. sorokinianum = H. sativum), Epicoccum nigrum, Exserohilum prolatum = Drechslera prolata (teleomorph: Setosphaeria prolata) Graphium penicillioides, Leptosphaeria maydis, Leptothyrium zeae, Ophiosphaerella herpotricha, (anamorph: Scolecosporiella sp.),Paraphaeosphaeria michotii, Phoma sp., Septoria zeae, S. zeicola, S. zeina*
Northern corn leaf blight (white blast, crown stalk rot, stripe)	*Setosphaeria turcica (anamorph: Exserohilum turcicum = Helminthosporium turcicum)*
Northern corn leaf spot Helminthosporium ear rot (race 1)	*Cochliobolus carbonum (anamorph: Bipolaris zeicola = Helminthosporium carbonum)*
Phaeosphaeria leaf spot	*Phaeosphaeria maydis = Sphaerulina maydis*
Rostratum leaf spot (Helmin- thosporium leaf disease, ear and stalk rot)	*Setosphaeria rostrata, (anamorph: xserohilum rostratum = Helminthosporium rostratum)*
Java downy mildew	*Peronosclerospora maydis = Sclerospora maydis*
Philippine downy mildew	*Peronosclerospora philippinensis = Sclerospora philippinensis*
Sorghum downy mildew	*Peronosclerospora sorghi = Sclerospora sorghi*
Spontaneum downy mildew	*Peronosclerospora spontanea = Sclerospora spontanea*
Sugarcane downy mildew	Peronosclerospora sacchari = Sclerospora sacchari

Disease	Pathogen
Sclerotium ear rot (southern blight)	*Sclerotium rolfsii Sacc. (teleomorph: Athelia rolfsii)*
Seed rot-seedling blight	*Bipolaris sorokiniana, B. zeicola = Helminthosporium carbonum, Diplodia maydis, Exserohilum pedicillatum, Exserohilum turcicum = Helminthosporium turcicum, Fusarium avenaceum, F. culmorum, F. moniliforme, Gibberella zeae (anamorph: F. graminearum), Macrophomina phaseolina, Penicillium spp., Phomopsis sp., Pythium spp., Rhizoctonia solani, R. zeae, Sclerotium rolfsii, Spicaria sp.*
Selenophoma leaf spot	*Selenophoma sp.*
Yellow leaf blight	*Ascochyta ischaemi, Phyllosticta maydis (teleomorph: Mycosphaerella zeae-maydis)*
Zonate leaf spot	*Gloeocercospora sorghi*

Other exemplary plant pathogens which are contemplated herein, including their host plant species, are described herein.

For example, the plant pathogen may belong to the group Plasmodiophoromycota, such as, for example, *Plasmodiophora brassicae* (clubroot of crucifers), *Spongospora subterranea*, or *Polymyxa graminis*.

For example, the plant pathogen may belong to the group Oomycota, such as, for example, *Bremia lactucae* (downy mildew of lettuce), *Peronospora* (downy mildew) in snapdragon (*P. antirrhini*), onion (*P. destructor*), spinach (*P. effusa*), soybean (*P. manchurica*), tobacco ("blue mold"; *P. tabacina*) alfalfa and clover (*P. trifolium*), *Pseudoperonospora humuli* (downy mildew of hops), *Plasmopara* (downy mildew in grapevines) (*P. viticola*) and sunflower (*P. halstedii*), *Sclerophthora macrospora* (downy mildew in cereals and grasses), *Pythium* (for example damping-off of Beta beet caused by *P. debaryanum*), *Phytophthora infestans* (late blight in potato and in tomato and the like) or *Albugo sp.*

For example, the plant pathogen may belong to the group Ascomycota, such as, for example, Microdochium nivale (snow mold of rye and wheat), *Fusarium, Fusarium graminearum, Fusarium culmorum* (partial ear sterility mainly in wheat), *Fusarium oxysporum* (Fusarium wilt of tomato), Blumeria graminis (powdery mildew of barley (f.*sp. hordei*) and wheat (*f.sp. tritici*)), *Erysiphe pisi* (powdery mildew of pea), *Nectria galligena* (Nectria canker of fruit trees), *Uncinula necator* (powdery mildew of grapevine), *Pseudopeziza tracheiphila* (red fire disease of grapevine), *Claviceps pur-purea* (ergot on, for example, rye and grasses), *Gaeumannomyces graminis* (take-all on wheat, rye and other grasses), *Magnaporthe grisea*,

Pyrenophora graminea (leaf stripe of barley), *Pyrenophora teres* (net blotch of barley), *Pyrenophora tritici-repentis* (leaf blight of wheat), *Venturia inaequalis* (apple scab), *Sclerotinia sclerotium* (stalk break, stem rot), or *Pseudopeziza medicaginis* (leaf spot of alfalfa, white and red clover).

5 For example, the plant pathogen may belong to the group Basidiomycetes, such as, for example, *Typhula incarnata* (typhula blight on barley, rye, wheat), *Ustilago maydis* (blister smut on maize), *Ustilago nuda* (loose smut on barley), *Ustilago tritici* (loose smut on wheat, spelt), *Ustilago avenae* (loose smut on oats), *Rhizoctonia solani* (rhizoctonia root rot of potato), *Sphacelotheca spp.* (head smut of sorghum), *Melampsora lini* (rust of flax), *Puccinia graminis* 10 (stem rust of wheat, barley, rye, oats), *Puccinia recondita* (leaf rust on wheat), *Puccinia dispersa* (brown rust on rye), *Puccinia hordei* (leaf rust of barley), *Puccinia coronata* (crown rust of oats), *Puccinia striiformis* (yellow rust of wheat, barley, rye and a large number of grasses), *Uromyces appendiculatus* (brown rust of bean), or *Sclerotium rolfsii* (root and stem rots of many plants).

For example, the plant pathogen may belong to the group Deuteromycetes (Fungi 15 imperfecti), such as, for example, *Septoria* (Stagonospora) *nodorum* (glume blotch) of wheat (*Septoria tritici*), *Pseudocercosporella herpotrichoides* (eyespot of wheat, barley, rye), *Rynchosporium secalis* (leaf spot on rye and barley), *Alternaria solani* (early blight of potato, tomato), *Phoma betae* (blackleg on Beta beet), *Cercospora beticola* (leaf spot on Beta beet), *Alternaria brassicae* (black spot on oilseed rape, cabbage and other crucifers), *Verticillium* 20 *dahliae* (verticillium wilt), *Colletotrichum, Colletotrichum lindemuthianum* (bean anthracnose), *Phoma lingam* (blackleg of cabbage and oilseed rape), or *Botrytis cinerea* (grey mould of grapevine, strawberry, tomato, hops and the like).

In one particular example, the plant pathogen is a fungal pathogen. For example, the fungal pathogen may be selected from the group consisting of *Magnaporthe oryzae*, *Botrytis* 25 *cinerea*, *Puccinia* spp., *Blumeria graminis*, *Mycosphaerella graminicola* (*Zymoseptoria tritici*), *Colletotrichum* spp., *Melampsora lini*, *Phakopsora pachyrhizi, Phyrenophora tritici-repentis, Leptosphaeria maculans, Phakopsora pachyrhizi, Phakopsora meibomiae, Puccinia striiformis f. sp. tritici, Puccinia recondite, Magnaporthe grisea, Cercospora zeae-maydis, Cercospora Zeina, Septoria lycopersici, Rhynchosporium commune, Phyrenophora teres-maculata,* 30 *Alternaria solani, Alternaria alternada, Septoria apiicola, Septoria glycines, and Thekospora minima.* For example, the fungal pathogen may be *Mycosphaerella graminicola* (*Zymoseptoria tritici*). For example, the fungal pathogen may be *Phyrenophora tritici-repentis*. For example, the fungal pathogen may be *Puccinia striiformis f. sp. tritici.* For example, the fungal pathogen may be *Rhynchosporium commune.* For example, the fungal pathogen may be *Septoria* 35 *apiicola.* For example, the fungal pathogen may be *Thekospora minima.* For example, the fungal pathogen may be *Leptosphaeria maculans.* For example, the fungal pathogen may be *Septoria lycopersici.*

In another particular example, plant pathogen is a oomycete pathogen. For example, the oomycete pathogen may be selected from the group consisting of *Phytophthora infestans, Hyaloperonospora arabidopsidis, Phytophthora ramorum, Phytophthora sojae, Phytophthora capsici, Plasmopara viticola, Phytophthora parasitica,* and *Albugo candida.*

5 As used herein, the term "plant" refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom). This includes familiar organisms such as but not limited to trees, herbs, bushes, grasses, veins, ferns, mosses and green algae. The term refers to both monocotyledonous plants, also called monocots, and dicotyledonous plants, also called dicots. By way of non-limiting examples, the plant may be a fruiting plant, a leguminous 10 plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant, a medicinal plant and a noxious plant or weed. In this regard, the choice of plant may vary depending on the plant pathogen of interest, geographic location and season.

In one particular example, the plant may be a cereal plant selected from the group consisting of wheat, durum, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa 15 and buckwheat.

Other examples of particular plants include but are not limited to potatoes, roses, apple trees, sunflowers, bananas, tomatoes, opo, pumpkins, squash, lettuce, cabbage, oak trees, guzmania, geraniums, hibiscus, clematis, poinsettias, sugarcane, taro, duck weed, pine trees, Kentucky blue grass, zoysia, coconut trees, brassica leafy vegetables (*e.g.,* broccoli, broccoli 20 raab, Brussels sprouts, cabbage, Chinese cabbage (Bok Choy and Napa), cauliflower, cavalo, collards, kale, kohlrabi, mustard greens, rape greens, and other brassica leafy vegetable crops), bulb vegetables (*e.g.,* garlic, leek, onion (dry bulb, green, and Welsh), shallot, and other bulb vegetable crops), citrus fruits (*e.g.,* grapefruit, lemon, lime, orange, tangerine, citrus hybrids, pummelo, and other citrus fruit crops), cucurbit vegetables (*e.g.,* cucumber, citron melon, edible 25 gourds, gherkin, muskmelons (including hybrids and/or cultivars of cucumis melons), watermelon, cantaloupe, and other cucurbit vegetable crops), fruiting vegetables (including eggplant, ground cherry, pepino, pepper, tomato, tomatillo, and other fruiting vegetable crops), grape, leafy vegetables (*e.g.,* romaine), root/tuber and corm vegetables (*e.g.,* potato), and tree nuts (almond, pecan, pistachio, and walnut), berries (*e.g.,* tomatos, barberries, currants, 30 elderberries, blueberries, gooseberries, honeysuckles, mayapples, nannyberries, Oregon-grapes, see-buckthorns, hackberries, bearberries, lingonberries, strawberries, sea grapes, blackberries, cloudberries, loganberries, raspberries, salmonberries, thimbleberries, and wineberries), cereal crops (*e.g.,* corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales, buckwheats, fonio, and quinoa), pome fruit (*e.g.,* apples, pears), stone fruits (*e.g.,* coffees, jujubes, mangos, olives, 35 coconuts, oil palms, pistachios, almonds, apricots, cherries, damsons, nectarines, peaches and plums), vein (*e.g.,* table grapes, wine grapes), fiber crops (*e.g.,* hemp, cotton), ornamentals, forestry plants and the like. For a more complete list of representative crop plants see, for example, Glossary of Crop Science Terms: III, Nomenclature, Common and Scientific Names,

Crop Science Society of America, July 1992, which is herein incorporated in its entirety. In one example, the crop plant is wheat. In another example, the crop plant is barley. In another example, the crop plant is canola. In another example, the crop plant is blueberry. In another example, the crop plant is tomato. In another example, the crop plant is celery or celeriac.

5 The term "plant" in a broad sense refers to differentiated plants as well as undifferentiated plant material, such as protoplasts, plant cells, seeds, plantlets, etc., that under appropriate conditions can develop into mature plants, the progeny thereof, and parts thereof. "Progeny" comprises any subsequent generation of a plant, plant cell, plant tissue, or plant organ.

Ornamental and horticulture plants include, for example, flowering plants, shrubs, grasses 10 and trees. Examples of Ornamental plants include, but not limited to, Frangipani Tree, Gardenia, Bougainvillea, Hibiscus Tree, Blue Water Lily, Ylang Ylang, Madagascar Rosy Periwinkle, Orchid, Croton, Spanish Jasmine, Red Ginger, Firecracker, Indian Coleus, Porcelain Rose, Geranium, Petunia, Begonia, Pelargonium, Fuchsia etc. However, any ornamental or horticultural plants may be screened using the method of the disclosure.

15 Forestry plants are ones that are used for conserving and repairing forests, woodlands. Non-limiting examples include gum tree, oak, Acacia, Callitris, Casuarina, Eucalyptus, Pinus, Mangrove, Melaleuca etc.

Aquatic plants include those that, under normal conditions, germinate and grow with at least their bases in the water and are large enough to be seen with the naked eye. Non-limiting 20 examples include parrot feather, creeping primrose, water mint, water hyacinth, duckweed, hydrilla etc.

Medicinal plants include those plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body. Medicinal plants typically comprise phytochemicals and other active compounds which have the potential to have 25 pharmacological activity, including alkaloids, glycosides, polyphenols and terpenes, for example. Examples of medicinal plants will be known to a person skilled in the art.

Noxious plants or weed can be monocotyledonous and dicotyledonous. Examples of weed species among the monocots, *Avena, Lolium, Alopecurus, Phalaris, Echinochloa, Digitaria, Setaria, Cyperus* species from the annual sector and from the perennial species *Agropyron,* 30 *Cynodon , Imperata, perennial Cyperus* species etc; and among the dicots, Galium, Viola, Veronica, Lamium, Stellaria, Amaranthus, Sinapis, Ipomoea, Sida, Matricaria and Abutilon among the annual, and Convolvulus, Cirsium, Rumex and Artemisia in the case of perennial weeds. Examples of weeds in crop fields include but not limited to Echinochloa, Sagittaria, Alisma, Eleocharis, Scirpus and Cyperus.

35 As used herein, the term "plant part" refers to any part of a plant including but not limited to the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, and the like.

Molecular markers and selection

The detection and exploitation of genetic variation has always been an integral part of plant breeding. In particular, DNA-based molecular markers are useful for detecting the genetic variation available in germplasm collections and/or breeding lines. These markers have been used extensively for the development of saturated molecular genetic maps and physical maps and for the identification of genes or quantitative trait loci (QTLs) controlling traits of economic importance which are in turns can be used for marker-assisted selection. During the past two decades, a number of new next-generation sequencing (NGS) technologies have been developed and subsequently deployed to generate DNA sequence data inexpensively and at a rate that is several orders of magnitude faster than that of traditional technologies. As a result, genomics-assisted breeding approaches have greatly advanced with the increasing availability of genome and transcriptome sequence data for several model plant and crop species. Examples of such genomics-assisted breeding approaches include marker assisted selection (MAS), genome selection (GS), and genome wide association studies (GWAS) (for review see, e.g. , Varshney *et al.* ,(2009) *Trends Biotechnol.* 27(9):522-530). GWAS methods in particular have attracted significant interest in plant breeders. The GWAS method typically involves an examination of many common genetic variants in different individuals to see if any variant is associated with a trait. GWAS technology focuses on associations between single- nucleotide polymorphisms (SNPs) and specific traits such as increased yield or disease resistance. These studies typically compare the DNA sequence information of two groups of plants that either displays or lacks the target trait (i.e., controls). Each plant gives a sample of DNA, from which millions of genetic variants are read using SNP arrays. If one type of the variant (one allele) is more frequent in plants with the disease resistance, the SNP is said to be "associated" with the disease resistance. The associated SNPs are then considered to mark a region of the genome which influences the risk of disease, for example. In contrast to methods which specifically test one or a few genetic regions, the GWAS studies investigate the entire genome. This approach is therefore considered to be non-candidate-driven in contrast to gene- specific candidate-driven studies. As such, GWAS identifies SNPs and other variants in DNA (described herein) which may be associated with a trait (such as a resistance or susceptibility to a plant pathogen), but which may not on their own specify which genes or genetic elements are causal. The present disclosure contemplates the use of such methods to identify molecular markers associated with resistance or susceptibility to plant pathogens present in plants identified as being resistant or susceptible to plant pathogens using the phenotypic screening method described herein. These molecular markers can then be used as tools for selection and breeding of plant varieties which are resistant or susceptible to plant pathogens, as desired.

Accordingly, the present disclosure also provides a method of identifying one or more molecular markers associated with resistance of a plant to a plant pathogen and/or identifying

one or more molecular markers associated with susceptibility of a plant to a plant pathogen, comprising:

(a) performing the phenotypic screening method described herein to identify one or more plants which exhibit a resistance phenotype to a plant pathogen and one or more plants which exhibit a susceptibility phenotype to the plant pathogen;

(b) identifying one or more molecular markers associated with resistance to the plant pathogen and/or identifying one or more molecular markers associated with susceptibility to the plant pathogen, comprising:

(i) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a resistance phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(ii) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a susceptibility phenotype to the plant pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the plant pathogen into the plant or part thereof,

(iii) comparing the sequence data at (i) and (ii) and identifying one or more molecular markers which are associated with:

a. a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof,
b. a resistance phenotype after entry of the plant pathogen into the plant or part thereof,
c. a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof, and/or
d. a susceptibility phenotype after entry of the plant pathogen into the plant or part thereof.

In each of the foregoing examples, the polynucleotide sequence data may be DNA sequence data and/or RNA sequence data. In one example, the polynucleotide sequence data is DNA sequence data. In one example, the polynucleotide sequence data is RNA sequence data.

The terms "molecular marker" and "genetic marker" are used interchangeably herein and refer to a region of a nucleotide sequence (e.g. , in a chromosome) that is subject to variability (i.e. , the region can be polymorphic for a variety of alleles). Genetic markers are typically used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion/deletion (INDEL) mutations, simple sequence repeats (SSRs or microsatellite) markers,

sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), isozyme markers, arbitrarily primed polymerase chain reaction (AP-PCR), DNA amplification fingerprinting (DAF), , kompetitive allele specific PCR (KASP)
5 markers or combinations of the markers described herein, which define a specific genetic and chromosomal region or chromosomal location. Genetic markers can, for example, be used to locate genetic loci containing alleles on a chromosome that contribute to variability of phenotypic traits, such as resistance or susceptibility to a plant pathogen. The phrase "genetic marker" can also refer to a polynucleotide sequence complementary to a genomic sequence, such as a
10 sequence of a nucleic acid used as probes. A genetic or molecular marker can be physically located in a position on a chromosome that is distal or proximal to the genetic loci with which it is linked (i.e. is intragenic or extragenic, respectively).

Genetic or molecular markers can be used during the breeding process for the selection of qualitative and/or quantitative traits. For example, markers closely linked to alleles, or markers
15 containing sequences within the actual alleles of interest, can be used to select plants that contain the alleles of interest during a backcrossing breeding program. The markers can also be used to select for the genome of the recurrent parent and against the genome of the donor parent. Using this procedure can minimize the amount of genome from the donor parent that remains in the selected plants. It can also be used to reduce the number of crosses back to the recurrent parent
20 needed in a backcrossing program. The use of genetic markers in the selection process is often called genetic marker enhanced selection or marker-assisted selection (MAS). Genetic molecular markers may also be used to identify and exclude certain sources of germplasm as parental varieties or ancestors of a plant by providing a means of tracking genetic profiles through crosses.

Molecular tools (e.g., oligonucleotide primers and/or detection probes) and molecular
25 assays may be designed to determine the genotype of a plant at the one or more molecular markers associated with resistance or susceptibility of a plant to a plant pathogen. As used herein, the term "genotype" refers to the genetic constitution of a cell or organism. An individual's "genotype" for one or more molecular markers includes the specific alleles for the one or more molecular markers present in the individual's haplotype. As is known in the art, a genotype can
30 relate to a single locus (e.g., a single molecular marker) or to multiple loci (e.g., multiple molecular marker), whether the loci are related or unrelated and/or are linked or unlinked. In some examples, a plant's genotype relates to one or more molecular markers within or associated with genes that are involved in the expression of the phenotype of interest (e.g. a resistance or susceptibility to a plant pathogen).

35 The one or more molecular markers associated with the resistance or susceptibility phenotype may comprise between one and ten markers. In some examples, the one or more molecular markers associated with the resistance or susceptibility phenotype comprise more than ten genetic markers.

In one example, the one or more molecular markers identified are associated with a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof. For example, the one or more molecular markers identified may be associated with a resistance phenotype prior to entry of the pathogen into the plant or part thereof. Alternatively, or in addition, the one or more molecular markers identified may be associated with a resistance phenotype during entry of the pathogen into the plant or part thereof.

In another example, the one or more molecular markers identified are associated with a resistance phenotype after entry of the pathogen into the plant or part thereof.

In one example, the one or more molecular markers identified are associated with a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof. For example, the one or more molecular markers identified may be associated with a susceptibility phenotype prior to entry of the pathogen into the plant or part thereof. Alternatively, or in addition, the one or more molecular markers identified may be associated with a susceptibility phenotype during entry of the pathogen into the plant or part thereof.

In another example, the one or more molecular markers identified are associated with a susceptibility phenotype after entry of the pathogen into the plant or part thereof.

The one or more molecular markers may be selected from the group consisting of a single nucleotide polymorphism (SNP) marker, an amplified fragment length polymorphism (AFLP) marker, a DNA amplification fingerprinting (DAF) marker, a random amplified polymorphic DNA (RAPD) marker, microsatellite markers (e.g. simple sequence repeats (SSRs)), insertion mutation markers, sequence-characterized amplified region (SCAR) markers, cleaved amplified polymorphic sequence (CAPS) markers, kompetitive allele specific PCR (KASP) and any combinations thereof.

In one example, comparing the sequence data at (iii) comprises performing one or more sequence alignment steps to identify the one or more molecular markers or sequence variants. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, (1981) *Adv. Appl. Math.* 2: 482; by the homology alignment algorithm of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48: 443; by the search for similarity method of Pearson and Lipman, (1988) *Proc. Natl. Acad. Sci.* 85: 2444; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California; GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, (1988) *Gene* 73: 237-244; Higgins and Sharp, (1989) *CABIOS*, 5: 151-153; Corpet *et al*, (1988) *Nucleic Acids Research* 16: 10881-90; Huang *et al*, (1992) *Computer Applications in the Biosciences* 8: 155-65, and Pearson *et al.*, (1994) *Methods in Molecular Biology* 24: 307-331.

The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); Altschul *et al*, (1990) *J. Mol. Biol.*, 215:403-410; and, Altschul *et al*, (1997) *Nucleic Acids Res.* 25:3389-3402. Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive- valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sd. USA* 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences {see e.g., Karlin & Altschul, (1993) *Proc. Nat. Acad. Sd.* USA 90:5873-5877). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

Multiple alignment of the sequences can be performed using the CLUSTAL method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=IO, GAP LENGTH PENALTY=IO). Default parameters for pairwise alignments

using the CLUSTAL method are KTUPLE 1, GAP PENALTY=3, WINDO W=5 and DIAGONALS SAVED=5.

One or more informative molecular biomarkers (e.g., polymorphisms) may be determined between corresponding polynucleotide sequences obtained from resistant and susceptible plants in the alignment. The alignment can be made such that the sequences derived from the resistant and susceptible plants can be compared. Differences can then be identified which are informative for a resistant phenotype or a susceptible phenotype (e.g., polymorphisms). In some examples, sequence differences at one or more molecular markers are associate with differing phenotypes of Classes 1-6 identified in the method of the disclosure.

Molecular selection tools (e.g., oligonucleotide primers and detection probes) may then be designed to discriminated between marker variants. For example, in the case of SNP markers, detection probes may be designed for detection of marker variants in a sample, for example, by hybridization on DNA chips or a beads-based detection platform. Detection probes may be designed such that a polymorphism or variant is reflected therein. In case of single nucleotide polymorphisms (SNPs) the detection probes typically contain the variant SNP alleles at the central position such as to maximize allele discrimination. Such probes can advantageously be used to screen test samples having a certain polymorphism. The probes can be synthesized using any method known in the art. The probes are typically designed such that they are suitable for high throughput screening methods e.g., such as the Affymetrix platform using chip-based detection of SNPs or bead technology provided by Illumina.

In some examples, the method comprises generating one or more polynucleotides sequences for the plant of the species or cultivar by performing one or more assays selected from a nucleic acid isolation assay, a nucleic acid purification assay, a digestion assay, an assay for enrichment of molecular markers, a nucleic acid amplification assay, nucleic acid sequencing and combinations thereof.

Plant breeding methods

The present disclosure also provides a method of breeding a plant which is resistant or susceptible to a plant pathogen (as desired). In some examples, the method comprises crossing or selfing a plant identified as being resistant or susceptible to the plant pathogen (as appropriate or desired) using the phenotyping method described herein to thereby produce one or more progeny plants which are resistant or susceptible to the plant pathogen. Alternatively, or in addition, the method may comprise: (i) obtaining a plant identified as being resistant or susceptible to the plant pathogen (as desired) using the method described herein; and (ii) introducing one or more foreign nucleic acids to the plant and/or modifying or editing one or more polynucleotides within the plant's genome, to produce a plant which is resistant or susceptible to the plant pathogen.

As used herein, the term "breeding", and grammatical variants thereof, refer to any process that generates a progeny individual. Breeding can be sexual or asexual, or any combination thereof. Exemplary non-limiting types of breeding include crossing, selfing, doubled haploid derivative generation, and combinations thereof. The method of breeding a plant as described
5 herein may comprise performance of the method described herein for identifying the plant which exhibits the resistance or susceptibility to the plant pathogen (as desired), and then selfing, crossing, backcrossing or genetically modifying the plant identified as exhibiting the resistance or susceptibility to the plant pathogen (as desired).

As used herein, the term “selfing”, “self-fertilization” “self-pollination” or variations
10 thereof means the fusion of male and female gametes from the same individual. Accordingly, in the context of the present disclosure, selfing refers to fusion of male and female gametes from a plant identified as being resistant or susceptible to the plant pathogen (as appropriate or desired) using the phenotyping method described herein.

As used herein, the term “crossing”, “cross-pollination” or variations thereof means a cross
15 between two different plants. Accordingly, in the context of the present disclosure, crossing refers to crossing a plant identified as being resistant or susceptible to the plant pathogen (as appropriate or desired) using the phenotyping method described herein with another plant of the same species and/or variety.

As used herein, the term "backcross", and grammatical variants thereof, refers to a process
20 in which a breeder crosses a progeny (e.g., a hybrid progeny) back to one of the parents, for example, a first generation hybrid F1 with one of the parental genotypes of the F1 hybrid. In some examples, a backcross is performed repeatedly, with a progeny individual of one backcross being itself backcrossed to the same parental genotype.

As used herein, the term “progeny” or “progeny plants” or similar includes the descendants
25 of a plant identified as being resistant or susceptible to the plant pathogen (as appropriate or desired) using the phenotyping method of the disclosure. The term “progeny” is intended to encompass "direct progeny" and “indirect progeny”. As used herein, the term "direct progeny" refers to plants that derive from the seed (or, sometimes, other tissue) of a plant identified as being resistant or susceptible to the plant pathogen (as appropriate or desired) using the
30 phenotyping method described herein and is in the immediately subsequent generation. For instance, for a given lineage, a T2 plant is the direct progeny of a T1 plant. As used herein, the "indirect progeny" refers to plants that derive from the seed (or other tissue) of the direct plant progeny produced by the method of breeding a plant as described herein, or from the seed (or other tissue) of subsequent generations in that lineage; for instance, a T3 plant is the indirect
35 progeny of a T1 plant.

In one example, the method of breeding a plant which is resistant to a plant pathogen comprises selecting the plant to be crossed or selfed based on the presence of one or more molecular markers associated with resistance to the plant pathogen and/or the absence of one or

more molecular markers associated with susceptibility to the plant pathogen, wherein the one or more molecular markers are identified using the method described herein. For example, the plant to be crossed or selfed is selected based on the presence of one or more molecular markers associated with resistance to the plant pathogen. For example, the plant to be crossed or selfed
5 is selected based on the absence of one or more molecular markers associated with susceptibility to the plant pathogen.

In one example, the method of breeding a plant which is resistant to a plant pathogen of interest comprises: (i) obtaining a plant which has been identified by the method of the disclosure as being capable of eliciting a defence response to the pathogen prior to or during entry of the
10 pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 1 or Class 2 response phenotype); and (ii) producing a plant which is resistant to the plant pathogen of interest by selfing, crossing or genetically modifying the plant obtained at (i).

In one example, the method of breeding a plant which is resistant to a plant pathogen of interest comprises: (i) obtaining a plant which has been identified by the method of the disclosure
15 as being capable of eliciting a defence response to the pathogen post entry of the pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 2, Class 3 or Class 4 response phenotype); and (ii) producing a plant which is resistant to the plant pathogen of interest by selfing, crossing or genetically modifying the plant obtained at (i).

In one example, the method of breeding a plant which is resistant to a plant pathogen of
20 interest comprises: (i) obtaining a plant which has been identified by the method of the disclosure as being capable of eliciting a defence response to the pathogen prior to, during and post entry of the pathogen into the plant tissue (*e.g.,* a plant exhibiting a Class 2 response phenotype); and (ii) producing a plant which is resistant to the plant pathogen of interest by selfing, crossing or genetically modifying the plant obtained at (i).

25 In one example, the method of breeding a plant which is resistant to a plant pathogen of interest comprises: (i) obtaining a plant which has been identified by the method of the disclosure as not being capable of eliciting a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue but which still shows evidence of resistance to the pathogen (*e.g.,* a plant exhibiting a Class 5 response phenotype); and (ii) producing a plant which is
30 resistant to the plant pathogen of interest by selfing, crossing or genetically modifying the plant obtained at (i).

In one example, the method of breeding a plant which is susceptible to a plant pathogen of interest comprises: (i) obtaining a plant which has been identified by the method of the disclosure as being susceptible to the plant pathogen (*e.g.,* a plant exhibiting a Class 6 response
35 phenotype); and (ii) producing a plant which is resistant to the plant pathogen of interest by selfing, crossing or genetically modifying the plant obtained at (i).

In each of the foregoing examples, the plant obtained at (i) may be crossed with a plant which has been identified by the method of the disclosure as having a response phenotype to the

pathogen which is different to the plant obtained at (i). In other examples, the plant obtained at (i) may be crossed with a plant which has been identified by the method of the disclosure as having a response phenotype to the pathogen which is the same as that of the plant obtained at (i). In other examples, the plant obtained at (i) may be crossed with a plant whose response phenotype to the pathogen is unknown.

The term "introducing", as used in the context of a nucleic acid, means presenting the nucleic acid to plant tissue in such a manner that the nucleic acid gains access to the interior of a cell in the plant tissue. Where more than one nucleic acid is to be introduced, these nucleic acids can be assembled as part of a single nucleic acid construct, or as separate nucleic acid constructs, and can be located on the same or different transformation vectors. Accordingly, multiple nucleic acid can be introduced into the plant tissue in a single transformation event, in separate transformation events, or, *e.g.,* as part of a breeding protocol.

The term "transformation" as used herein refers to the introduction of a nucleic acid into plant tissue. Transformation of the cell may be stable or transient.

Four general methods for direct delivery of nucleic acids into cells have been described: (1) chemical methods (Graham *et al.*, 1973); (2) physical methods such as microinjection (Capecchi, 1980); electroporation (see, for example, WO 87/06614, US 5,472,869, US 5,384,253, WO 92/09696 and WO 93/21335); and the gene gun (see, for example, US 4,945,050 and US 5,141,131); (3) viral vectors (Clapp, 1993; Lu *et al.*, 1993; Eglitis *et al.*, 1988); and (4) receptor-mediated mechanisms (Curiel *et al.*, 1992; Wagner *et al.*, 1992).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts, nor the susceptibility of *Agrobacterium* infection are required.

In another alternative example, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (U.S. 5, 451,513, U.S. 5,545,818, U.S. 5,877,402, U.S. 5,932479, and WO 99/05265).

Agrobacterium-mediated transfer is another widely applicable system for introducing nucleic acids into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art (see, for example, US 5,177,010, US 5,104,310, US 5,004,863, US 5,159,135). Further, the integration of the T-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome.

In a preferred example, the nucleic acid molecule introduced to the plant tissue is stably introduced. As used herein, the term "stably introducing" or "stably introduced", in the context of a nucleic acid introduced into plant tissue, is intended to mean that the introduced nucleic acid is stably incorporated into the genome of the plant, and thus the plant cells are stably transformed
5 with the nucleic acid. The integrated nucleic acid is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations. As referred to herein, the term "genome" includes the nuclear and the plastid genome, and therefore includes integration of a nucleic acid into, for example, the chloroplast genome. Stable transformation as used herein can also refer to a nucleic acid that is maintained extrachromasomally, for example,
10 as a minichromosome.

A "nucleic acid" as used herein shall be understood to mean a polynucleotide such as, for example, DNA, RNA or oligonucleotides. The one or more nucleic acids introduced to the plant tissue may be present in one or more nucleic acid constructs, such as expression cassettes, capable of directing expression of a particular polynucleotide sequence in the nucleic acid in the plant,
15 and will generally comprise a promoter operatively-linked to the polynucleotide sequence and/or one or more other regulatory elements required for proper expression and translation of a protein or polypeptide encoded thereby.

Preferably, the nucleic acid introduced into the plant tissue comprises at least one gene of interest. The gene of interest may increase or decrease the endogenous level of activity of a
20 protein in the genetically modified plant, or may introduce a new protein to the plant. For example, the gene of interest may encode a protein or functional polynucleotide which; increases yield, confers enhanced animal and/or human nutrition, confers herbicide tolerance (*e.g.*, glyphosate resistance or glufosinate resistance), affects carbohydrate biosynthesis or modification (*e.g.*, starch branching enzyme, starch debranching enzyme, starch synthases, ADP-
25 glucose pyrophosphorylase), is involved in fatty acid biosynthesis or modification (*e.g.*, a desaturase, elongase, hydroxylase, epoxidase, conjugase, acetylase, TAG assembly), confers insect resistance (*e.g.* crystal toxin protein of *Bacillus thuringiensis*), confers viral resistance (*e.g.* viral coat protein); confers fungal resistance (*e.g.*, chitinase, β-1,3-glucanase, moricin-related peptides or phytoalexins), alters sucrose metabolism (*e.g.*, invertase or sucrose synthase), confers
30 reduced allergenicity, increases digestibility, confers environmental stress tolerance, confers nematode resistance, is a gene encoding a pharmaceutical (*e.g.*, antibiotics, antibodies, secondary metabolites, pharmaceutical peptides or vaccines), is a gene encoding an industrial enzyme, or increases the use of the sorghum plant or part thereof as a biofuel.

Other techniques known in the art for genetically modifying or genetically editing plants
35 are also contemplated, including, but not limited to, TILLING, zinc finger nuclease (ZFN), TAL effector nuclease (TALEN), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR).

In one particular example, it is contemplated that the method of breeding a plant as described herein may comprise introducing one or more nucleic acid(s) related to the clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas system for inducing targeted genetic alterations.

5 The CRISPR system can be portable to plant cells by co-delivery of plasmids expressing the Cas endonuclease and the necessary crRNA components. The Cas endonuclease may be converted into a nickase to provide additional control over the mechanism of DNA repair (Cong *et al.*, 2013).

Thus, in one embodiment, loci of the CRISPR system may be transformed into the plant to 10 enable CRISPR mediated gene editing thereof.

The plants which are bred in the method of the disclosure may be of any species or variety. Exemplary groups of plants and plant species are described herein in the context of the phenotypic screening method of the disclosure and shall be taken to apply *mutatis mutandis* to each of the following and foregoing examples describing methods of breeding a plant, unless 15 specifically stated otherwise

In one particular example, the plant which is bred is selected from the group consisting of a fruiting plant, a leguminous plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant and a noxious plant or weed.

In one example, the plant which is bred is a cereal plant. For example, the cereal plant 20 may be selected from the group consisting of wheat, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa and buckwheat. In one example, the cereal plant is wheat. In one example, the cereal plant is barley.

In one example, the plant which is bred is an oil plant. Exemplary oil plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each 25 and every example describing plant breeding methods. For example, the oil plant may be canola, soybean, sunflower, olive, coconut or palm. In one example, the oil plant is canola.

In one example, the plant which is bred is a vegetable plant. Exemplary vegetable plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods. In one example, the 30 vegetable plant is a tomato plant, or celery or celeriac plant.

In one example, the plant which is bred is a fruiting plant. Exemplary fruiting plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods. In one example, the fruiting plant is a blueberry plant.

35 In one example, the plant is an ornamental plant. Exemplary ornamental plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods.

In one example, the plant is a forestry plant. Exemplary forestry plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods.

In one example, the plant is a fibre plant. Exemplary fibre plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods.

In one example, the plant is an aquatic plant. Exemplary aquatic plants are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods.

In one example, the plant is an noxious plant or weed. Exemplary noxious plants and weeds are described herein in the context of screening methods and shall be taken to apply *mutatis mutandis* to each and every example describing plant breeding methods.

The present disclosure also provides a plant or plant part which has been bred by performing the method of breeding a plant as described herein. The term "plant" as used herein as a noun refers to whole plants, but as used as an adjective refers to any substance which is present in, obtained from, derived from, or related to a plant, such as for example, plant organs (*e.g.,* leaves, stems, roots, flowers), single cells (*e.g.,* pollen), seeds, plant cells and the like. The term "plant part" refers to all plant parts that comprise the plant DNA, including vegetative structures such as, for example, leaves or stems, roots, floral organs or structures, pollen, seed, seed parts such as an embryo, endosperm, scutellum or seed coat, plant tissue such as, for example, vascular tissue, cells and progeny of the same, as long as the plant part is the product of a method of the disclosure.

In one example, the plant part is a plant propagation material. For example, the plant propagation material may be a seed, a plant cutting or scion.

EXAMPLES

Example 1: Protocol for the preparation of *Zymoseptoria tritici* inoculum

Preparation for YMS medium

YMS (Yeast Malt Sucrose) medium was prepared as a solid medium with agar on petri dishes (90 x 15 mm, Thermo Fisher), or liquid without agar in flasks. 1 litre of YMS medium contained: 4 g yeast extract, 4 g malt extract, 4 g sucrose, 15 g technical agar, and 25 mg Gentamicin. Generally, liquid YMS is a more convenient inoculum preparation for large number of plants, while solid YMS agar is better for small number of plants. Since the infiltration method requires a minimal amount of inoculum, the solid YMS agar was prepared.

Isolation of pathogen and growth of inoculum

The asexual fruiting body of *Z. tritici* is called pycnidium (pl. pycnidia). These fruiting bodies appear as tiny black spots on the surface of infected leaves (see Figure 2). Infected pieces of leaf were collected, then surface sterilized with 70% ethanol and placed flat on a piece of wet Whatman® Filter Paper in a petri dish humid chamber. Leaf pieces were incubated at 25°C for 3 hrs or overnight (16 hrs). Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. The pycnidium on the infected leaf pieces produces conidia as a cirrhus or ooze-like structure. All conidia belonging to a sporulating pycnidium are genetically identical, so they are recognized as an isolate with a designated individual name. The cirrhus of the sporulating pycnidium were picked out with a sterilized needle and sub-cultured on solid YMS agar petri dishes under aseptic conditions in a Laminar Air Flow cabinet. After 4-10 days, each sub-cultured isolate grew into approximate 1 mm pinkish cream cluster of spores (blastospores).

Conditions for inoculum storage

The pinkish isolate clusters were also sub-cultured for future experiments. Briefly, 500 µl of sterile water was added to the solid YMS petri dish agar surface using a sterile pipette to keep the solid YMS petri dish agar surface moist. This step was repeated, as required to maintain a moist agar surface. The petri dishes were incubated under white fluorescent lights (800-1000 lumens) at 18°C. After 5-6 days, a pinkish cream growth was observed in the plate. Spores can be maintained in this form under cool moist conditions with frequent subculturing, ensuring that the petri dish agar surface does not dry out. Petri dishes were also monitored constantly for contaminants. A fresh culture was started every 30 days to minimise the risk of contamination and loss of pathogenicity.

Blastospores collected from the YMS agar surface can also be mixed with a solution of 0.5% glycerol. This spore solution is added to a 2 ml cryogenic tube containing 1-2 mm diameter sterilised glass or plastic beads, and stored at -80 °C for up to 5 years. Blastospores can be recovered from cryogenic storage and regenerated on solid YMS agar petri dishes.

Calculation of inoculum spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer (Neubauer, Counting Chambers, BLAUBRAND®, Cell culture) was used to count and then adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was

less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 2: Experimental procedures for *Z. tritici* inoculation

Preparation of the inoculum suspension

5 The *Z. tritici* isolates in the liquid YMS medium were diluted to the desired concentration with the addition of gelatin (0.5%), which acts as a surfactant helping spores to adhere to the leaf surface. The spore concentration used was approximately 4 x 10^6 spores/ml. Gelatin solution was prepared by dissolving 5 g of gelatin (Mckenzie's, AUS) in 100 ml of hot water, for every litre of final inoculum. The water and gelatin powder were placed on a magnetic stirrer and stirred 10 until dissolved. Once the solution has cooled to less than 30°C (a large amount of hot water can decrease viability of inoculum), the gelatin solution was added to the inoculum suspension.

In preparation of inoculum for the infiltration method, approximately 5 µl of dense blastospores was scraped from the petri dish into 50 ml of clean distilled water and adjusted for a final concentration of approximately 1 x 10^6 spores/ml without adding gelatin.

15 Plant growth

Twenty pots were organized in a 5 x 4 matrix on a plastic rack, filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 67 mm (L) x 67 mm (W) x 155mm (D). One or two wheat seeds were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. A cultivar known to be 20 susceptible (and/or previously identified as exhibiting a Class 6 using the method described herein) was included as a reference for disease assessments. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with 25 temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached the two-leaf stage 10-16 days after sowing. To maintain the healthy growth of plants, regularly watering them is required until the finish of the experiments.

Septoria inoculation procedures

The 'spray method'

30 In the spray method, the final spore concentrations were adjusted to 4 x 10^6 spores/ml, supplemented with 0.5% gelatin. The spore suspension was applied as a fine mist to the leaf surface of 2-3 weeks-old plants with two fully emerged leaves, using an airbrush (Anest Iwata, Japan). However, similar application of the spore suspension to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the 35 spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by

immersion. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 16 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C). A negative control (mock) treatment was included for the spray which consisted of the carrier liquid for the spray method applied without fungal spores to ensure the response phenotypes were not due to background effects. This was applied to all varieties sown in the experiment.

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 0.5-1 x 10^6 spores/ml. Gelatin was not required for this method. When the plants were grown an additional 4-7 days after seeing the shoot of the second leaf (not fully expanded, normally lighter green colour), 10 µL of inoculum (about 100 spores) was infiltrated on the second leaf blade using a 3 ml Syringe (Terumo, Japan). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring. A negative control (mock) treatment was included for the infiltration which consisted of the carrier liquid for the infiltration method applied without fungal spores to ensure the response phenotypes were not due to background effects. This was applied to all varieties sown in the experiment.

As mentioned above, leaf infiltration was carried out 4-7 days after the emergence of the 2nd leaf, this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into the leaves of different genotypes, as it is very common that leaves of some genotypes are much more difficult to infiltration than others. After the infiltration, a thin mist of water was sprayed on the plants within the first 24 hours.

Plant assessment

Plants were scored 4-42 days after the inoculation, depending on the progress of experiments. Septoria tritici blotch (STB) scores (1-5) were then assigned to each plant according to the method described in Zwart *et al* (2010) *Molecular Breeding*, 26:107-124. Briefly, STBs were assigned based on the visually estimated percentage of necrotic lesions containing pycnidia on the infected leaves, where 1 = no symptoms; 2 = occasional pycnidia in a few lesions, affecting < 25% of leaf area; 3 = a low density of pycnidia in many or most lesions, affecting 26-50% leaf area; 4 = an even distribution of pycnidia at moderate density over most of the lesions, affecting 51-75% leaf area; 5 = maximum number of pycnidia distributed over most lesions, affecting 76-100% leaf area. Other traits taken in consideration during assessment were the percentage of necrosis area (Nec, 0-100%) on the infected leaf and the percentage of pycnidia covered on the necrosis area (Pyc, 0-100%).

Example 3: Different Stages of infection in plants resulting from *Z. tritici* spores

This example describes the nine stages of interaction between *Z. tritici* and wheat plants which have been used in the phenotyping method of the disclosure to classify plants according to their defence and resistance phenotypes.

5 Stage 1 –Attachment of spores to plant surface (*e.g.*, leaves) (0-12 hr)

Primary infection can be caused by ascospores or pycnidia spores, which attach themselves to the surface of a plant (*e.g.,* a plant leaf). Ascospores are, in general, responsible for primary infection at the start of a growing season. Some plants possess a physical barrier, comprised of a thin waxy cuticle and epidermis, which restricts attachment of spores to the host 10 plant leaves.

Stage 2 – Germination of spores (0-24 hr)

Once the spores have successfully attached to the surface of a plant (*e.g.,* a leaf surface), the spores degrade the epidermis in anticipation of germination. Water acts as an important factor for the successful pathogen infection and surfactants (*e.g.,* Tween 20) are frequently added to 15 inoculum buffer to assist in this process. The plant initiates the first stage of host defence i.e., effector triggered immunity (ETI). The first screening method of the disclosure (i.e., the surface contact method) starts at this point.

Stage 3 - Hyphae entry into the stomatal cavity (1-3 day)

Fungus hyphae grow and penetrate the leaf surface via stomata. During this stage, hyphae 20 undergo slow growth in the stomatal cavity. Plant pathogenesis-related (PR) genes are typically expressed at this stage of the infection cycle.

Stage 4 - Substomatal colonization (2-5 day)

Hyphae enlarges in the substomatal cavity and branch into intercellular space. Symptoms are typically not evident at this stage and the latent period varies depending on the plant genotype 25 and environmental conditions. Expressions of PR genes increases at this stage. As the inoculum directly infiltrates into the leaf surface, the infection process using the second screening method (i.e., the infiltration method) starts at this point.

Stage 5 - Apoplastic colonization (4-7 day)

Hyphae elongate through the mesophyll to the surrounding substomatal cavities. Hyphae 30 are still growing slowly at this point of the infection, and fungal biomass is very low. No macro symptoms are observable.

Stage 6 - Host cell death (7-11 day)

Growth rate of hyphae increases significantly in susceptible host plants. Water-soaked lesions or tattered grayish/brownish lesions are evident on the leaf surface (7-12 day). A hypersensitive response (HR)-like reaction starts at this point, which is characterized by rapid cell death localised to the area of infection in order to restrict the growth and spread of hyphae to other leaf areas. Resistance genes, such as NBS-LRR and cysteine kinase, may function here as receptors and interact with the effectors from the pathogen. The plant can induce another stage of defence, 'ETI', if the appropriate resistant genes are present.

Stage 7 - Increase in fungal biomass (9-14 day)

Faster hyphal growth and hyphal aggregation in the substomatal cavities is evident. Failure of the host to recognize pathogen effectors results in bursting of mesophyll cells. The pathogen feeds on the efflux of nutrients released by host cells. Macro symptoms of chlorosis and necrosis begin to appear in the leaf tissue.

Stage 8 - Reproduction asexual/sexual structure (13-21 day)

As more mesophyll cells die, the leaf loses its physical integrity and collapses, establishing the necrotic leaf area where the asexual pycnidiospores are produced to form small, black pycnidia,. Sexual ascospores may also be produced in pseudothecium.

Stage 9 - Reinfection of new sites (17 day onward)

A new cycle of infection commences. With the assistance of water splash dispersal, pycnidiospores disperse and spread the disease up through the leaf layers of the host.

Example 4: A phenotyping selection tool to classify plant defence responses to fungal pathogens

This example relates to a multi-modal screening method for classifying plants based on their defence response phenotypes to fungal pathogens (designated the 'Defend system'). Critically, the Defend system is able to distinguish between events occurring during the early phases of infection (i.e., pre-entry or during entry) versus those occurring after the pathogen has successfully entered into the leaf or other plant tissue (i.e., post-entry). In this regard, the method is capable of identifying when a plant defence (to a plant pathogen) is being triggered at different points along the infection pathway by separating those events which occur during the early phases of infection, to those which occur after the pathogen has successfully entered into the plant tissue. Using this method, the inventors have characterised six distinct Classes of response phenotype (also referred to herein as "Defend Classes"), which are as follows:

Class 1: a response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method (i.e., the contact inoculation method), and chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the second screening method (i.e., the infiltration inoculation method). A plant which exhibits a Class 1 response phenotype elicits a defence response to the pathogen prior to or during entry of the pathogen into the plant tissue, but not post entry This response phenotype is only effective during the very early stages of the infection cycle.

Class 2: a response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method and the second screening method. The plant's defence response prevents/restricts the colonisation phase from proceeding to chlorosis and prevents fruiting structures from forming. A plant which exhibits a Class 2 response phenotype elicits a defence response to the pathogen prior to or during and post entry of the pathogen into the plant tissue

Class 3: a response phenotype characterised by chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and an absence of chlorosis, necrosis and pathogen fruiting bodies form on the plant(s) or plant part(s) following performance of the second screening method. The plant's defence response prevents/restricts the colonisation phase from proceeding to chlorosis and prevents fruiting structures from forming. A plant which exhibits a Class 3 response phenotype elicits a defence response to the pathogen post entry of the pathogen into the plant tissue, but not prior to or during entry.

Class 4: a response phenotype characterised by colonization of the plant(s) or plant part(s) by the pathogen, but infection is halted with minimal chlorosis, following performance of the first screening method and/or the second screening method. A plant which exhibits a Class 4 response phenotype does not elicit a defence response to the pathogen prior to entry or during of the pathogen into the plant tissue, but does elicit a defence response to the pathogen post entry of the pathogen into the plant tissue and colonisation of the plant by the pathogen still occurs. Class 4 is typically identified in the second screening method (i.e., the infiltration inoculation method) during the latent period and at the end of the experiment.

Class 5: a response phenotype characterised by chlorosis, necrosis and formation of pathogenic fruiting structures with evidence of host resistance, wherein host resistance is characterised by a reduction in number of lesion and/or lesion area compared to a plant or plant part in which there is no evidence of host resistance. A plant which exhibits a Class 5 response phenotype does not elicit a defence response to the pathogen prior to or post entry of the pathogen into the plant tissue, but shows evidence of resistance to the pathogen represented by a reduced

number of lesion and/or reduced lesion area compared to a plant or plant part in which there is no evidence of host resistance (*e.g.*, a plant classified as Class 6). Class 5 is typically identified in the second screening method (i.e., the infiltration inoculation method) during the latent period and at the end of the experiment.

5 Class 6: a response phenotype characterised by chlorosis, necrosis and formation of pathogen fruiting structures with no evidence of host resistance. A plant which exhibits a Class 6 response phenotype does not elicit a defence response to the pathogen prior to or post entry of the pathogen into the plant tissue, and shows no evidence of resistance to the pathogen. Class 6 is typically identified following completion of the first screening method (i.e., the contact 10 inoculation method) and the second screening method (i.e., the infiltration inoculation method), at the end of the experiment.

The critical step for the 'Defend' system is the distinction between Class 1 and Classes 2-5 phenotypes. This allows for the selection and combination of genotypes from plants which trigger defence responses during different stages of the infection process. The 'Defend' 15 phenotyping system is illustrated in Figure 1 and Table 3.

Table 3. 'Defend' uses a comparison of eight phases during the infection of leaves by a fungal pathogen using two different inoculation methods (i.e., the contact inoculation method and the infiltration inoculation method). The system combines the phenotypes observed in both 20 phenotyping methods to reveal information that would not otherwise be available if either inoculation method was used on its own. The first separation occurs between defence being triggered at or before the pathogen enters the leaf (Class 1).

Stage	**Phases of infection**	**Apply spores to leaf surface (Traditional method)**		**Infiltrate spores**		**'Defend' Classes (C1-C6)**
		Effective	Detected	Effective	Detected	
1	Spore attachment	No	No	No	No	
2	Spore germination	No	No	No	No	
3	Entry into leaf via a) stomata, b) between epidermal cells, c) direct penetration of epidermal cells	**Yes C1 Yes C2 No C3**	No	**No C1 Yes C2 Yes C3**	No	**C1, C2 and C3 distinguished**

Stage	Phases of infection	Apply spores to leaf surface (Traditional method)		Infiltrate spores		'Defend' Classes (C1-C6)
4	Apoplastic colonization	Yes	**No**	Yes	**Yes**	
5	Latent period (time from inoculation to first symptoms)	Yes	Yes	Yes	Yes	
6	Host cell death (Effector mediated or other)	Yes	**No**	Yes	**Yes**	C1-C6 distinguished
7	Increase in fungal biomass (systemic movement beyond primary infection site)	Yes	**No**	Yes	**Yes**	
8	Reproduction asexual/sexual structure	Yes	Yes	Yes	Yes	

"Effective" means if the pathogen is detected by the host at this stage disease is stopped or slowed.

"Detected" means this phenotype can be separated from other stages of the infection process using this method alone.

The new 'Defend' method has been used to discover new sources of resistance in plants.

Example 5: Evaluation of 'Defend' system and traditional screening methods for selecting wheat cultivars resistant and susceptible to *Z. tritici*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on several wheat varieties with genotypes with known resistance to *Z. tritici* was designed to, *inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are known to be resistant to *Z. tritici*.

Twenty bread wheat cultivars were selected for inclusion in the experiment, including 'Lorikeet' with the gene Stb19, 'Currawong' with the genes Stb7 and Stb12, and 'Summit' which is a susceptible cultivar. Briefly, the twenty cultivars were sown in a fixed order of 5 x 4 tubes in a rack, each rack was replicated four times and arranged in a zig-zag format on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 2. The *Z. tritici* isolate used for the experiment was WAI332.

The experiment indicated no difference between the spray and the infiltration methods on 'Lorikeet' as a resistant genotype (Defend Class 2) and 'Summit' as a susceptible genotype (Defend Class 6 (Figure 2). However, the initial experiment differentiated the type of resistance as shown in 'Currawong', in which the spray method gave a resistant phenotype but a susceptible phenotype in the infiltration method (Defend Class 1) (Figure 2), suggesting the gene(s) resistant to the isolate WAI332 had a high chance of providing resistance at the stages of spore germination and hyphae entry into stomata, but not at later stages after the latent period (Table 3). By comparison, the Stb19 in Lorikeet is proposed to at least function at Stage 4 and Stage 5 and/or stages after, but its resistant functions at Stage 2 and Stage 3 could not be excluded.

Example 6: Protocol for the preparation of *Septoria apiicola* inoculum

Preparation for V8-PDA medium

V8-PDA (Potato-Dextrose Agar)medium was prepared as a solid medium with agar on polystyrene petri dishes (90 x 15 mm, Thermo Fisher). 1 litre of V8-PDA medium contained: 150 ml Campbells V8 juice, 10 g PDA, 1.5 g calcium carbonate ($CaCO_3$), 15 g technical agar, 850 ml of distilled water and 25 mg Gentamicin.

Isolation of pathogen and growth of inoculum

The asexual fruiting body of *S. apiicola* is called pycnidium (pl. pycnidia). These fruiting bodies appear as tiny black spots on the surface of infected leaves (see Figure 3). Infected pieces of leaves were collected, then surface sterilized with 70% ethanol and placed flat on a piece of wet Whatman® Filter Paper in a petri dish for 3 hrs or overnight (16 hrs). Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. The pycnidium produces conidia as a cirrhus or ooze-like structure. Because all conidia belonging to a sporulating pycnidium are genetically identical, they are recognized as an isolate with a designated name. The germinated pycnidium were picked up with a sterilized needle and sub-cultured on a petri dish under aseptic conditions in a Laminar Air Flow cabinet. After 10-15 days, the sub-cultured isolate grew into approximate 5-7 mm cluster of sporulating hyphae.

Conditions for inoculum storage

The spores from each isolate were also sub-cultured for future experiments. Briefly, sterile water was added to the V8-PDA plate surface using a sterile pipette to keep the V8 plate surface moist. This step was repeated, whenever necessary. The plates were incubated under the white fluorescent lights (800-1000 lumens) at 18°C. After 5-6 days, a dark hyphae with spore masses was observed in the plate. Spores can be maintained in this form under cool moist conditions with frequent subculturing, ensuring that the plates do not dry out. Plates were also monitored

constantly for contaminants. A fresh culture was started once a month to minimise the risk of contamination and loss of pathogenicity.

Spores can also be mixed with 0.5% glycerol, and collected with 1-2 mm plastic beads in the 2 ml tube, and stored at -80 °C for long term storage.

5 Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x
10 and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

15 **Example 7: Experimental procedures for *S. apiicola* inoculation**

Preparation of the inoculum suspension

The *S. apiicola* isolates on the solid V8 medium were flushed with sterile water and the agar surface was gently agitated to facilitate dislodgement of spores. Approximately 5 µl of spores was scraped from the petri dish into 50 ml of clean distilled water and adjusted for a final
20 concentration of approximately 7 x 10^5 spores/ml. Inoculation preparation was the same for the spray and the infiltration method,

Plant growth

Eighteen celery and celeriac (*Apium graveolens*) genotypes were planted as a single plant per pot, and each genotype was replicated three times. Pots were filled with Osmocote Premium
25 Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 160 mm (dia) x 172 mm (D) (3 litre volume). One transplanted 10 day old seeding were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature
30 was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached harvest maturity 90 days after sowing.

Septoria inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 7 x 10^5 spores/ml. The spore suspension was applied as a fine mist to the leaf surface of 90-100 day old plants using an airbrush. However, similar application of the spore suspension to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated plants were placed in a dark chamber at 90-100% relative humidity at 16 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 7 x 10^6 spores/ml. 90-100 day old plants were used. 10 µL of inoculum (about 100 spores) was infiltrated on two leaflets of a fully expanded leaf using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the leaf, and marked for scoring. Sterile water was infiltrated into one adjacent leaflet as the control. After the infiltration, a thin mist of water was sprayed on the plants within the first 24 hours.

Plant assessment

Plants were scored 14-28 days after the inoculation, depending on the progress of experiments. Scores were assigned to each plant using a modified version of the Septoria tritici blotch (STB) scoring (1-5) method described in Zwart *et al* (2010) *Molecular Breeding*, 26:107-124. Briefly, Scores were assigned based on the visually estimated percentage of necrotic lesions containing pycnidia on the infected leaves, where 0 = no symptoms (no chlorosis and no disease reaction) for either infiltration or spray methods; 1 = chlorosis but no pycnidia for either infiltration or spray methods; 2 = chlorosis, occasional pycnidia, no necrosis in infiltration lesions, for the spray method: occasional pycnidia, lesions affecting < 25% of leaf area; 3 = chlorosis, a low density of pycnidia, no necrosis in infiltration lesions, for the spray method: a low density of pycnidia in many or most lesions, lesions affecting 26-50% leaf area; 4 = spreading chlorosis past the infiltration point, necrosis at the infiltration point, an even distribution of pycnidia in infiltration lesions, for the spray method: an even distribution of pycnidia at moderate density over most of the lesions, affecting 51-75% leaf area; 5 = maximum number of pycnidia, spreading chlorosis and necrosis past the infiltration point in infiltration lesions, for the spray method: maximum number of pycnidia distributed over most lesions, affecting 76-100% leaf area. Other traits taken in consideration during assessment were the percentage of necrosis area (Nec, 0-100%) on the infected leaf and the percentage of pycnidia covered on the necrosis area (Pyc, 0-100%).

Example 8: Different Stages of infection in plants resulting from pathogenic *S. apiicola* spores

The nine stages of interaction between *S. apiicola* and celery plants used in the
5 phenotyping method of the disclosure to classify plants according to their defence and resistance phenotypes are the same as Stages 1-9 described in the context of *Z. tritici* and wheat plants in Example 3.

Example 9: Evaluation of the 'Defend' system and traditional screening methods for
10 **selecting celery and celeriac varieties resistant and susceptible to *S. apiicola***

In this example, the inventors evaluated the new 'Defend' phenotypic screening method described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on eighteen celery and celeriac varieties with genotypes with unknown resistance to *S. apiicola* was designed to, *inter alia*, determine whether the 'Defend' method could
15 differentiate plants harbouring genes that are unknown to be resistant to *S. apiicola*.

Eighteen celery and celeriac cultivars were selected for inclusion in the experiment, listed in Table 4. Briefly, the 18 cultivars were sown in a fixed order of 18 x 3 pots, each variety was replicated three times on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 7. The *S. apiicola* isolate used
20 for the experiment was WAI4287 which has unknown virulence on the celery and celeriac varieties.

Table 4: Celery and celeriac varieties (*Apium graveolens*) used to demonstrate the 'Defend' method.

Variety	Genus/species	Crop type
Amsterdam	*Apium graveolens subsp. secalinum*	Leaf Celery
Balena	*Apium graveolens subsp. dulce*	Celery
Chinese	*Apium graveolens subsp. dulce*	Celery
Conga	*Apium graveolens subsp. dulce*	Celery
Dorata_D_Asti	*Apium graveolens subsp. dulce*	Celery
Giant_Pascal	*Apium graveolens subsp. dulce*	Celery
Ilona	*Apium graveolens subsp. dulce*	Celery
Jive	*Apium graveolens subsp. dulce*	Celery
Laboni	*Apium graveolens subsp. dulce*	Celery
Light_Green	*Apium graveolens subsp. dulce*	Celery
Mambo	*Apium graveolens subsp. dulce*	Celery
Peppermint_Stick	*Apium graveolens subsp. dulce*	Celery
Sanjit_RZ_F1	*Apium graveolens subsp. dulce*	Celery
Stringless_Organic	*Apium graveolens subsp. dulce*	Celery

Tall_Utah	*Apium graveolens subsp. dulce*	Celery
Tango	*Apium graveolens subsp. dulce*	Celery
Verona	*Apium graveolens subsp. rapaceum*	Celeriac
White_Alabaster	*Apium graveolens subsp. rapaceum*	Celeriac

The experiment indicated no difference between the spray and the infiltration methods on 'Conga', 'Jive', 'Stringless Organic', 'Tall Utah' and 'Tango' as a susceptible genotypes, Defend Class 6. Representative images for 'Jive', 'Stringless Organic' and 'Tall Utah' are presented in Figures 4A, 4B and 4C respectively.

The experiment indicated a difference between the infiltration method (intermediate phenotype) and the spray method (resistant phenotype) for the variety 'Amsterdam' (Defend Class 1 or 4/5) (Figure 4D).

The experiment indicated an intermediate phenotype for both infiltration and spray methods for the variety 'Peppermint Stick' (Defend Class 4/5) (Figure 4E).

The experiment differentiated the type of resistance as shown in varieties 'Balena' (Figure 4F) and 'White Alabaster' (not shown), in which the infiltration method gave an intermediate or resistant phenotype and the spray method gave a susceptible phenotype (Defend Class 3), suggesting the gene(s) resistant to the isolate WAI4287 had a high chance of providing resistance at least in the stages of post entry into the plant, but not complete resistance (Table 5).

The remaining varieties tested displayed symptoms from the infiltration method consistent with Class 6 or Class 4/5. *See*, for example, Figures 4G and 4H for Giant Pascal and Laboni, respectively.

Table 5: Average score of phenotypes from two different application of inoculum applied to identify the Defend Class each variety belongs to. Each treatment was replicated 3 times per variety. Mock treatments = where no fungal spores were added to the inoculum suspension. Spore treatment = fungal spores added to the inoculum suspension. Scale 1= resistant 5 = susceptible

	Treatment				
Variety	**Mock_infiltrate**	**Mock_spray**	**Spore_infiltrate**	**Spore_spray**	**Defend Class**
Sanjit_RZ_F1	0	0	3	5	6
Verona	0	0	2	4	6
Stringless_Organic	0	0	3	3	6
Dorata_D_Asti	0	0	3	4	6
Jive	0	0	3	5	6
Mambo	0	0	3	5	6
Conga	0	0	3	5	6
Tango	0	0	2	5	6
Ilona	0	0	2	2	4/5

Balena	0	0	1	4	3
Laboni	0	0	2	3	4/5
Light_Green	0	0	3	5	6
Giant_Pascal	0	0	2	4	4/5
White_Alabaster	0	0	1	4	3
Chinese	0	0	3	4	6
Amsterdam	0	0	1	2	1
Peppermint_Stick	0	0	2	3	4/5
Tall_Utah	0	0	3	4	6

Example 10: Protocol for the preparation of *Puccinia striiformis f. sp. tritici* inoculum

Isolation, growth and storage of *Pst* inoculum

The asexual fruiting body of *Pst* is called a uredinium (pustules) which produce asexual urediniospores. These are thin-walled spores produced by the uredium on the surface of infected leaves (see Figure 5). Critically, the fresh rust spores from a single pustule, which can be recognized a single race with a designated name, are carefully picked up and mixed with 0.05% Tween 20, and then applied on young susceptible cultivars, i.e. 'Tammarin Rock'. Briefly, the spore suspension was gently painted on the fully emerged leaf blades of 2-3 weeks-old plants, using a cotton swab or brush. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 8-12 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C). After 14 days, new cluster of pustules should be observed on the plant leaf blades. New pustules were collected from the plant. Then repeat the process described above on more plants, in order to obtain sufficient amount of rust spores for future experiments, or to collect sufficient amount of spores for long-term storage. Rust spores are collected in 2 ml tubes, dried with silica gel at 4 °C for 2-3 days then into -80 °C , and stored at -80 °C for long term usage.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration in suspended cultures. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 11: Experimental procedures for *Puccinia striiformis f. sp. tritici* inoculation

Preparation of the inoculum suspension

The spore concentration of *Pst* used for the spray method was approximately 6 x 10^5 spores/ml. Dissolvents used for the spray method can be distilled water with Tween 20 or light mineral oil such as Isopar. In preparation of inoculum for the infiltration method, a final concentration was adjusted to 10^4 spores/ml. To mix the rust spores well in the liquid, 50 µl of
5 Tween 20 was dissolved in 100 ml of water.

Plant growth

Twenty pots were organized in a 5 × 4 matrix on a plastic rack, filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 67 mm (L) × 67 mm (W) × 155mm (D). One or two wheat seeds were sown in each pot and the pots
10 were then placed in a glasshouse to grow under controlled conditions. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached the two-leaf stage 10-16
15 days after sowing.

Puccinia striiformis f. sp. tritici inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 6 x 10^5 spores/ml, supplemented with 0.05% Tween 20. The spore suspension was applied as a fine mist to the leaf
20 surface of 2-3 weeks-old plants with two fully emerged leaves, using an airbrush. However, similar application of the spore suspension to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 8-12 °C for 48 hrs in the dark
25 prior to being transferred to the glasshouse (18-22 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 10^4 spores/ml. When the plants were grown an additional 4-7 days after seeing the shoot of the 2nd leaf (not fully expanded, normally lighter green colour), 10 µL of inoculum (about 100 spores) was
30 infiltrated on the second leaf blade using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring.

As mentioned above, leaf infiltration was carried out 4-7 days after the emergence of the 2nd leaf, this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into
35 the leaves of different genotypes, as it is very common that leaves of some genotypes are much

tougher to infiltrate than others. After the infiltration, a thin mist of water was sprayed on the plants within the first 24 hours.

Plant assessment

Plants were scored 14-28 days after the inoculation, depending on the progress of experiments. *Puccinia striiformis f. sp. tritici* (*Pst*) scores (1-5) were then assigned to each plant using 0–4 scale (McIntosh et al., 1995) based on the infection types. Low infection types (LITs= 0–2) were considered resistant, and infection type =2+ as intermediate while high infection types (HITs= 3–4) were rated susceptible.

Example 12: Different Stages of infection in plants resulting from pathogenic spores

This example describes the nine stages of interaction between *Pst* and wheat plants which have been used in the phenotyping method of the disclosure to classify plants according to their defence and resistance phenotypes.

Stage 1 –Attachment of spores to plant surface (*e.g.*, leaves) (0-3 h)

Primary infection can be caused by teliospores or urediniospores, which attach themselves to the surface of a plant (*e.g.*, a plant leaf). Teliospores are, in general, responsible for primary infection at the start of a growing season, where the alternate host is present allowing full disease life cycle to occur. Some plants possess a physical barrier, comprised of a thin waxy cuticle and epidermis, which restricts attachment of spores to the host plant leaves.

Stage 2 – Germination of spores (3-24 h)

Once the spores have successfully attached to the surface of a plant (*e.g.*, a leaf surface), the spores degrade the epidermis in anticipation of germination. Water acts as an important factor for the successful pathogen infection and surfactants (*e.g.*, tween-20) are frequently added to inoculum buffer to assist in this process. The plant initiates the first stage of host defence i.e., effector triggered immunity (ETI). The first screening method of the disclosure (i.e., the surface contact method) starts at this point.

Stage 3 - Hyphae entry into the plant cell (6-48 h)

Fungus hyphae grow and form germ tube, which produces an appressorium and a penetration peg that enters epidermal cells directly or through stomata and invades the plant cell by forming a haustorium. During this stage, Plant pathogenesis-related (PR) genes are typically expressed at this stage of the infection cycle.

Stage 4 - Apoplastic colonization (2-4 day)

Fungal growth develops from the vesicle and proceeds intercellularly in the mesophyll layer. Symptoms are typically not evident at this stage. Expressions of PR genes increases at this stage. As the inoculum directly infiltrates into the leaf surface, the infection process using the second screening method (i.e., the infiltration method) starts at this point.

5 Stage 5 - Apoplastic colonization (4-7 day)

Hyphae grows and proliferates in plasmodesmata and mesophyll cells. No macro symptoms or weak sign of infection may be observed.

Stage 6 - Host cell death (6-8 day)

Damage of cellular organelles beyond the advancing hyphae implies the involvement of
10 a toxin in the infection process. Small brown-yellow pustules may be evident on the leaf surface. A hypersensitive response (HR)-like reaction starts at this point, which is characterized by rapid cell death localised to the area of infection to restrict the growth and spread of hyphae to other leaf areas. Resistance genes, such as NBS-LRR and cysteine kinase, may function here as receptors and interact with the effectors from the pathogen. The plant can induce another stage
15 of defence, ‘ETI’, if the appropriate resistant genes are present.

Stage 7 - Increase in fungal biomass (10-14 day)

Faster hyphal growth and hyphal aggregation in the mesophyll cells are evident. Failure of the host to recognize pathogen effectors results in bursting of mesophyll cells. The pathogen feeds on the efflux of nutrients released by host cells. Macro symptoms of clusters of brown-
20 yellow pustules begin to appear in the leaf tissue.

Stage 8 – Increase in fungal biomass (14-21 day)

As more mesophyll cells die, the leaf loses its physical integrity and collapses, necrotic leaf area is frequently observed in susceptible cultivars. The pustules fade the yellow color and show up with dark brown spots in the leaf tissue.

5 Stage 9 - Reinfection of new sites (12-14 day onward)

A new cycle of infection commences. Coalescing chlorotic or necrotic leaf area are otherwise extended larger and larger. With the assistance of wind and water splash dispersal, more teliospores or urediniospores disperse and spread the disease up through the leaf layers of the host.

10

Example 13: Evaluation of the 'Defend' system and traditional screening methods for selecting wheat varieties resistant and susceptible to *Puccinia striiformis f. sp. tritici*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method described in Example 4 relative to a traditional spray method of phenotypic screening. An 15 experiment on several wheat varieties with genotypes with known resistance to was designed to *Puccinia striiformis f. sp. tritici, inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are known to be resistant to *Puccinia striiformis f. sp. tritici*.

Twenty bread wheat cultivars were selected for inclusion in the experiment, including 20 'Lancer' with the gene *Yr6*+, 'McVey' with unknown Yr gene , and 'Tammarin Rock' which is a susceptible cultivar. Briefly, the twenty cultivars were sown in a fixed order of 5 x 4 tubes in a rack, each rack was replicated three times and arranged in a zig-zag format on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 11. The *Pst* isolate used for the experiment was race #198.

25 The experiment indicated no difference between the spray and the infiltration methods on 'Lancer' as a resistant genotype (Defend Class 2) and 'Tammarin Rock' as a susceptible genotype (Defend Class 6) (Figure 5). The *Yr6*+ in Lancer is proposed to at least function at Stage 4 and Stage 5 and/or stages after, but its resistant functions at Stage 2 and Stage 3 could not be excluded. However, the initial experiment differentiated the type of resistance as shown in 'McVey', in 30 which the spray method gave a susceptible phenotype but a resistant phenotype in the infiltration method (Defend Class 3) (Figure 5).

Example 14: Protocol for the preparation of *Septoria lycopersici* inoculum

Preparation for V8-PDA medium

35 V8-PDA medium was prepared as a solid medium with agar on polystyrene petri dishes (90 x 15 mm, Thermo Fisher). 1 litre of V8-PDA medium contained: 150 ml Campbells V8 juice,

10 g PDA, 1.5 g calcium carbonate ($CaCO_3$), 15 g technical agar, 850 ml of distilled water and 25 mg Gentamicin.

Isolation of pathogen and growth of inoculum

The asexual fruiting body of *S. lycopersici* is called pycnidium (pl. pycnidia). These fruiting bodies appear as tiny black spots on the surface of infected leaves (see Figure 3). Infected pieces of leaves were collected, then surface sterilized with 70% ethanol and placed flat on a piece of wet Whatman® Filter Paper in a petri dish for 3 hrs or overnight (16 hrs). Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. The pycnidium produces conidia as a cirrhus or ooze-like structure. Because all conidia belonging to a sporulating pycnidium are genetically identical, they are recognized as an isolate with a designated name. The germinated pycnidium were picked up with a sterilized needle and sub-cultured on a petri dish under aseptic conditions in a Laminar Air Flow cabinet. After 7-30 days, the sub-cultured isolate grew a mycelia mat on the plate and formed pycnidia which produce conidia as above..

Conditions for inoculum storage

The conidia isolate were also sub-cultured for future experiments. The plates were incubated under the white fluorescent lights (800-1000 lumens) at 18°C. After 7-30 days, mycelium covered the plate and pycnidia were observed forming. Spores can be maintained in this form under cool moist conditions with frequent subculturing, ensuring that the plates do not dry out. Plates were also monitored constantly for contaminants. A fresh culture was started once a month to minimise the risk of contamination and loss of pathogenicity.

Conidia spores can also be mixed with 0.5% glycerol, and collected with 1-2 mm plastic beads in the 2 ml tube, and stored at -80 °C for long term storage.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 15: Experimental procedures for *S. lycopersici* inoculation

Preparation of the inoculum suspension

The *S. lycopersici* isolates on the V8-PDA medium were diluted to the desired concentration. The spore concentration used was approximately 3 x 10^6 spores/ml for spray and 3 x 10^5 spores/ml for infiltration. In preparation of inoculum for the infiltration method, plates were flooded with approximately 3 ml of water conidia spores was scraped from the petri dish into 50 ml of clean distilled water and adjusted for a final concentration of approximately 3 x 10^5 and 3 x 10^6.

Plant growth

Sixty pots were organized in a 15 x 4, filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 160 mm (dia) x 172 mm (D) (3 litre cyclone pot). Pre-germination of seeds was conducted, eight tomato seeds were sown in fifteen pots each of 67 (L) x 67 (W) x 72 (D) mm and the pots were then placed in a glasshouse to grow under controlled conditions. Seedlings were transplanted to the larger pots at 14-21 days after sowing. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 25°C (±3°C) to 29°C (±3°C).

S. lycopersici inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 3 x 10^6 spores/ml, supplemented with 0.1% Tween20. The spore suspension was applied as a fine mist to the leaf surface of 3-5 weeks-old plants with fully emerged leaves, using an airbrush. However, similar application of the spore suspension to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated seedlings were placed in a growth chamber at 90-100% relative humidity at 25 °C for 48 hrs in with 12 hr light/dark period prior to being transferred to the glasshouse (25-29 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 3 x 10^5 spores/ml. Tween 20 was not required for this method, approximately 10 µL of inoculum (about 100 spores) was infiltrated on the abaxial side of the second leaf blade using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring.

As mentioned above, leaf infiltration was carried out 4-7 days after the emergence of the 2nd vegetative leaf, this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into the leaves of different genotypes, as it is very common that leaves of some
5 genotypes are much tougher to infiltrate than others.

Plant assessment

Plants were scored 4-42 days after the inoculation, depending on the progress of experiments. *S. lycopersici* (Tomato Septoria) scores (1-5) were then assigned to each plant according to the method described in Table 6. Briefly, scores were assigned based on the visually
10 estimated percentage of necrotic lesions containing pycnidia on the infected leaves, where 0 = no symptoms; 1 = No necrosis, anthocyanin or chlorosis at site, no disease; 2 = Small dark necrotic spots at site, no expansion beyond site of infiltration, infiltration site covered less than 20% by spots; 3 = Increased density of necrosis up to 50% of site; 4 = Density of necrosis increased 60-90%; 5 = Large necrotic areas expanding beyond the site of infiltration containing
15 pycnidia.

Table 6: Disease assessment scale applied infiltration inoculations

Scale	Details for assessment of necrosis
0	No symptoms
1	No necrosis, anthocyanin or chlorosis at site, no disease
2	Small dark necrotic spots at site, no expansion beyond site of infiltration, infiltration site covered less than 20% by spots
3	Increased density of necrosis up to 50% of site
4	Density of necrosis increased 60-90%, pycnidia abundant
5	Large necrotic areas expanding beyond the site of infiltration, pycnidia abundant
Symbols	P= pycnidia present, C= chlorosis, N= necrosis, - = absence of symptom + = extended beyond infiltration site, ++ =extended far beyond infiltration site

Example 16: Different Stages of infection in plants resulting from pathogenic spores

20 The nine stages of interaction between *S. lycopersici* and tomato plants used in the phenotyping method of the disclosure to classify plants according to their defence and resistance phenotypes are the same as Stages 1-9 described in the context of *Z. tritici* and wheat plants in Example 3.

25

Example 17: Evaluation of the 'Defend' system and traditional screening methods for selecting tomato varieties resistant and susceptible to *S. lycopersici*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on fifteen tomato varieties with genotypes with unknown resistance to *S. lycopersici* was designed to, *inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are unknown to be resistant to *S. lycopersici*.

Fifteen tomato cultivars were selected for inclusion in the experiment, listed in Table 7. Briefly, the 15 cultivars were sown in a fixed order of 15 x 4 pots, each variety was replicated four times and arranged in a zig-zag format on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 15. The *S. lycopersici* isolate used for the experiment was WAI5112 which has unknown virulence on the tomato varieties.

Table 7: Tomato varieties (*Solanum lycopersicum*) used to demonstrate the 'Defend' method.

Variety
Tiny Tom
Grosse lisse
Red cherry
Money Maker
Honeybee
Roma VF
Siberian
Rouge de Marmande
Sweet100
UG16112
HMX58811
H1311
H3402
Black Cherry
Black Krim

The experiment indicated no difference between the spray and the infiltration methods on 'Honeybee' as a susceptible genotype, Defend class 6 (Figure 6). However, the initial experiment differentiated the type of resistance as shown in varieties 'Black Cherry' and 'Black Krim', in which the infiltration method gave an intermediate phenotype (Defend class 1 or 5) (Figure 6), suggesting the gene(s) resistant to the isolate WAI5112 had a high chance of providing resistance at least in the stages of post entry into the plant, but not complete resistance (Table 8). The remaining varieties tested displayed symptoms from the infiltration method consistent with Class 6.

Table 8: Average score of phenotypes from two different application of inoculum applied to identify the Defend Class each variety belongs to. Each treatment was replicated 4 times per variety. Mock treatments = where no fungal spores were added to the inoculum suspension. Spore treatment = fungal spores added to the inoculum suspension. Scale 1= resistant 5 = susceptible

	Treatment				
Variety	**Mock_infiltrate**	**Mock_spray**	**Spore_infiltrate**	**Spore_spray**	**Defend Class**
Tiny Tom	0	0	5	na	6
Grosse lisse	0	0	4c++n+p	na	6
Red cherry	0	0	4	na	6
Money Maker	0	0	4c+n+p	na	6
Honeybee	0	0	4c+n+p	5	6
Roma VF	0	0	4.5	na	6
Siberian	0	0	5	na	6
Rouge De Marmande	0	0	4c+n+p	na	6
Sweet100	0	0	3.5	na	6
UG16112	0	0	5	na	6
HMX58811	0	0	4.5	na	6
H1311	0	0	4.5	na	6
H3402	0	0	5	na	6
Black Cherry	0	0	3-c	na	5/1
Black Krim	0	0	3-c	na	5/1

5 #Symbols are p= pycnidia present, c= chlorosis, N= necrosis, - = absence of symptom,+ = extended beyond infiltration site, ++ =extended far beyond infiltration site.

Example 18: Protocol for the preparation of *Thekospora minima* inoculum

Isolation, growth and storage of *T. minima* inoculum

10 The asexual fruiting body of *T. minima* is called a uredinium (pustules) which produce asexual urediniospores. These are thin-walled spores produced by the uredium on the surface of infected leaves (see Figure 7). Critically, the fresh rust spores from a single pustule, which can be recognized a single race with a designated name, are carefully picked up and mixed with 0.05% Tween 20, and then applied on young susceptible cultivars, i.e. 'Pink Icing'. Briefly, the 15 spore suspension was gently painted on the back of young fully emerged leaf surface, using a cotton swab or brush. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 17 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C). After 14 days, new cluster of pustules should be observed on the back of plant leaf. New pustules were collected from the plant. Then repeat the process described above on more plants, in order 20 to obtain sufficient amount of rust spores for future experiments, or to collect sufficient amount

of spores for long-term storage. Rust spores are collected in 2 ml tubes, dried with silica gel at 4 °C for 2-3 days then into -80 °C , and stored at -80 °C for long term usage.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust
5 the spore concentration in suspended cultures. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number
10 of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 19: Experimental procedures for *T. minima* inoculation

Preparation of the inoculum suspension

15 The spore concentration of *T. minima* used for the spray method was approximately 3 x 10^5 spores/ml. Dissolvents used for the spray method can be distilled water with Tween 20 or light mineral oil such as Isopar. In preparation of inoculum for the infiltration method, a final concentration was adjusted to 10^4 spores/ml. To mix the rust spores well in the liquid, 50 µl of Tween 20 was dissolved in 100 ml of water.

20 Plant growth

Twenty pots were organized in a 5 × 4 matrix on a plastic rack, filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 67 mm (L) × 67 mm (W) × 155mm (D). One or two blueberry seeds were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. Since sunlight is
25 important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants produce new leaves every 7-10 days.

30 *T. minima* inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 3 x 10^5 spores/ml, supplemented with 0.05% Tween 20. The spore suspension was gently painted on the back of leaf surface, using a paint brush. However, similar application of the spore suspension to the leaf

surface could be achieved by the spray method using an air brush or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 17 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C).

5 *The 'infiltration method'*

In the infiltration method, final spore concentrations were adjusted to 10^4 spores/ml. 10 µL of inoculum (about 100 spores) was infiltrated on the back of leaf surface using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring.

10 As mentioned above, leaf infiltration is more easily applied on young leaves other than old leaves, this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into the leaves of different genotypes. After the infiltration, a thin mist of water was sprayed on the plants within the first 24 hours.

15 *Plant assessment*

Plants were scored 14-28 days after the inoculation, depending on the progress of experiments. *T. minima* scores (1-5) were then assigned to each plant using 0–4 scale (McIntosh et al., 1995), based on the infection types. Low infection types (LITs= 0–2) were considered resistant, and infection type = 2+ as intermediate while high infection types (HITs= 3–4) were 20 rated susceptible.

Example 20: Different Stages of infection in plants resulting from pathogenic spores

The nine stages of interaction between *T. minima* and blueberry plants used in the phenotyping method of the disclosure to classify plants according to their defence and resistance 25 phenotypes are the same as Stages 1-9 described for *Pst* and wheat plants in Example 12.

Example 21: Evaluation of the 'Defend' system and traditional screening methods for selecting blueberry varieties resistant and susceptible to *T. minima*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method 30 as described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on several blueberry varieties was designed to *T. minima, inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are resistant to *T. minima*.

Five blueberry cultivars were initially selected for inclusion in the experiment, including 35 'Peach Sorbent' which exhibits some symptoms and 'Pink Icing' which is the most susceptible

cultivar. Further, the two cultivars were sown in 20 cm pots. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 19. The *T. minima* isolate used for the experiment was bulk of rust collected from a blueberry commercial nursery at Coffs Harbour.

5 The experiment indicated no difference between the spray and the infiltration methods on 'Pink Icing' as a susceptible genotype classified into Defend class 6 and 'Peach Sorbent' which might be classified as Defend class 3 (Figure 7). The initial experiment differentiated the type of resistance as shown in 'Peach Sorbent', in which the spray method gave a susceptible phenotype but a resistant phenotype in the infiltration method (Defend class 3) (Figure 7).

10

Example 22: Protocol for the preparation of *Leptosphaeria maculans* inoculum

Preparation for V8-PDA medium

V8-PDA medium was prepared as a solid medium with agar on polystyrene petri dishes (90 x 15 mm, Thermo Fisher). 1 litre of V8-PDA medium contained: 150 ml Campbells V8 juice, 15 10 g PDA, 1.5 g calcium carbonate ($CaCO_3$), 15 g technical agar, 850 ml of distilled water and 25 mg Gentamicin.

Isolation of pathogen and growth of inoculum

The asexual fruiting body of *L.maculans* is called pycnidium (pl. pycnidia). These fruiting bodies appear as tiny black spots on the surface of infected leaves (see Figure 3). Infected pieces 20 of leaves were collected, then surface sterilized with 70% ethanol and placed flat on a piece of wet Whatman® Filter Paper in a petri dish for 3 hrs or overnight (16 hrs). Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. The pycnidium produces conidia as a cirrhus or ooze-like structure. Because all conidia belonging to a sporulating pycnidium are genetically identical, they are 25 recognized as an isolate with a designated name. The germinated pycnidium were picked up with a sterilized needle and sub-cultured on a petri dish under aseptic conditions in a Laminar Air Flow cabinet. After 7-30 days, the sub-cultured isolate grew a mycelia mat on the plate and formed pycnidia which produce conidia as above.

Conditions for inoculum storage

30 The conidia isolate were also sub-cultured for future experiments. The plates were incubated under the white fluorescent lights (800-1000 lumens) at 18°C. After 7-30 days, mycelium covered the plate and pycnidia were observed forming. Spores can be maintained in this form under cool moist conditions with frequent subculturing, ensuring that the plates do not dry out. Plates were also monitored constantly for contaminants. A fresh culture was started once 35 a month to minimise the risk of contamination and loss of pathogenicity.

Conidia spores can also be mixed with 0.5% glycerol and collected with 1-2 mm plastic beads in the 2 ml tube and stored at -80 °C for long term storage.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 23: Experimental procedures for *L.maculans* inoculation

Preparation of the inoculum suspension

The *L. maculans* isolates on the V8-PDA medium were diluted to the desired concentration. The spore concentration used was approximately 3.5 x 10^5 spores/ml for wound, spray and infiltration. In preparation of inoculum for the infiltration method, plates were flooded with approximately 3 ml of water conidia spores was scraped from the petri dish into 50 ml of clean distilled water and adjusted for a final concentration of approximately 3.5 x 10^5.

Plant growth

Thirty two pots were organized in a 8 x 4 , filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 160 mm (Diameter) x 172 mm (Depth) (3 litre cyclone pot). Eight canola seeds were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached the full cotyledon expansion stage 10-16 days after sowing, the first true leaves were removed from seedlings every 2 days during the experiment. This ensures the cotyledons do not prematurely senesce.

Blackleg inoculation procedures

The 'wound method'

In the wound method, the final spore concentrations were adjusted to 3.5 x 10^5 spores/ml. Wounding of the cotyledons was performed using a sterile needle, pin or punch to create a hole of approximately 1 mm diameter through the cotyledon at approximately the centre of the left and right sides. The spore suspension was applied as a droplet of inoculum to the wound site on cotyledons of 2-3 weeks-old plants with two fully emerged cotyledons, using an pipette. However, similar application of the spore suspension to the surface of the wound site could be achieved by the wound method using an eye dropper, or a syringe, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated seedlings were placed in a dark chamber at 90-100% relative humidity at 16 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 3.5 x 10^5 spores/ml. The spore suspension was applied as a to cotyledons of 2-3 weeks-old plants with two fully emerged cotyledons, approximately 10 µL of inoculum (about 100 spores) was infiltrated on the abaxial side of the cotyledons using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the cotyledon and marked for scoring.

As mentioned above, cotyledon infiltration was carried out 4-7 days after the cotyledons reached full expansion , this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into the cotyledons of different genotypes, as it is very common that cotyledons of some genotypes are much tougher to infiltrate than others.

Plant assessment

Plants were scored 4-42 days after the inoculation, depending on the progress of experiments. *Leptosphaeria maculans* (blackleg) scores (1-5) were then assigned to each plant according to the method described in Table 9. Briefly, scores were assigned based on the visually estimated percentage of necrotic lesions containing pycnidia on the infected cotyledons, where 0 = no symptoms; 1 = No necrosis, anthocyanin at site, no disease; 2 = Small dark necrotic spots at site, no expansion beyond site of infiltration or wound, infiltration site covered less than 20% by spots; 3 = Increased density of necrosis up to 50% of site; 4 = Density of necrosis increased 60-90%; 5 = Large necrotic areas expanding beyond the site of infiltration or wounding.

Table 9: Disease assessment scale applied to wound and infiltration inoculations

Scale	Details
0	No symptoms
1	No necrosis, anthocyanin at site, no disease
2	Small dark necrotic spots at site, no expansion beyond site of infiltration or wound, infiltration site covered less than 20% by spots
3	Increased density of necrosis up to 50% of site
4	Density of necrosis increased 60-90%, pycnidia abundant
5	Large necrotic areas expanding beyond the site of infiltration or wounding, pycnidia abundant

Wound score (modified 1-5 used here instead of 1-9) Symptoms were assessed at 17 days post-inoculation (dpi) on a scale from 0 (no darkening around wounds) to 9 (large grey-green lesions with prolific sporulation) (Koch *et al.* 1991).

5

Example 24: Different Stages of infection in plants resulting from pathogenic *L. maculans* spores

The nine stages of interaction between *L. maculans* and canola plants used in the phenotyping method of the disclosure to classify plants according to their defence and resistance
10 phenotypes are the same as Stages 1-9 described in the context of *Z. tritici* and wheat plants in Example 3.

Example 25: Evaluation of the 'Defend' system and traditional screening methods for selecting canola varieties resistant and susceptible to *L. maculans*

15 In this example, the inventors evaluated the new 'Defend' phenotypic screening method as described in Example 4 relative to a traditional wounding method of phenotypic screening. An experiment on several canola varieties with genotypes with known resistance to *L. maculans* was designed to, *inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are known to be resistant to *L. maculans*.

20 Eight canola cultivars were selected for inclusion in the experiment, including 'Bristol' with the genes Rlm2 and Rlm9, 'ATR-Beacon' with the genes Rlm3, Rlm4 and Rlm9, and ''Westar' which is a susceptible cultivar (Table 10). Briefly, the eight cultivars were sown in a fixed order of 8 x 4 pots, each variety was replicated four times and arranged in a zig-zag format on the same bench. Experiment conditions using the wound method and the infiltrated method
25 were followed as described above in Example 23. The *L. maculans* isolate used for the experiment was WAI4364 which has at least virulence on Rlm1/LepR3, LepR1 using the wounding method.

Table 10: Canola varieties used to demonstrate the 'Defend' method and resistance genes present where known.

Species	Variety	Known resistance genes	Source
Brassica napus	Westar	Susceptible	Van de Wouw 2018
Brassica napus	AV-Garnet	Rlm1/LepR3	Van de Wouw 2018
Brassica napus	AG-Saphire		
Brassica napus	ATR-Beacon	Rlm3, Rlm4, Rlm9	Van de Wouw 2018
Brassica napus	Pivot		
Brassica napus f. *biennis*	Bristol	Rlm2, Rlm9	Van de Wouw 2018
Brassica napus	Hua You 8		
Brassica napus	Egra		

The experiment indicated no difference between the wound and the infiltration methods on 'Westar', 'AG-Saphire', 'Pivot', 'Bristol', 'Hua You 8' as a susceptible genotypes (Defend class 6 (Figure 8). However, the initial experiment differentiated the type of resistance as shown in varieties 'Egra', 'ATR-Beacon' and 'AV-Garnet', in which the wound method gave a resistant phenotype but a susceptible phenotype in the infiltration method (Defend class 1) (Figure 2), suggesting the gene(s) resistant to the isolate WAI4364 had a high chance of providing resistance at the stages of spore germination and hyphae entry into the plant, but not at later stages after the latent period (Table 11).

Table 11: Average score of phenotypes from two different application of inoculum applied to identify the Defend Class each variety belongs to. Each treatment was replicated 4 times per variety. Mock treatments = where no fungal spores were added to the inoculum suspension. Spore treatment = fungal spores added to the inoculum suspension. Scale 1= resistant 5 = susceptible

	Treatment				
Variety	**Mock_infiltrate**	**Mock_wound**	**Spore_infiltrate**	**Spore_wound**	**Defend Class**
AG-Saphire	0	0	5	3	1
AV-Garnet	0	0	5	2	1
ATR-Beacon	0	0	5	3.25	1
Bristol	0	0	5	4.5	6
Egra	0	0	5	2.5	1
Hua You 8	0	0	5	4.5	6
Pivot	0	0	5	3.5	6
Westar	0	0	5	5	6

Example 26: Protocol for the preparation of *Rhyncosporium commune* inoculum

Preparation for LBA medium

LBA medium was prepared as a solid medium with agar on polystyrene petri dishes (90 x 15 mm, Thermo Fisher). 1 litre of LBA medium contained: 500 g dried Lima bean extract, 15 g technical agar, 1000 ml of distilled water and 25 mg Gentamicin.

Isolation of pathogen and growth of inoculum

Infected pieces of barley leaves were collected, then surface sterilized for 15 sec with 70% ethanol followed by 30 sec in 0.5% Sodium Hypochlorite solution. Leaf pieces were placed flat in petri dishes containing water agar (15 g technical agar, 1000 ml distilled water) and sealed with parafilm. Petri dishes were incubated for 5 days (120 hrs) at 15 °C in the dark. Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. After incubation for 2-10 days scald lesions on the surface sterilised leaf pieces produce conidia that exuded in small clear whitish droplets. The conidiospores were picked up with a sterilized needle and sub-cultured on a petri dish containing LBA medium under aseptic conditions in a Laminar Air Flow cabinet. After 15-30 days culture at 15 °C in the dark, the sub-cultured isolate grew into approximate 5-7 mm cluster of sporulating hyphae. Conidiospores from the sub-cultured isolate were streaked on a fresh petri dish containing LBA medium under aseptic conditions in a Laminar Air Flow cabinet and after 3-7 days culture at 15 °C in the dark individual germinated spores were cut from the agar and transferred to a fresh petri dish containing LBA medium. Because all conidia derived from a single spore are genetically identical, cultures derived from a single germinated spore are recognized as an isolate with a designated name.

Conditions for inoculum storage

The spores from each isolate were also sub-cultured for future experiments. Briefly, sterile water was added to the LBA plate surface using a sterile pipette to keep the LBA plate surface moist. This step was repeated, whenever necessary. The plates were incubated in the dark at 15 °C. After 10-20 days, a white to pinkish spore mass with hyphae was observed in the plate. Spores can be maintained in this form under cool moist conditions with frequent subculturing, ensuring that the plates do not dry out. Plates were also monitored constantly for contaminants. A fresh culture was started every 14-21 days to minimise the risk of contamination and loss of pathogenicity.

Spores can also be mixed with 0.5% glycerol, and collected with 1-2 mm plastic beads in the 2 ml tube, and stored at -80 °C for long term storage.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by
5 rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

10

Example 27: Experimental procedures for *R. commune* inoculation

Preparation of the inoculum suspension

The *R. commune* isolates on the solid LBA medium were flushed with sterile water and the agar surface was gently agitated to facilitate dislodgement of spores. Approximately 5 µl of
15 spores was scraped from the petri dish into 50 ml of clean distilled water and adjusted for a final concentration of approximately 5 x 10^5 spores/ml. Inoculation preparation was the same for the spray and the infiltration method,

Plant growth

Thirty-six barley (*Hordeum vulgare*) genotypes were planted as a single plant per pot, and
20 each genotype was replicated three times. Pots were filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Three lots of 36 pots organized in a 9 x 4 matrix on a plastic rack were filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 67 mm (L) x 67 mm (W) x 155mm (D). Two seeds were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. Since sunlight
25 is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached the two-leaf stage 10-16 days after sowing.

30 *R. commune* inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 5 x 10^5 spores/ml. The spore suspension was applied as a fine mist to the leaf surface of 2-3 weeks-old plants with two fully emerged leaves using an airbrush. However, similar application of the spore suspension

to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated plants were placed in a dark chamber at 90-100% relative humidity at 16 °C for 48 hrs in the dark prior to being transferred to the glasshouse (18-
5 22 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 5 x 10^5 spores/ml. 2-3 weeks-old plants with two fully emerged leaves old plants were used. 10 µL of inoculum (about 100 spores) was infiltrated on a fully expanded leaf blade using a 3 ml Syringe (Terumo,
10 DVR-3416). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring. After the infiltration, a thin mist of water was sprayed on the plants within the first 24 hours.

Plant assessment

Plants were scored 14-28 days after the inoculation, depending on the progress of
15 experiments. Scores were assigned to each plant using a modified version of the Barley Leaf Scald scoring method described in Wallwork and Grcic (2011) *Australian Plant Pathology*, 40:490-496. Briefly, Scores were assigned based on the visually estimated percentage of necrotic lesions on the infected leaves, where 0 = no symptoms (resistant, R) for either infiltration or spray methods; 1 = scattered or small minor lesions (resistant- moderately resistant, R-MR); 2 = minor
20 lesions only, mostly on leaf sheaths or leaf margins (moderately resistant, MR); 3 = few and/or late appearing susceptible leaf lesions (moderately susceptible, MS); 4 = many or large susceptible leaf lesions (moderately susceptible - susceptible, MS-S); 5 = many large lesions or death of seedling leaves (susceptible, S). Other traits taken in consideration during assessment were the percentage of necrosis area (Nec, 0-100%) on the infected leaf or whole seedling.

25

Example 28: Different Stages of infection in plants resulting from pathogenic *R. commune* spores

The nine stages of interaction between *R. commune* and barley plants used in the phenotyping method of the disclosure to classify plants according to their defence and resistance
30 phenotypes are the same as Stages 1-9 described in the context of *Z. tritici* and wheat plants in Example 3, with the exception that primary infection at stage 1 can be caused by ascospores or conidiospores (as opposed to ascospores or pyconidia spores).

Example 29: Evaluation of 'Defend' system and traditional screening methods for selecting barley cultivars resistant and susceptible to *R. commune*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method as described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on fifteen tomato varieties with genotypes with unknown resistance to *R. commune* was designed to, *inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are unknown to be resistant to *R. commune*.

Thirty six barley cultivars were selected for inclusion in the experiment, listed in Table 12. Briefly, the 36 cultivars were sown in a fixed order of 9 x 4 pots, each variety was replicated three times on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 27. The *R. commune* isolate used for the experiment was WAI1310 which has unknown virulence on the barley varieties.

Table 12: Barley varieties (*Hordeum vulgare*) used to demonstrate the 'Defend' method.

Entry	Variety	Entry	Variety
1	AB245	19	Tilga
2	AB249	20	Sloop
3	ICARDA4	21	W13284
4	ICARDA9	22	Orge
5	Jet	23	Zavilla
6	Brier	24	WABAR2147
7	Sultan	25	WA9621
8	Ciho8618	26	Ethiopia
9	C14364	27	WA9718
10	AB240	28	Quina
11	Chieftain	29	WA8834
12	Hudson	30	Turk
13	Skiff	31	Atlas
14	Tantangara	32	Atlas46
15	AB6	33	Osiris
16	Franklin	34	Ciho3515
17	Keel	35	Litmus
18	VB9104	36	Steptoe

The experiment indicated no difference between the spray and the infiltration methods on 'Brier' and 'Atlas46' as susceptible genotypes, Defend Class 6 (Figure 9A and 9B respectively). However, the initial experiment differentiated the type of resistance for varieties 'Franklin' and 'Steptoe' (as shown in Figure 9C for 'Franklin'), in which the infiltration method gave a resistant phenotype and the spray method gave a susceptible phenotype (Defend Class 3), suggesting the

gene(s) resistant to the isolate WAI1310 had a high chance of providing resistance at least in the stages of post entry into the plant (Table 13). The experiment also indicated little difference between the spray and infiltration methods on 'ICARDA4' as a resistant genotype (Defend Class 2) (Figure 9D). The remaining varieties tested displayed symptoms from the infiltration and spray methods consistent with Class 6, Class 2 or Classes 4 and 5.

Table 13: Average score of phenotypes from two different application of inoculum applied to identify the Defend Class each variety belongs to. Each treatment was replicated 3 times per variety. Mock treatments = where no fungal spores were added to the inoculum suspension. Spore treatment = fungal spores added to the inoculum suspension. Scale 0 = resistant 5 = susceptible

	Treatment				
Variety	**Mock_infiltrate**	**Mock_spray**	**Spore_infiltrate**	**Spore_spray**	**Defend Class**
AB245	0	0	0	NA	NA
AB249	0	0	0	NA	NA
ICARDA4	0	0	2	0	2
ICARDA9	0	0	NA	4	NA
Jet	0	0	3	0	4/5
Brier	0	0	4	5	6
Sultan	0	0	NA	2	NA
Ciho8618	0	0	NA	0	NA
C14364	0	0	0	1	2
AB240	0	0	1	0	2
Chieftain	0	0	0	2	2
Hudson	0	0	2	4	4/5
Skiff	0	0	0	1	2
Tantangara	0	0	NA	0	NA
AB6	0	0	0	1	2
Franklin	0	0	1	5	3
Keel	0	0	2	1	2
VB9104	0	0	2	2	4/5
Tilga	0	0	3	2	4/5
Sloop	0	0	5	3	
W13284	0	0	0	3	2
Orge	0	0	2	4	4/5
Zavilla	0	0	NA	0	NA
WABAR2147	0	0	0	2	2
WA9621	0	0	1	0	2
Ethiopia	0	0	1	0	2
WA9718	0	0	NA	1	NA
Quina	0	0	NA	0	NA
WA8834	0	0	1	1	2
Turk	0	0	1	0	2
Atlas	0	0	2	5	4/5

	Treatment				
Variety	**Mock_infiltrate**	**Mock_spray**	**Spore_infiltrate**	**Spore_spray**	**Defend Class**
Atlas46	0	0	5	5	6
Osiris	0	0	1	0	2
Ciho3515	0	0	3	0	4/5
Litmus	0	0	NA	NA	NA
Steptoe	0	0	1	4	3

Example 30: Protocol for the preparation of *Pyrenophora tritici-repentis* inoculum

Preparation for V8-PDA medium

V8-PDA medium was prepared as a solid medium with agar on polystyrene petri dishes (90 x 15 mm, Thermo Fisher), containing 150 ml/l Campbells V8 juice, 410 g/l PDA, 41.5 g/l sucrose calcium carbonate (CaCO3), 15 g/l technical agar, 850 ml/l of distilled water and 25 mg/l Gentamicin.

Isolation of pathogen and growth of inoculum

The asexual fruiting body of *P. tritici-repentis* is called conidium (pl. conidia). Infected pieces of leaves were collected, then surface sterilized with 70% ethanol and placed flat on a piece of wet Whatman® Filter Paper in a petri dish for 3 hrs or overnight (16 hrs). Infected leaves from different host plants were placed in different petri dishes and marked with identifiers to allow the tracking of individual plants. The conidiophore is erect, simple (not branched) and may have one to nine transverse septa, with a swollen and olive-black base. On V8-PDA agar, the conidia spore grows and forms a white to light gray, fluffy mycelium which does not produce conidia, which can be recognized as an isolate with a designated name. The dense mycelium produces conidiophores and conidia after exposure to near-ultraviolet light for 12 to 24 hours followed by 12 to 24 hours of darkness.

Conditions for inoculum storage

0.5 cm-diameter-mycelial plugs can be carved from the growth plate, freeze-dried, and stored in the 2 ml tube at -80 °C for long term storage. Otherwise, a mycelial plug is sub-cultured for the experiment. Briefly, a single plug was placed in the middle of V8-PDA plate and incubated under the white fluorescent lights (800-1000 lumens) at 22-24 °C. After 4-7 days, a light gray, fluffy mycelium was observed in the plate. 8-10 agar plugs were carved from the plate and placed upside down on a new V8-PDA plate. The mycelium produces conidiophores and conidia after continuous exposure to near-ultraviolet light for 3-4 days. 1 ml of sterile water with Tween 20 (0.02%) was added on the plate to harvest the conidia spores using a soft brush. The process can be repeated to increase the yield of conidia spores.

Calculation of the spore concentration using the haemocytometer

To minimise variation in cell numbers, a haemocytometer was used to count and adjust the spore concentration. When the cover-glass was applied to the glass slide, the chamber was approximately 0.1 millimetres deep. Briefly, the cover-glass was pushed on the slide firmly by rubbing along the cover-glass supports using moist fingers. The spore culture was diluted 5x-10x and added to the haemocytometer using a Pasteur pipette and checked to see if the spores were evenly distributed. The 'L' squares were used for counting the number of cells. If the count was less than 200 cells in a selected square, then all four squares were counted and the mean cell count determined.

Example 31: Experimental procedures for *P. tritici-repentis* inoculation

Preparation of the inoculum suspension

The *P. tritici-repentis* inoculum in the sterile water was diluted to the desired concentration in addition with Tween 20 (0.02%), approximately 1 x 10^4 spores/ml for both the spray method and the infiltration method. In particularly for the infiltration method, only sterile water was added to adjust the working concentration of inoculum.

Plant growth

Twenty pots were organized in a 5 x 4 matrix on a plastic rack, filled with Osmocote Premium Plus Superior Potting Mix (Scotts, AUS). Each pot had the following dimensions: 67 mm (L) x 67 mm (W) x 155mm (D). One or two wheat seeds were sown in each pot and the pots were then placed in a glasshouse to grow under controlled conditions. Since sunlight is important for the development of symptoms of infection, care was taken to ensure that all pots were exposed to sufficient sunlight and appropriate temperatures. The glasshouse temperature was controlled using an Evaporative Cooler/Gas Heater Air conditioning system (Bonaire, AUS), with temperatures ranging from 18°C (±3°C) to 22°C (±3°C). Plants reached the two-leaf stage 10-16 days after sowing.

P. tritici-repentis inoculation procedures

The 'spray method'

In the spray method, the final spore concentrations were adjusted to 1 x 10^4 spores/ml, supplemented with 0.02% Tween 20. The spore suspension was applied as a fine mist to the leaf surface of 2-3 weeks-old plants with two fully emerged leaves, using an airbrush. However, similar application of the spore suspension to the leaf surface could be achieved by the spray method using a pressurised spray can or spray bottle, or by applying the spore suspension to the leaf surface as a thin film using a paint brush, cotton swab or by immersion. Inoculated seedlings

were placed in a dark chamber at 90-100% relative humidity at 16 °C for 24-48 hrs in the dark prior to being transferred to the glasshouse (18-22 °C).

The 'infiltration method'

In the infiltration method, final spore concentrations were adjusted to 1 x 10^4 spores/ml.
5 Tween 20 was not required for this method. When the plants were grown an additional 4-7 days after seeing the shoot of the 2nd leaf (not fully expanded, normally lighter green colour), 10 µL of inoculum (about 100 spores) was infiltrated on the second leaf blade using a 3 ml Syringe (Terumo, DVR-3416). The infiltration range was easily seen by the progression of water within the leaf blade, and marked for scoring.

10 As mentioned above, leaf infiltration was carried out 4-7 days after the emergence of the 2nd leaf, this allowed the spore suspension to more easily enter via the stomata and spread within the intercellular space minimizing variations of the amount of inoculum that were infiltrated into the leaves of different genotypes, as it is very common that leaves of some genotypes are much tougher to infiltrate than others. After the infiltration, a thin mist of water was sprayed on the
15 plants within the first 24 hours.

Plant assessment

Plants were scored 10-14 days after the inoculation, depending on the progress of experiments. *P. tritici-repentis* (*Ptr*) scores (1-5) were then assigned to each plant according to the method described in Lamari and Bernier (1989) *Can. J. Plant Pathol.* 11:49–56: 1 (resistant)
20 = small dark brown to black spots without any surrounding chlorosis or tan necrosis; 2 (moderately resistant) = small dark brown to black spots with very little chlorosis or tan necrosis; 3 (moderately resistant or moderately susceptible) = small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing; 4 (moderately susceptible) = small dark brown to black spots completely surrounded with chlorotic
25 or tan necrotic zones; some of the lesions coalescing; 5 (susceptible) = dark brown or black centres may or may not be distinguishable; most lesions consist of coalescing chlorotic or tan necrotic zones.

Example 32: Different Stages of infection in plants resulting from *P. tritici-repentis* spores

30 This example describes the nine stages of interaction between *P. tritici-repentis* and wheat plants which have been used in the phenotyping method of the disclosure to classify plants according to their defence and resistance phenotypes.

Stage 1 –Attachment of spores to plant surface (*e.g.*, leaves) (0-6 h)

Primary infection can be caused by ascospores or conidia spores, which attach themselves to the surface of a plant (*e.g.*, a plant leaf). Ascospores are, in general, responsible for primary infection at the start of a growing season. Some plants possess a physical barrier, comprised of a thin waxy cuticle and epidermis, which restricts attachment of spores to the host plant leaves.

5 Stage 2 – Germination of spores (0-6 h)

Once the spores have successfully attached to the surface of a plant (*e.g.*, a leaf surface), the spores degrade the epidermis in anticipation of germination. Water acts as an important factor for the successful pathogen infection and surfactants (*e.g.*, Tween 20) are frequently added to inoculum buffer to assist in this process. The plant initiates the first stage of host defence i.e.,
10 effector triggered immunity (ETI). The first screening method of the disclosure (i.e., the surface contact method) starts at this point.

Stage 3 - Hyphae entry into the plant cell (6-24 h)

Fungus hyphae grow and form germ tube, which produces an appressorium and a penetration peg that enters epidermal cells directly or through stomata and forms a vesicle.
15 During this stage, Plant pathogenesis-related (PR) genes are typically expressed at this stage of the infection cycle.

Stage 4 - Apoplastic colonization (1-2 day)

Fungal growth develops from the vesicle and proceeds intercellularly in the mesophyll layer. Symptoms are typically not evident at this stage. Expressions of PR genes increases at this
20 stage. As the inoculum directly infiltrates into the leaf surface, the infection process using the second screening method (i.e., the infiltration method) starts at this point.

Stage 5 - Apoplastic colonization (3-5 day)

Hyphae grows and proliferates via plasmodesmata . No macro symptoms or weak sign of infection may be observed.

25 Stage 6 - Host cell death (3-5 day)

Damage of cellular organelles beyond the advancing hyphae implies the involvement of a toxin in the infection process. Small dark brown to black spots without any surrounding chlorosis or tan necrosis may be evident on the leaf surface. A hypersensitive response (HR)-like reaction starts at this point, which is characterized by rapid cell death localised to the area of
30 infection in order to restrict the growth and spread of hyphae to other leaf areas. Resistance genes, such as NBS-LRR and cysteine kinase, may function here as receptors and interact with the effectors from the pathogen. The plant can induce another stage of defence, 'ETI', if the appropriate resistant genes are present.

Stage 7 - Increase in fungal biomass (5-10 day)

Faster hyphal growth and hyphal aggregation in the mesophyll cells are evident. Failure of the host to recognize pathogen effectors results in bursting of mesophyll cells. The pathogen feeds on the efflux of nutrients released by host cells. Macro symptoms of chlorosis and necrosis begin to appear in the leaf tissue. Small dark brown to black spots with surrounding chlorosis or tan necrosis is evident on the leaf surface.

Stage 8 – Increase in fungal biomass (10-14 day)

As more mesophyll cells die, the leaf loses its physical integrity and collapses, dark brown lesions with coalescing chlorotic or necrotic leaf area is frequently observed in susceptible cultivars.

Stage 9 - Reinfection of new sites (14 day onward)

A new cycle of infection commences. Coalescing chlorotic or necrotic leaf area are otherwise extended larger and larger. With the assistance of water splash dispersal, more conidia spores disperse and spread the disease up through the leaf layers of the host.

Example 33: Evaluation of 'Defend' system and traditional screening methods for selecting wheat cultivars resistant and susceptible to *P. tritici-repentis*

In this example, the inventors evaluated the new 'Defend' phenotypic screening method as described in Example 4 relative to a traditional spray method of phenotypic screening. An experiment on several wheat varieties with genotypes with known resistance to *P. tritici-repentis* was designed to, *inter alia*, determine whether the 'Defend' method could differentiate plants harbouring genes that are known to be resistant to *P. tritici-repentis*.

Twenty bread wheat cultivars were selected for inclusion in the experiment, including 'Oasis' with the gene *Tsn1*, and 'Spear' which is a susceptible cultivar. Briefly, the twenty cultivars were sown in a fixed order of 5 × 4 tubes in a rack, each rack was replicated four times and arranged in a zig-zag format on the same bench. Experiment conditions using the spray method and the infiltrated method were followed as described above in Example 31. The *P. tritici-repentis* race 1 isolate used for the experiment was YLS WAI240.

The experiment indicated no difference between the spray and the infiltration methods on 'Oasis' as a resistant genotype (Defend Class 2) and 'Spear' as a susceptible genotype Defend Class 6 (Figure 10). However, the initial experiment differentiated the type of resistance as shown in 'ISIS', in which the spray method gave a resistant phenotype but a susceptible phenotype in the infiltration method (Defend Class 1) (Figure 10), suggesting the gene(s) resistant to race 1 had a high chance of providing resistance at the stages of spore germination and appressorium entry into mesophyll cells, but not at later stages (Table 3). By comparison, the Tsn1 in 'Oasis'

is suggested to function at Stage 6 from the published literature, interacting with the effector ToxA.

Editorial Note

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Description pages are missing pages 79 to 104

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CLAIMS:

1. A method of identifying a plant which exhibits a resistance phenotype to a fungal or oomycete pathogen, the method comprising:

(a) performing a first screening method comprising:

(i) contacting the surface of a first plant or part thereof, or first group of plants or parts thereof, with an inoculum of the fungal or oomycete pathogen;

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the fungal or oomycete pathogen to infect the plant(s) or plant part(s); and

(iii) determining one or more responses of the plant(s) or plant part(s) to the fungal pathogen at a plurality of time points during step (ii);

(b) performing a second screening method comprising:

(i) infiltrating a second plant or part thereof, or second group of plants of parts thereof, with an inoculum of the fungal or oomycete pathogen, wherein the second plant(s) or plant part(s) are of the same species and/or cultivar as the first plant(s);

(ii) growing the plant(s) or plant part(s) for a time and under conditions suitable for the fungal or oomycete pathogen to infect the plant(s) or plant part(s) and

(iii) determining one or more responses of the plant(s) or plant part(s) to the fungal or oomycete pathogen at a plurality of time points during step (ii); and

(c) determining whether or not the plant species or cultivar exhibits a resistance phenotype to the fungal or oomycete pathogen based on the one or more responses of the plants(s) or plant part(s) to the fungal or oomycete pathogen in the first and second screening methods.

2. The method of claim 1, comprising determining whether or not the plant species or cultivar exhibits a resistance phenotype to the fungal or oomycete pathogen prior to and/or during entry of the fungal or oomycete pathogen into the plant or part thereof, and/or after entry of the fungal or oomycete pathogen into the plant or part thereof, based on the one or more responses of the plants(s) or plant part(s) to the fungal or oomycete pathogen in the first and second methods.

3. The method of claim 1 or 2, wherein the first screening method comprises contacting the surface of the first plant(s) or plant part(s) with the inoculum by spraying, swiping and/or swabbing the inoculum onto the surface of the plant(s) or plant part(s), and/or dipping or soaking the plant(s) or plant part(s) in the inoculum.

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4. The method of any one of claims 1 to 3, wherein the second screening method comprises infiltrating the tissue of the second plant(s) or plant part(s) with the inoculum by syringe infiltration, syringe pressure infiltration and/or vacuum infiltration.

5. The method of any one of claims 1 to 4, wherein the one or more responses of the plant(s) or plant part(s) to the pathogen comprise one or more phenotypic responses to the pathogen.

6. The method according to claim 5, wherein the method comprises classifying the plant(s) or plant part(s) into response phenotypes comprising:

(i) a Class 1 response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the second screening method;

(ii) a Class 2 response phenotype characterised by an absence of chlorosis, necrosis and pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method and the second screening method;

(iii) a Class 3 response phenotype characterised by chlorosis, necrosis and/or formation of pathogen fruiting structures on the plant(s) or plant part(s) following performance of the first screening method, and an absence of chlorosis, necrosis and pathogen fruiting bodies form on the plant(s) or plant part(s) following performance of the second screening method;

(iv) a Class 4 response phenotype characterised by colonization of the plant(s) or plant part(s) by the pathogen, but infection is halted with minimal chlorosis, following performance of the first screening method and/or the second screening method;

(v) a Class 5 response phenotype characterised by chlorosis, necrosis and formation of pathogenic fruiting structures with evidence of host resistance, wherein host resistance is characterised by a reduction in number of lesion and/or lesion area compared to a plant or plant part in which there is no evidence of host resistance; or

(vi) a Class 6 response phenotype characterised by chlorosis, necrosis and formation of pathogen fruiting structures with no evidence of host resistance.

7. The method according to claim 6, wherein:

a plant or plant part which exhibits a Class 1 response phenotype elicits a defence response to the pathogen prior to and/or during entry of the pathogen into the plant tissue, but not post entry;

a plant or plant part which exhibits a Class 2 response phenotype elicits a defence response to the pathogen prior to, during and post entry of the pathogen into the plant tissue;

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a plant or plant part which exhibits a Class 3 response phenotype elicits a defence response to the pathogen post entry of the pathogen into the plant tissue, but not prior to and/or during entry;

a plant or plant part which exhibits a Class 4 response phenotype does not elicit a defence response to the pathogen prior to and/or during entry of the pathogen into the plant tissue, but does elicit a defence response to the pathogen post entry of the pathogen into the plant tissue and colonisation of the plant by the pathogen still occurs;

a plant or plant part which exhibits a Class 5 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, but shows evidence of resistance to the pathogen represented by a reduced number of lesion and/or reduced lesion area compared to a plant or plant part in which there is no evidence of host resistance (Class 6); and

a plant or plant part which exhibits a Class 6 response phenotype does not elicit a defence response to the pathogen prior to, during or post entry of the pathogen into the plant tissue, and shows no evidence of resistance to the pathogen.

8. The method of any one of claims 1 to 7, comprising selecting one or more plants or plant parts classified as being resistant to the fungal or oomycete pathogen based on a defence response to the pathogen at one or more time points in the infection cycle.

9. The method of claim 8, comprising:

selecting one or more plants or plant parts classified as being resistant to the fungal or oomycete pathogen based on a defence response to the pathogen prior to and/or during entry of the fungal or oomycete pathogen to the plant tissue;

selecting one or more plants or plant parts classified as being resistant to the fungal or oomycete pathogen based on a defence response to the pathogen post entry of the fungal or oomycete pathogen to the plant tissue; or

selecting one or more plants or plant parts classified as being resistant to the fungal or oomycete pathogen based on a defence response to the pathogen prior to, during and post entry of the fungal or oomycete pathogen to the plant tissue.

10. The method of any one of claims 1 to 9, wherein the one or more responses of the plant(s) or plant part(s) to the pathogen comprises a change of expression in one or more immune response genes.

11. The method of any one of claims 1 to 10, wherein the pathogen is a fungal pathogen.

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12. The method of claim 11, wherein the fungal pathogen is selected from the group consisting of *Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp., *Blumeria graminis*, *Mycosphaerella graminicola* (*Zymoseptoria tritici*), *Colletotrichum* spp., *Melampsora lini*, *Phakopsora pachyrhizi, Zymoseptoria tritici, Phyrenophora tritici-repentis, Leptosphaeria maculans, Phakopsora pachyrhizi* (Asian Soybean Rust)*, Phakopsora meibomiae* (New world Soybean Rust)*, Puccinia striiformis f. sp. tritici, Puccinia recondite, Magnaporthe grisea, Cercospora zeae-maydis, Cercospora Zeina, Septoria lycopersici, Rhynchosporium commune, Phyrenophora teres-maculata, Alternaria solani, Alternaria alternada, Septoria apiicola, Septoria glycines, and Thekospora minima.*

13. The method of any one of claims 1 to 10, wherein the pathogen is an oomycete pathogen.

14. The method of claim 13, wherein the oomycete pathogen is selected from the group consisting of *Phytophthora infestans, Hyaloperonospora arabidopsidis, Phytophthora ramorum, Phytophthora sojae, Phytophthora capsici, Plasmopara viticola, Phytophthora parasitica,* and *Albugo candida*.

15. The method of any one of claims 1 to 14, wherein:

the plant is a gymnosperm, a monocot or a dicot;

the plant is selected from the group consisting of a fruiting plant, a leguminous plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant, a medicinal plant and a noxious plant or weed; and/or

the plant is a cereal plant.

16. The method of claim 15, wherein the plant is a cereal plant selected from the group consisting of wheat, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa and buckwheat.

17. The method of claim 16, wherein the cereal plant is wheat.

18. The method of any one of claims 1 to 17, wherein identifying a plant which exhibits a resistance phenotype to a fungal or oomycete pathogen comprises identifying one or more molecular markers associated with resistance to the fungal or oomycete pathogen and/or identifying a plant which exhibits a susceptibility phenotype to a fungal or oomycete pathogen comprises identifying one or more molecular markers associated with susceptibility to the

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fungal or oomycete pathogen, wherein identifying the one or more molecular markers comprises:

(i) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a resistance phenotype to the fungal or oomycete pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the fungal or oomycete pathogen into the plant or part thereof,

(ii) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a susceptibility phenotype to the fungal or oomycete pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the fungal or oomycete pathogen into the plant or part thereof,

(iii) comparing the sequence data at (i) and (ii) and identifying one or more molecular markers which are associated with:

a. a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof,
b. a resistance phenotype after entry of the fungal or oomycete pathogen into the plant or part thereof,
c. a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof, and/or
d. a susceptibility phenotype after entry of the fungal or oomycete pathogen into the plant or part thereof.

19. A method of identifying one or more molecular markers associated with resistance of a plant to a fungal or oomycete pathogen and/or identifying one or more molecular markers associated with susceptibility of a plant to a fungal or oomycete pathogen, said method comprising:

(a) performing the method of any one or claims 1 to 17 to identify one or more plants which exhibit a resistance phenotype to a fungal or oomycete pathogen and one or more plants which exhibit a susceptibility phenotype to the fungal or oomycete pathogen;

(b) identifying one or more molecular markers associated with resistance to the fungal or oomycete pathogen and/or identifying one or more molecular markers associated with susceptibility to the fungal or oomycete pathogen, comprising:

(i) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a resistance phenotype to the fungal or oomycete pathogen prior

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to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the fungal or oomycete pathogen into the plant or part thereof,

(ii) obtaining polynucleotide sequence data for a plant of the species or cultivar which exhibits a susceptibility phenotype to the fungal or oomycete pathogen prior to and/or during entry of the pathogen into the plant or part thereof, and/or after entry of the fungal or oomycete pathogen into the plant or part thereof,

(iii) comparing the sequence data at (i) and (ii) and identifying one or more molecular markers which are associated with:

1) a resistance phenotype prior to and/or during entry of the pathogen into the plant or part thereof,
2) a resistance phenotype after entry of the fungal or oomycete pathogen into the plant or part thereof,
3) a susceptibility phenotype prior to and/or during entry of the pathogen into the plant or part thereof, and/or
4) a susceptibility phenotype after entry of the fungal or oomycete pathogen into the plant or part thereof.

20. The method of claim 18 or 19, wherein the one or more molecular markers may be selected from the group consisting of a single nucleotide polymorphism (SNP) marker, an amplified fragment length polymorphism (AFLP) marker, a DNA amplification fingerprinting (DAF) marker, a random amplified polymorphic DNA (RAPD) marker, microsatellite markers (SSRs), insertion mutation markers, sequence-characterized amplified region (SCAR) markers, cleaved amplified polymorphic sequence (CAPS) markers, kompetitive allele specific PCR (KASP) markers and any combinations thereof.

21. A method of breeding a plant which is resistant to a fungal or oomycete pathogen, comprising crossing or selfing a plant identified as being resistant to the fungal or oomycete pathogen using the method of any one of claims 1 to 17.

22. The method of claim 21, wherein the plant to be crossed or selfed has been identified as being resistant to the fungal or oomycete pathogen based on the presence of one or more molecular markers associated with resistance to the fungal or oomycete pathogen and/or the absence of one or more molecular markers associated with susceptibility to the fungal or oomycete pathogen, wherein the one or more molecular markers are identified using the method of claim 19 or 20.

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23. A method of breeding a plant which is resistant to a fungal or oomycete pathogen, comprising:

obtaining a plant identified as being resistant to the fungal or oomycete pathogen using the method of any one of claims 1 to 17; and

introducing one or more foreign nucleic acids to the plant and/or modifying or editing one or more polynucleotides within the plant's genome.

24. The method of any one of claims 21 to 23 comprising performing the method of any one of claims 1 to 17.

25. The method of any one of claims 21 to 24, wherein:

the plant is a gymnosperm, a monocot or a dicot;

the plant is selected from the group consisting of a fruiting plant, a leguminous plant, an oil plant, a vegetable plant, a cereal plant, a fibre plant, an ornamental plant, a forestry plant, an aquatic plant, a medicinal plant and a noxious plant or weed; and/or

the plant is a cereal plant.

26. The method of claim 25, wherein the plant is a cereal plant selected from the group consisting of wheat, barley, oats, rye, triticale, rice, millet, sorghum, corn (maize), quinoa and buckwheat.

27. The method of claim 26, wherein the cereal plant is wheat.

Figure 1

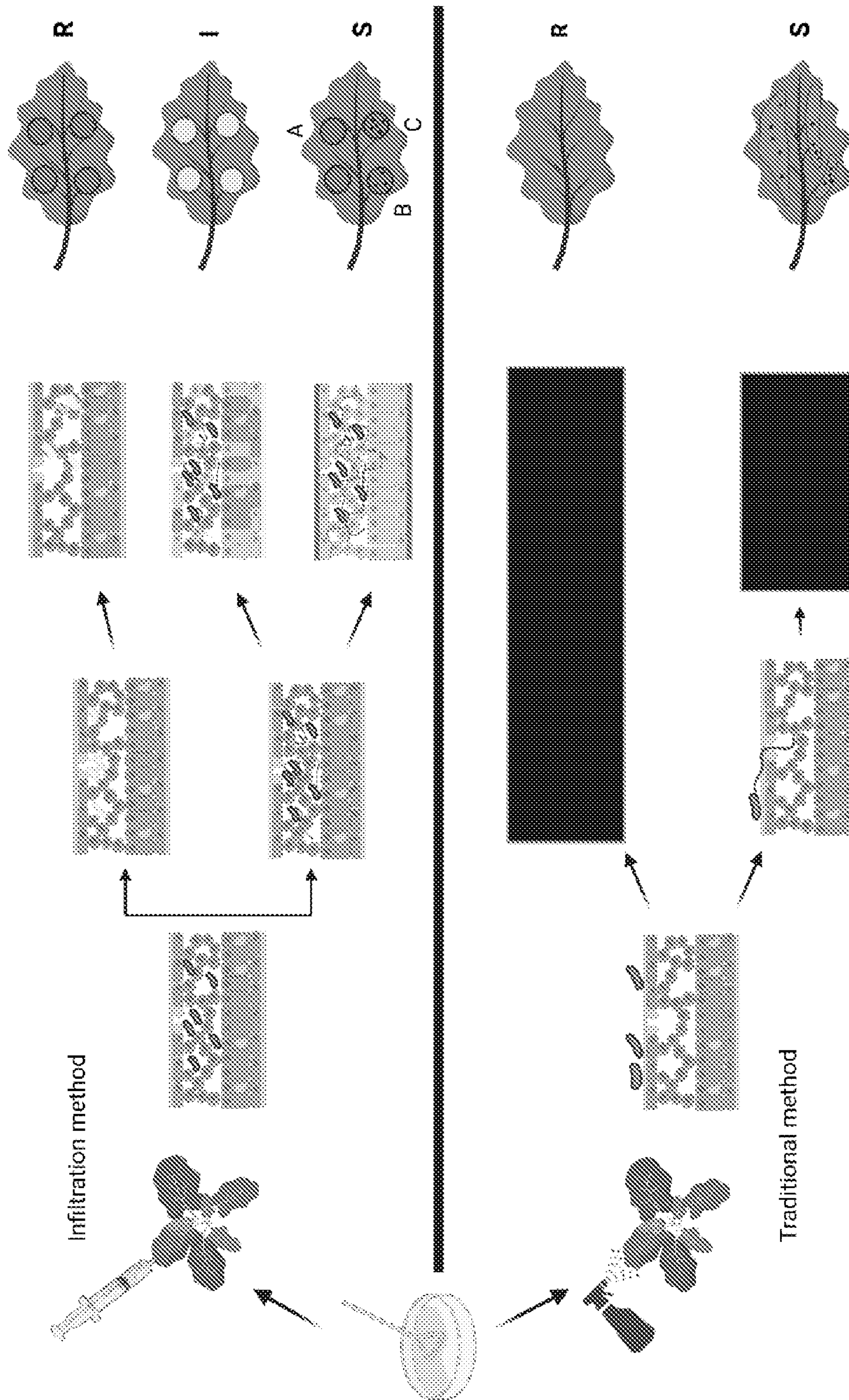

Figure 2

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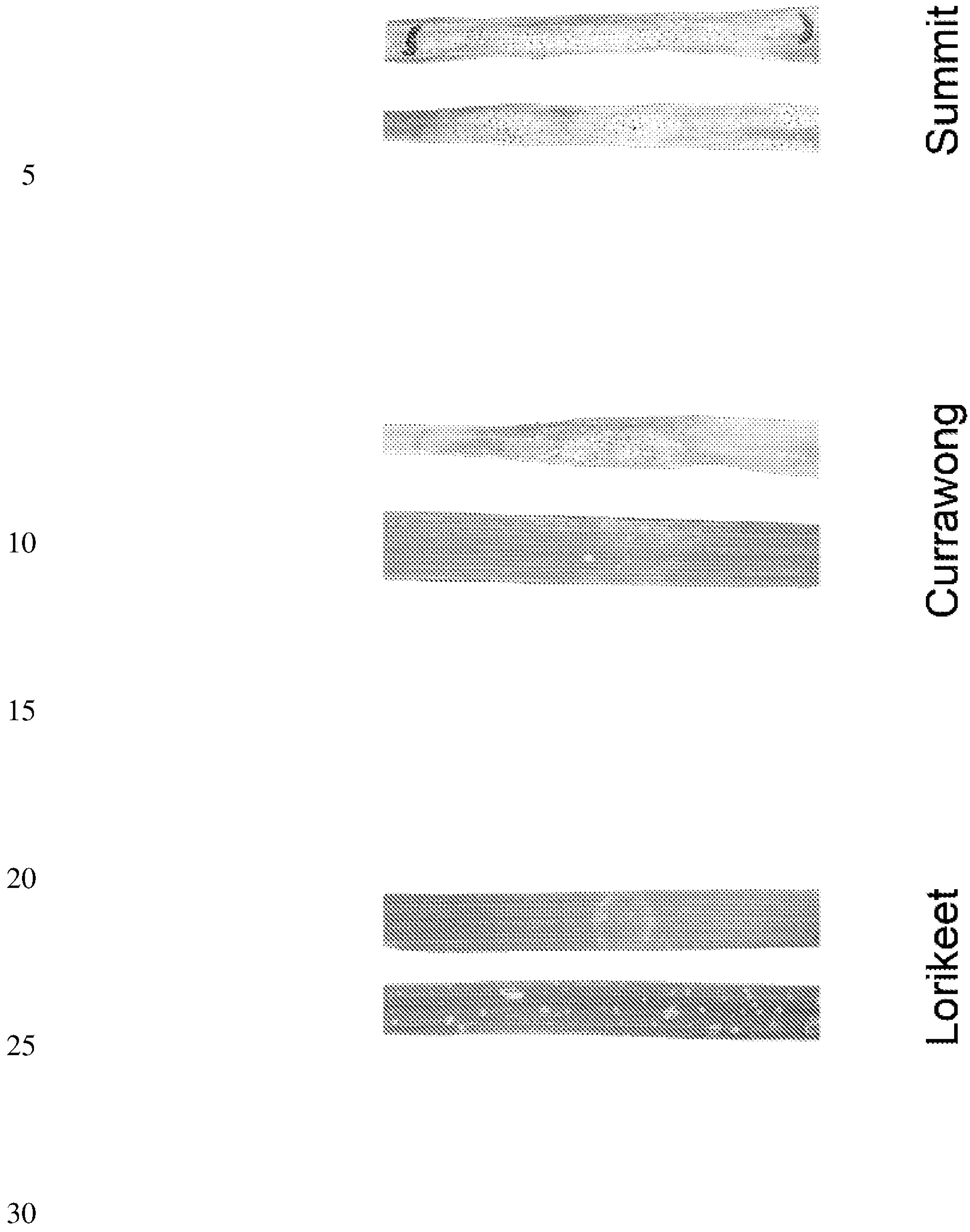

Figure 3

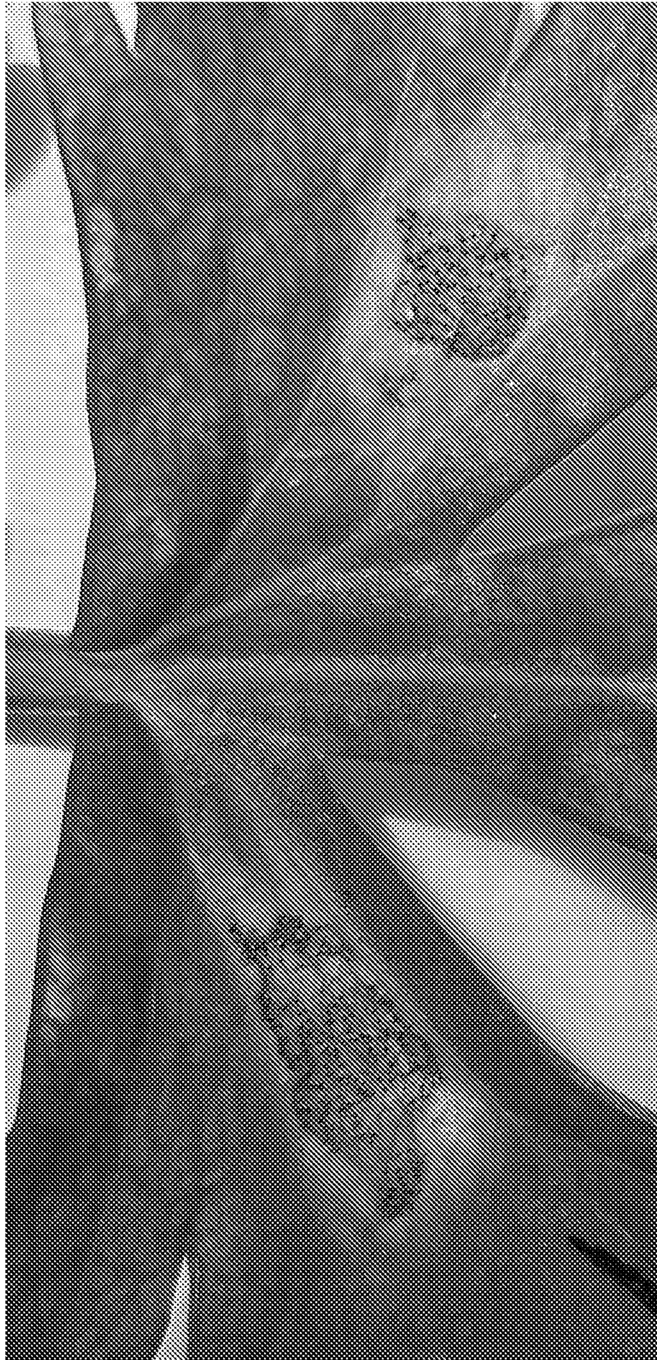

Figure 4

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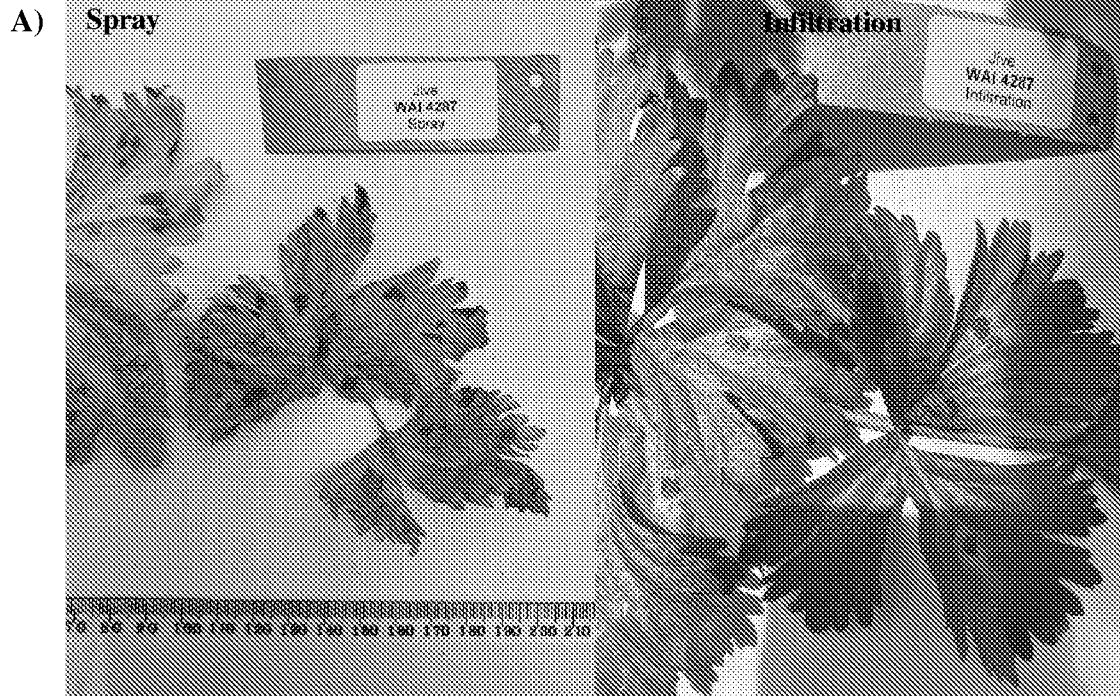
A)



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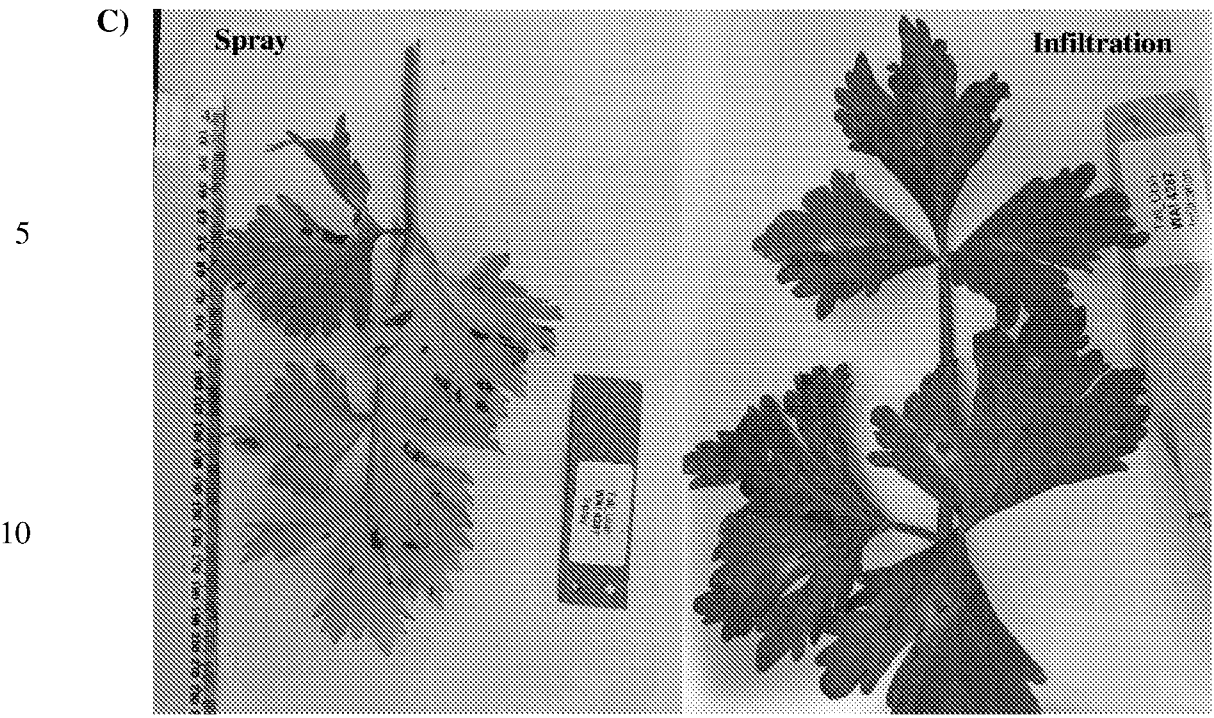
B)

Figure 4 (continued)



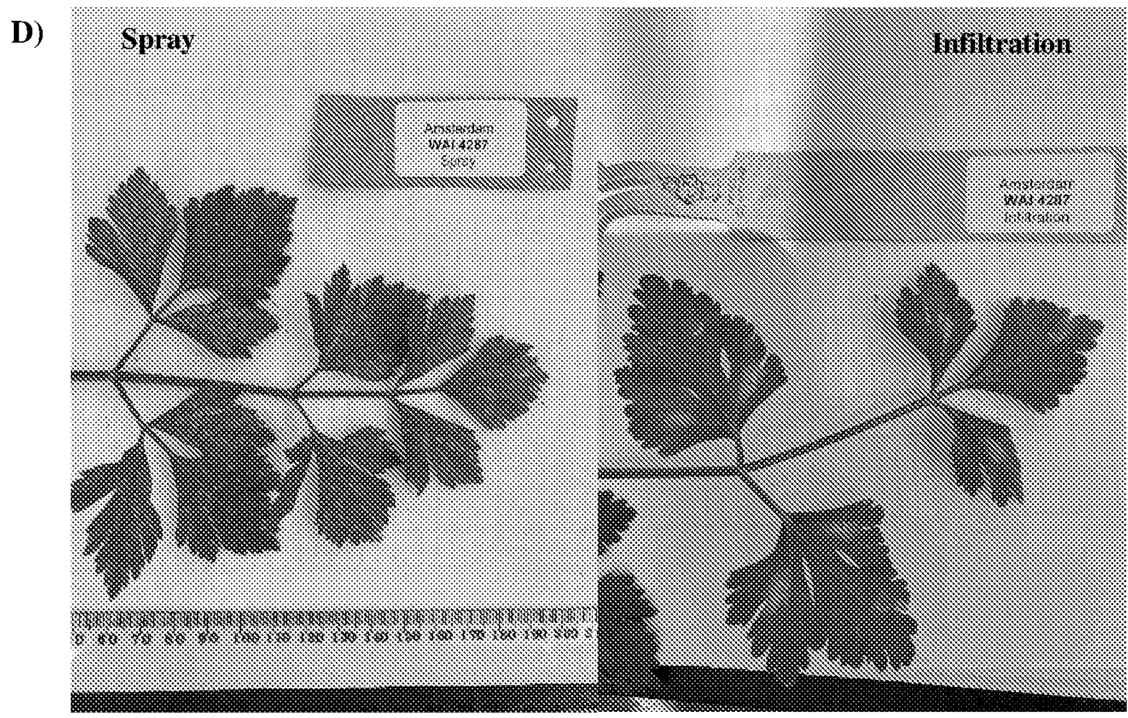
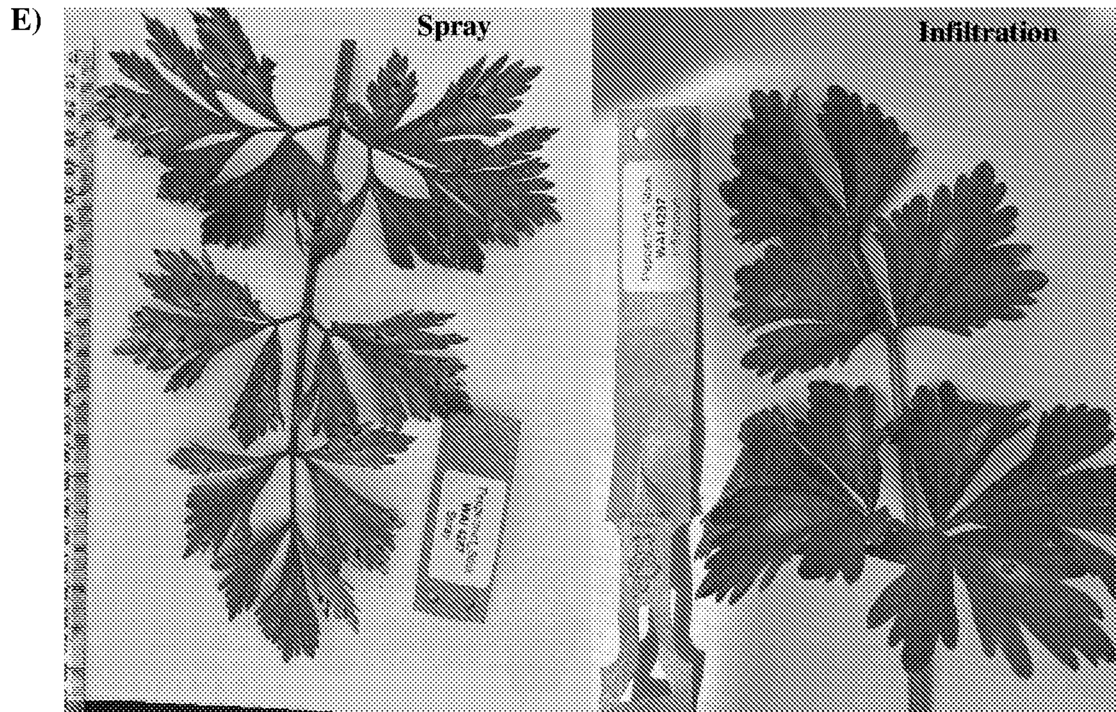


Figure 4 (continued)

E)



F)

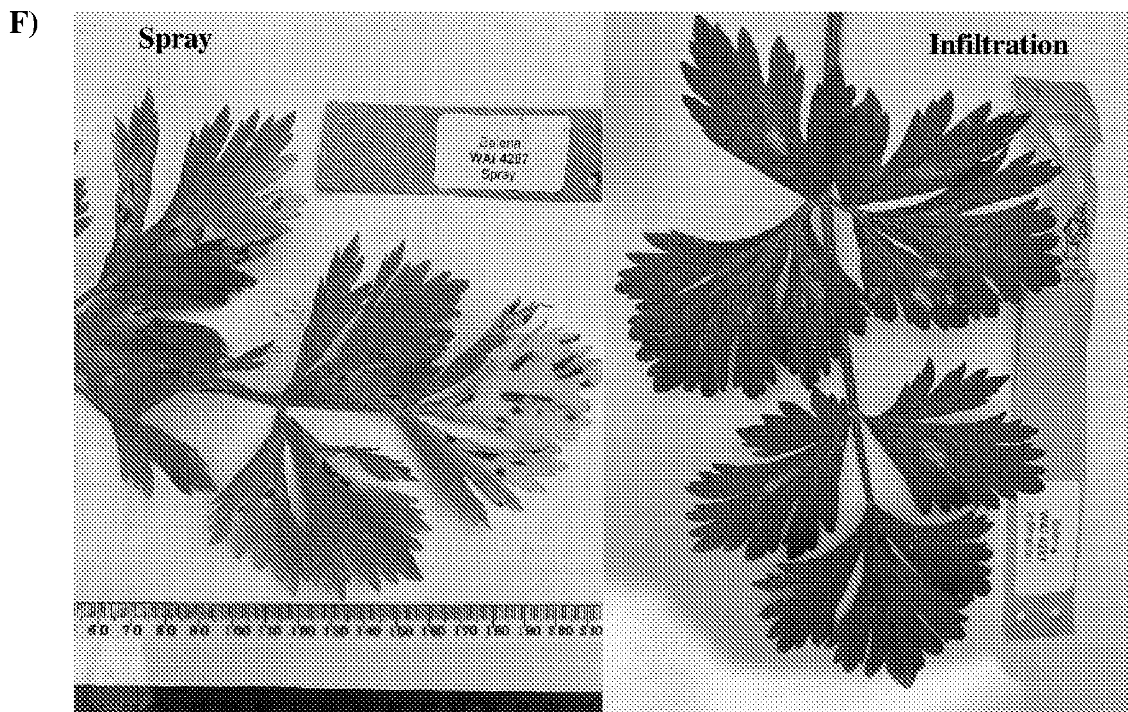
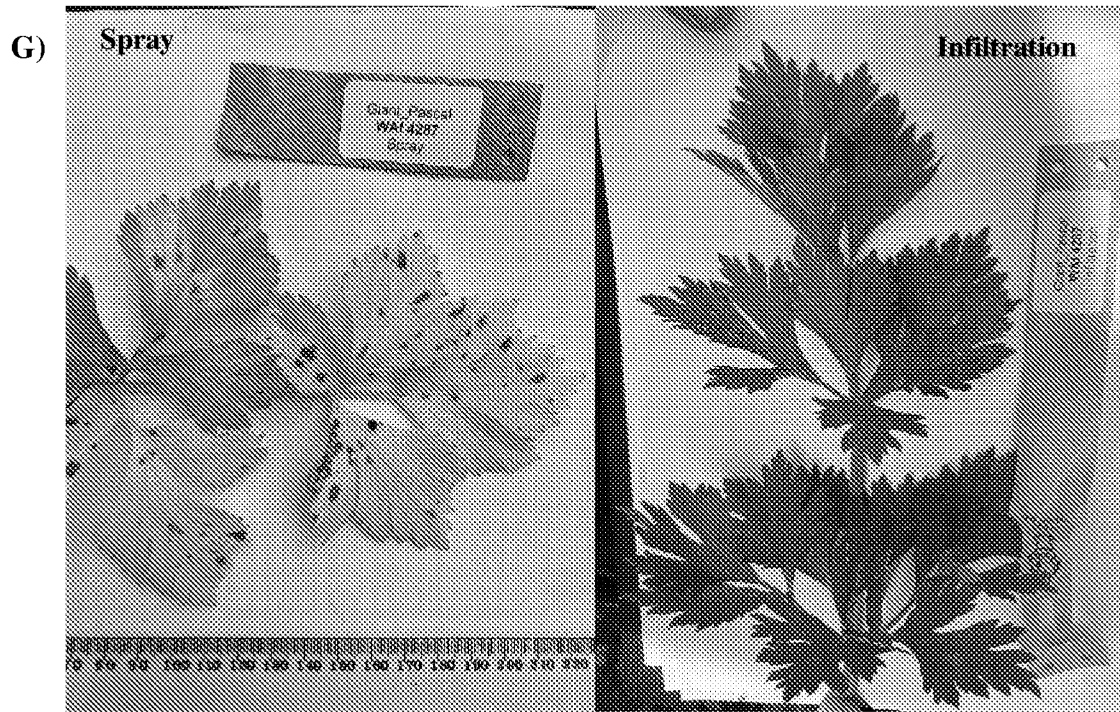


Figure 4 (continued)

G)



H)

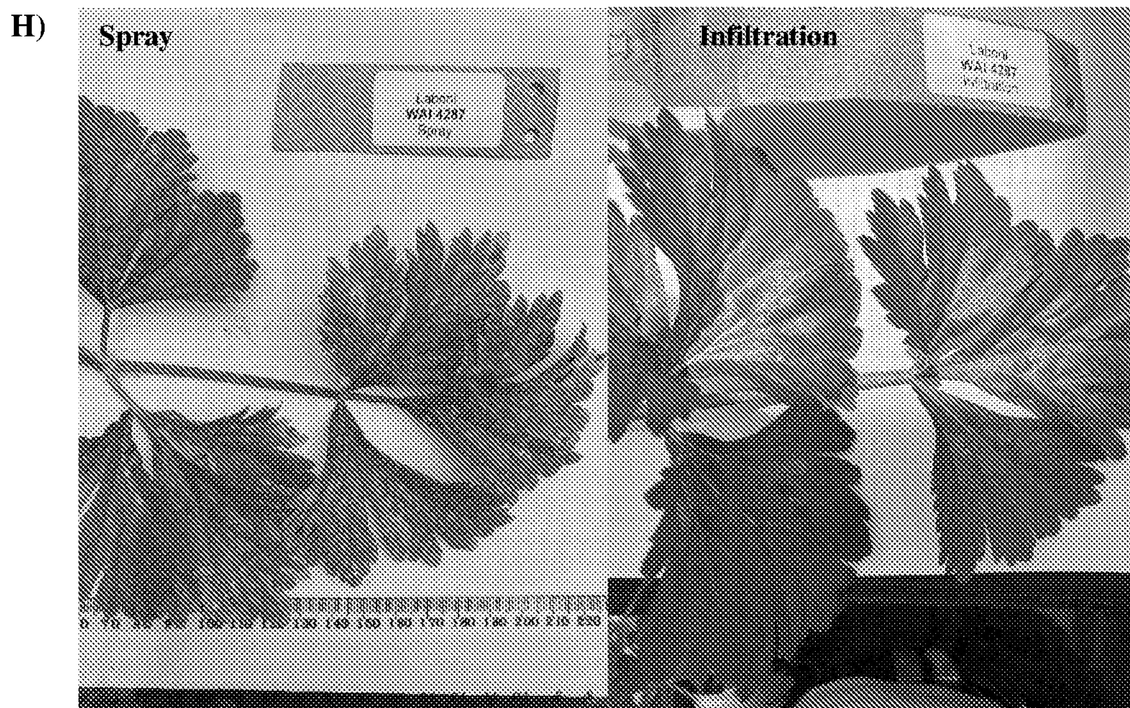


Figure 5

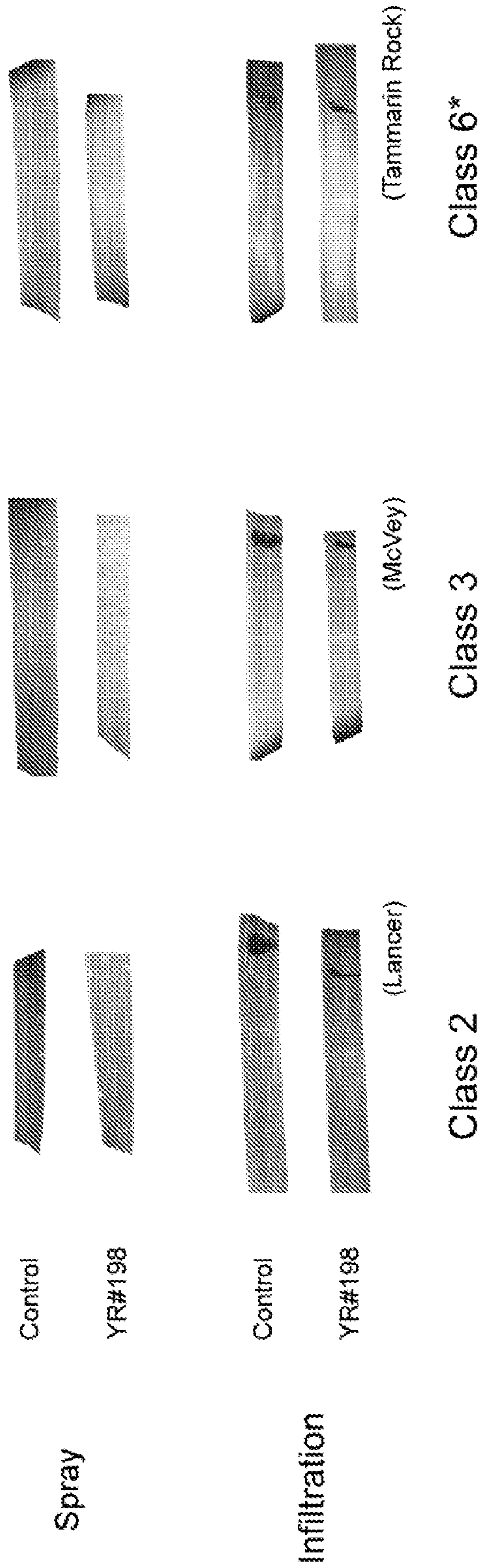


Figure 6

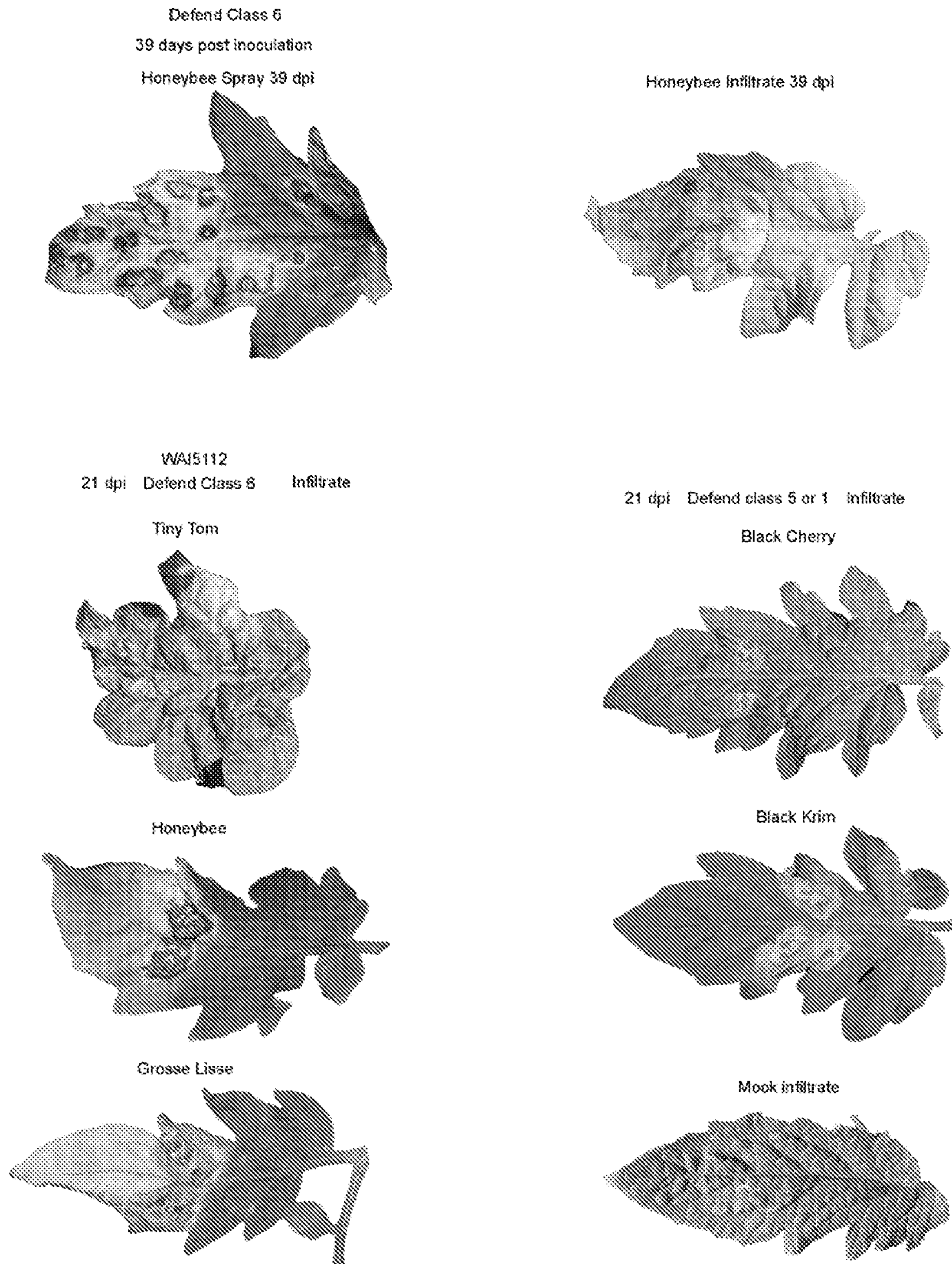


Figure 7

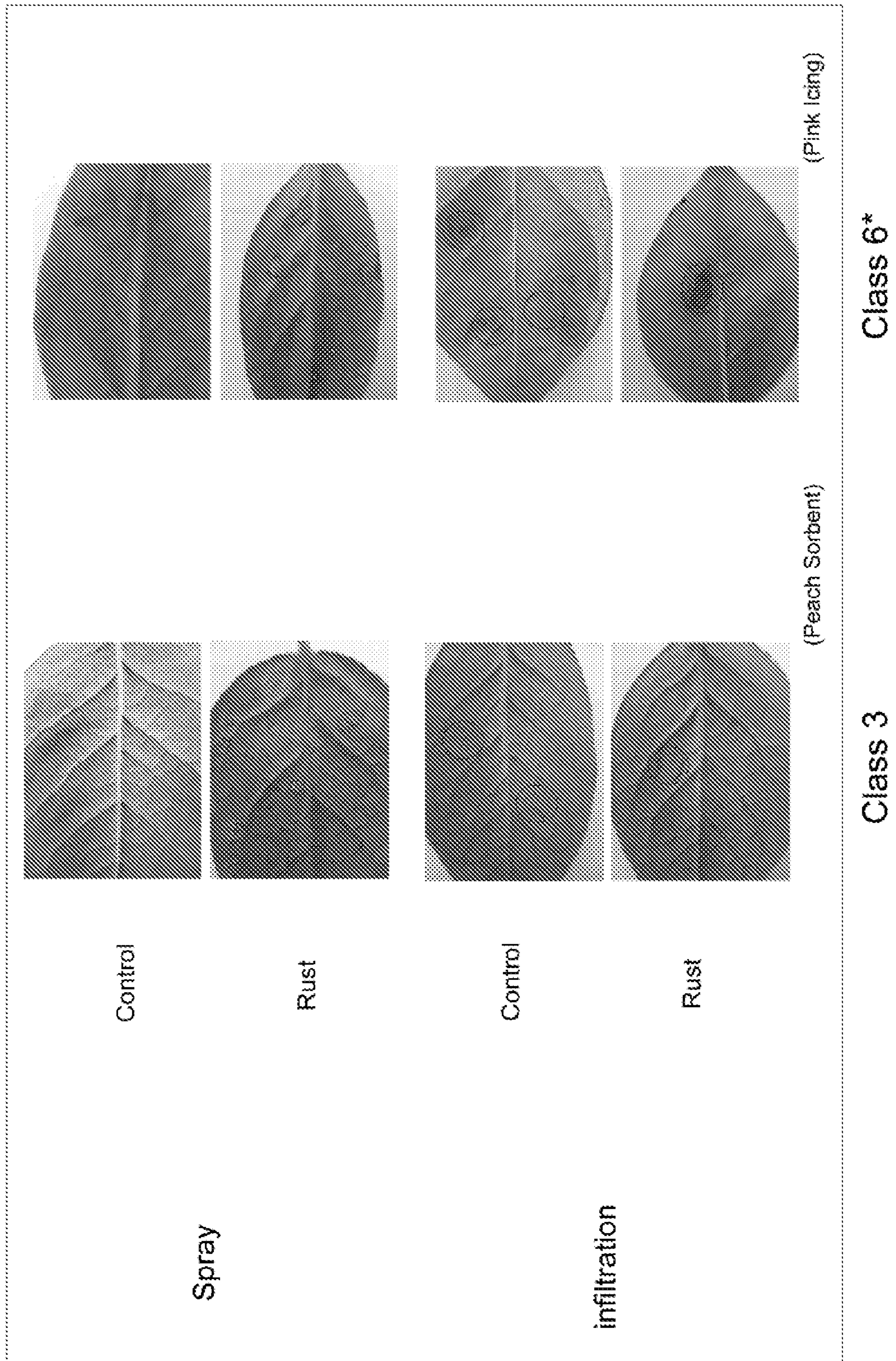


Figure 8

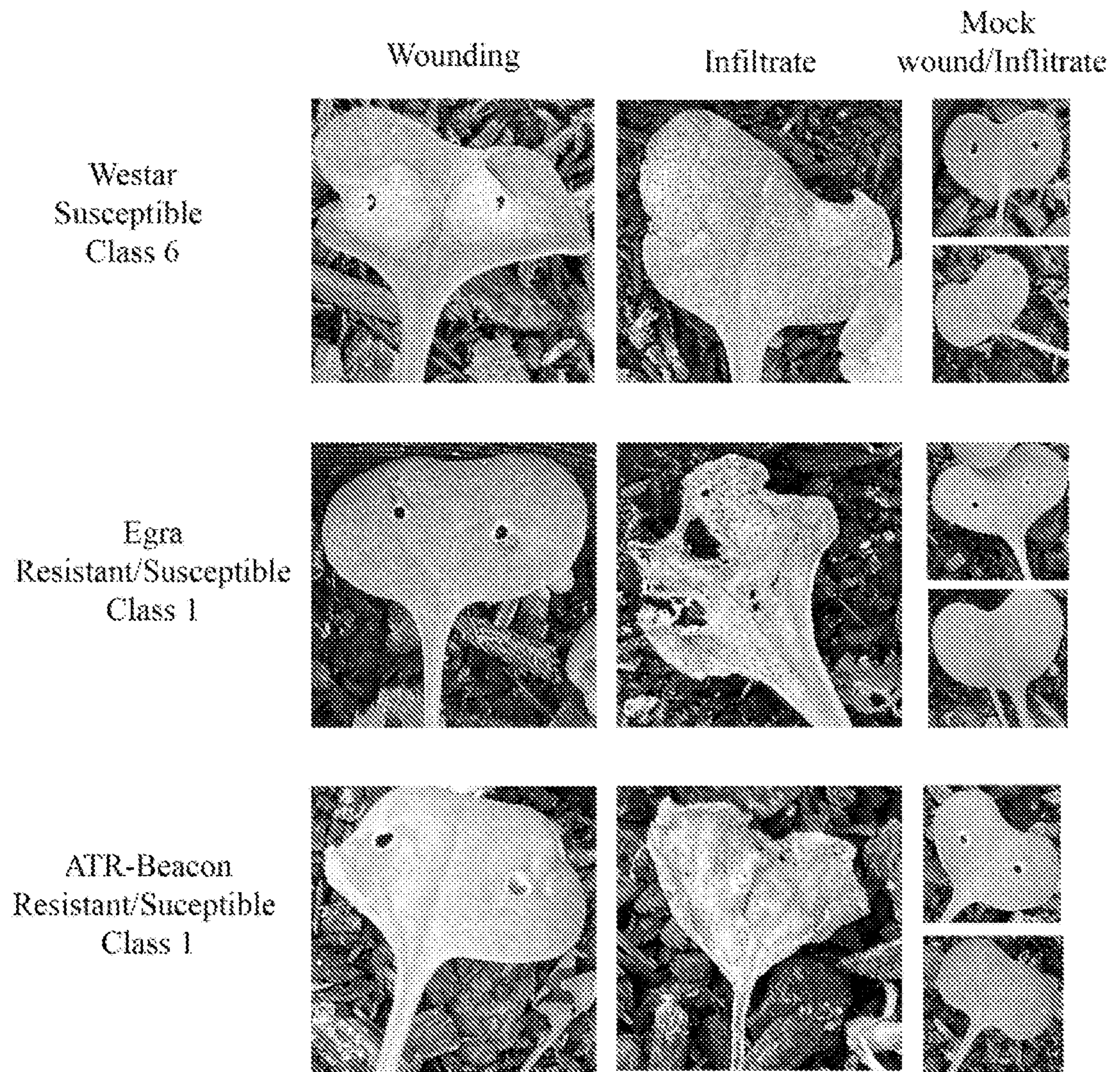


Figure 9

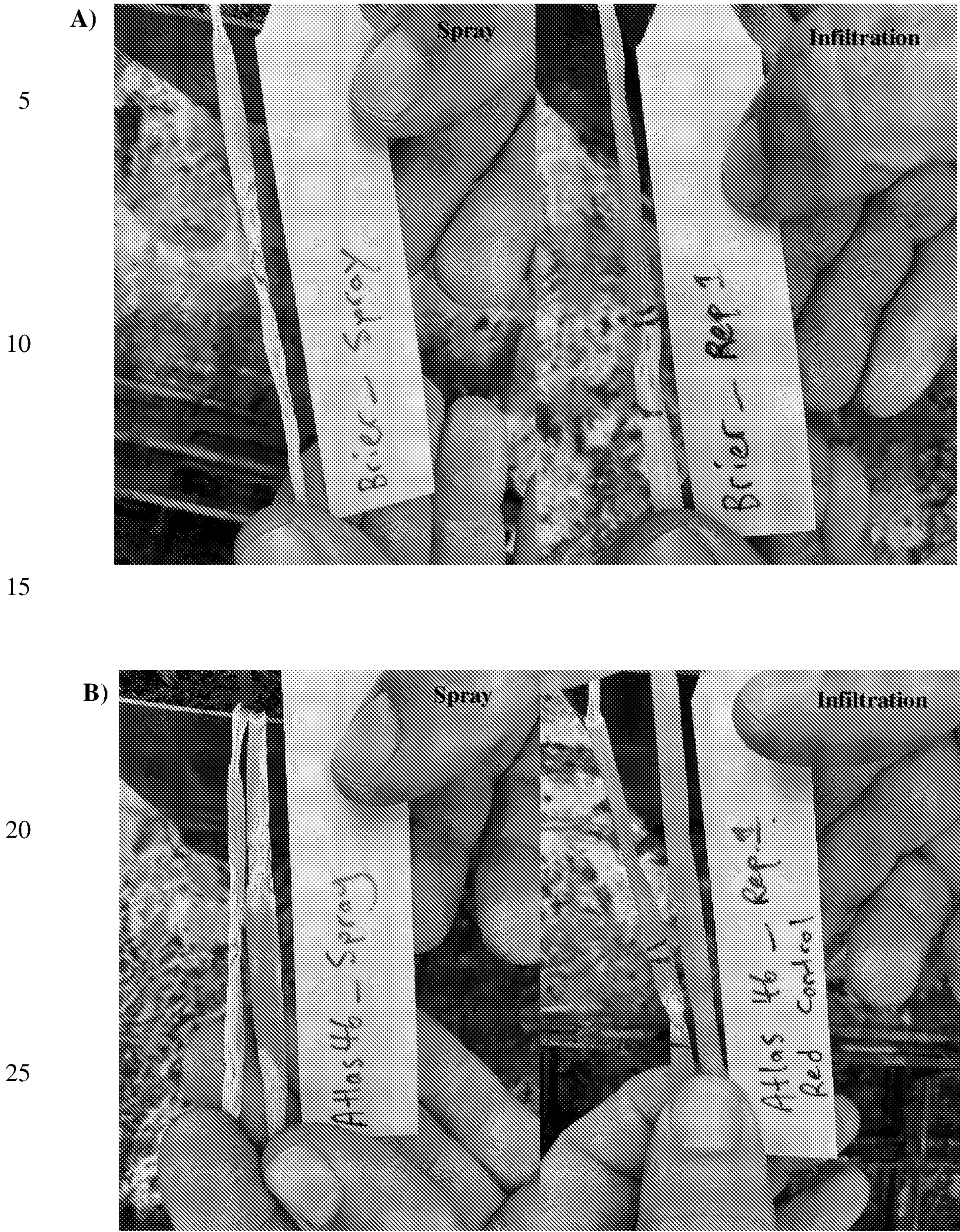


Figure 9 (Continued)

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C)

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D)

Figure 10