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(71) Applicant: **FORTIPHYTE, INC.** [US/US]; 2151 Berkeley Way, Berkeley, California 94704 (US).

(72) Inventors: **SCHULTINK, Alexander Christiaan**; c/o Fortiphyte, Inc., 2151 Berkeley Way, Berkeley, California 94704 (US). **WETZEL, Laura A.**; c/o Fortiphyte, Inc., 2151 Berkeley Way, Berkeley, California 94704 (US).

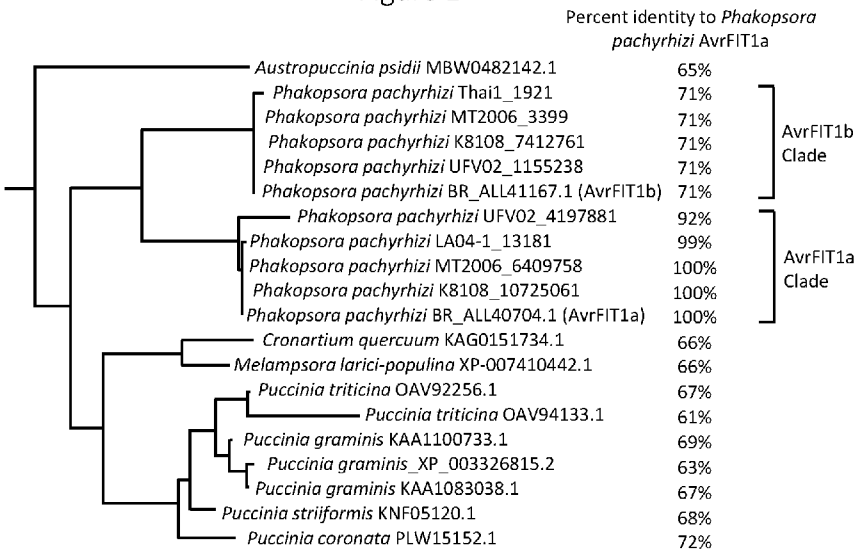
(74) Agent: **ROAHRIG, Bethany** et al.; Cooley LLP, 1299 Pennsylvania Avenue, NW, Suite 700, Washington, District of Columbia 20004 (US).

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(54) Title: PATHOGEN RESISTANCE IN PLANTS

Figure 1



(57) Abstract: The present disclosure provides an isolated, recombinant, or synthetic polynucleotide comprising a FIT1 protein, and homologs, fragments, and variations thereof. disclosure further relates to transgenic plants, plant parts, and plant cells comprising one or more of these polynucleotides, and exhibit resistance or tolerance to a pathogen, such as *Phakopsora pachyrhizi*. disclosure further relates to methods of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising targeted gene editing of a FIT1 homolog, and plants produced therefrom. The disclosure further relates to methods for identifying new functional FIT1 genes and/or alleles thereof.

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IN THE UNITED STATES PATENT & TRADEMARK RECEIVING OFFICE

INTERNATIONAL PCT PATENT APPLICATION

PATHOGEN RESISTANCE IN PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/108,023 filed on October 30, 2020, which is hereby incorporated by reference in its entirety for all purposes.

STATEMENT REGARDING FEDERALLLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under 1844088 by the National Science Foundation. The Government has certain rights to this invention.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: FOTI_001_01WO_SeqList_ST25.txt, date recorded: October 18, 2021, file size 238 kilobytes).

TECHNICAL FIELD

The disclosure relates to the identification and use of nucleic acid sequences for pathogen resistance in plants.

BACKGROUND

Pathogen effector proteins often convergently evolve to target the same or similar plant host proteins to promote virulence. Such proteins may be present in a diverse range of plant pathogens including but not limited to fungi, oomycetes, bacteria, nematodes or viruses.

An example of one such pathogen that causes harm to plants by secreting an effector protein is the obligate biotrophic fungus *Phakopsora pachyrhizi* (and to a lesser extent, the closely related fungus *Phakopsora meibomiae*), which causes Asian soybean rust (ASR). While soybeans make up the primary commercial crop affected by ASR, *Phakopsora* infects

leaf tissue from a broad range of leguminous plants, including at least 17 genera (Slaminko et al., 2008). In general, rust fungi (order Pucciniales) constitute one of the most economically important groups of plant pathogens because of their larger range of host and genetic diversity. There are more than 6000 species of rust fungi that cause harm to many plant species, such as wheat (*Puccinia* spp.), common bean (*Uromyces appendiculatus*), soybean (*Phakopsora pachyrhizi*), and coffee (*Hemileia vastatrix*) (Aime et al., 2006).

Infection in commercial crops requires application of various fungicides, which are costly and not always effective. In Brazil alone, control of ASR costs $2 billion annually. Thus, there is a need for plant varieties that are resistant to fungal pathogens.

The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification.

SUMMARY

The present disclosure provides for an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a functional FIT1 protein homologous to SEQ ID NO: 2. In some embodiments, the polynucleotide encodes a protein having at least 70% identity to SEQ ID NO: 2. In some aspects, the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto. The disclosure further relates to genetic constructs comprising one or more of these sequences, and transgenic plants, plant parts, or plant cells comprising one or more of these sequences, wherein the plant, plant part, or plant cell is resistant or tolerant to a pathogen.

In another embodiment, the disclosure teaches a method of producing a plant, plant part, or plant cell having resistance or tolerance to a pathogen, wherein the method comprises transforming a plant, plant part, or plant cell with a polynucleotide encoding a functional FIT1 protein. In some aspects, the nucleotide sequence encoding the FIT1 protein has been codon optimized. In some aspects, the FIT1 protein comprises a selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof,

or is encoded by an isolated, recombinant, or synthetic polynucleotide selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, complements thereof, fragments thereof, and sequences at least 70% identical thereto.

In another embodiment, the disclosure teaches a method of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising providing a plant species that is susceptible to a pathogen, identifying within the genome of the plant species a homolog of FIT1, wherein said homolog does not mediate AvrFIT1 recognition; and genetically modifying a plant, plant part, or plant cell from the susceptible plant species with targeted gene editing, wherein said targeted gene editing is directed at the FIT1 homolog, and wherein said targeted gene editing enables the FIT1 homolog to recognize AvrFIT1 and confers resistance or tolerance to a pathogen.

The disclosure further relates to genetically modified plants, plant parts, or plant cells produced by the methods disclosed herein, wherein the plant, plant part, or plant cell exhibits resistance or tolerance to a pathogen. In some aspects, the pathogen is a fungus from the order Cantharellales or Pucciniales. In some aspects, the fungal pathogen is *Rhizoctonia solani*, *Melampsora spp.*, *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora spp.*, *Puccinia* spp., *Uromyces spp.*, *Austropuccinia spp.*, *Cronartium spp.* or *Hemileia vastatrix*. In some aspects, the plant, plant part, or plant cell is in the subfamily Papilionoideae. In some aspects, the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

The disclosure further relates to methods for identifying a functional FIT1 gene and/or allele thereof comprising isolating a FIT1 homolog or allele thereof; expressing said FIT1 homolog or allele thereof in combination with an effector protein produced by *Phakopsora pachyrhizi* in a plant, plant part, or plant cell; and assaying said plant, plant part, or plant cell for an immune response. In some aspects, the effector protein comprises SEQ ID NO: 24, SEQ ID: 26, or sequences at least 90% identical thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying figures, which are incorporated herein and form a part of the specification, illustrate some, but not the only or exclusive, example embodiments and/or features. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than limiting.

Figure 1 shows a phylogenetic tree of AvrFIT1 homologs identified by performing a BLAST® search of the NCBI protein database. Additional AvrFIT1 sequences were obtained from published *Phakopsora pachyrhizi* transcriptomes and proteomes. The obtained sequences were aligned using Clustal Omega and manually filtered to remove redundant and incomplete sequences. A maximum likelihood phylogenetic tree was constructed from the aligned protein sequences.

Figure 2 shows photographs of plants inoculated with *Phakopsora* spores at two to four weeks of age. The images were taken at 14 to 21 days post inoculation. Presence of FIT1 in the plant genome correlates with resistance to ASR.

Figure 3A shows a phylogenetic tree of homologs of *Vigna unguiculata* Vu01g041300.1 (VuFIT1) (SEQ ID NO: 2) identified by performing a BLAST® search and conducting a protein alignment. This figure shows putative FIT1 orthologs in *Vigna radiata* XP_014501487.1 (SEQ ID NO: 4), *Vigna angularis* XP_017423114.1 (SEQ ID NO: 12), *Phaseolus acutifolius* 005G036500.1 (SEQ ID NO: 10), *Phaseolus lunatus* Pl05g0000042900.1 (SEQ ID NO: 6), *Phaseolus vulgaris* PvUI111.05g037200.1 (SEQ ID NO: 8), *Lablab purpureus* c22535_g1_i3 (SEQ ID NO: 14), *Mucuna pruriens* RDX66260.1 (SEQ ID NO: 16), *Cajanus cajan* XP_020211972.1 (SEQ ID NO: 18), and *Abrus precatorius* XP_027357710.1 (SEQ ID NO: 20). Also shown is a paralog of FIT1 from *Vigna unguiculata* Vu01g041400.1 (SEQ ID NO: 22) believed to be the result of a recent duplication which accumulated mutations and is thus non-functional. The phylogenetic tree was rooted using paralogs of FIT1 that do not function in AvrFIT1 perception. Activity of each FIT1 homolog to function for recognition of AvrFIT1a and AvrFIT1b, based on transient assays, is indicated. nt = not tested.

Figure 3B shows a protein alignment of the amino acid sequences listed in Figure 3A, specifically of the *Vigna unguiculata* allele of FIT1 (VuFIT1) (SEQ ID NO: 2), the *Vigna unguiculata* close paralog of FIT1 (VuFIT1b) (SEQ ID NO: 22), *Vigna angularis* allele of FIT1 (VaFIT1) (SEQ ID NO: 12), the *Vigna radiata* allele of FIT1 (VrFIT1) (SEQ ID NO: 4), the *Phaseolus acutifolius* allele of FIT1 (PaFIT1) (SEQ ID NO: 10), the *Phaseolus*

vulgaris allele of FIT1 (PvFIT1) (SEQ ID NO: 8), the *Phaseolus lunatus* allele of FIT1 (PlFIT1) (SEQ ID NO: 6), the *Lablab purpureus* allele of FIT1 (LpFIT1) (SEQ ID NO: 14), the *Cajanus cajun* allele of FIT1 (CcFIT1) 1 (SEQ ID NO: 18), the *Mucuna pruriens* allele of FIT1 (MpFIT1) (SEQ ID NO: 16), and the *Abrus precatorius* allele of FIT1 (ApFIT1) (SEQ ID NO: 20).

Figure 3C shows a phylogenetic tree of homologs of *Vigna unguiculata* FIT1 in *Glycine* identified by performing a BLAST® search and constructing a protein alignment and phylogenetic tree of the resulting sequences. The tree was rooted using an outgroup of distantly related TIR-NLR proteins from non-legumes.

Figure 4 shows a map of an example DNA construct comprising VuFIT1 (SEQ ID NO: 1) that can be used for transformation of a plant cell, selection of transformed cells, and expression of FIT1.

Figures 5A – 5D depict results showing transient expression of various FIT1 alleles in leaf tissue from a plant lacking an endogenous FIT1. **Fig. 5A** shows the *Vigna unguiculata* allele of FIT1 (VuFIT1), the *Phaseolus lunatus* allele of FIT1 (PlFIT1), the *Vigna radiata* allele of FIT1 (VrFIT1), the *Vigna unguiculata* close paralog of FIT1 (VuFIT1b), and AvrFIT1a. **Fig. 5B** shows *Vigna angularis* allele of FIT1 (VaFIT1), the *Phaseolus vulgaris* allele of FIT1 (PvFIT1), the *Lablab purpureus* allele of FIT1 (LpFIT1), the *Abrus precatorius* allele of FIT1 (ApFIT1), and AvrFIT1a. **Fig. 5C** shows *Vigna unguiculata* allele of FIT1 (VuFIT1), the *Phaseolus lunatus* allele of FIT1 (PlFIT1), the *Vigna radiata* allele of FIT1 (VrFIT1), the *Vigna unguiculata* allele of FIT1 (VuFIT1b), and AvrFIT1b. Fig. 5D shows *Vigna angularis* allele of FIT1 (VaFIT1), the *Phaseolus vulgaris* allele of FIT1 (PvFIT1), the *Lablab purpureus* allele of FIT1 (LpFIT1), the *Abrus precatorius* allele of FIT1 (ApFIT1), and AvrFIT1b.

Figures 6A – 6H depict wild type soybean leaves (lacking FIT1) (Fig. 6A-6D) and leaves from soybean plants expressing FIT1 (Fig. 6E-6H) inoculated with *Phakopsora pachyrhizi*. The leaves were photographed at 13- and 44-days post inoculation as indicated.

Figures 7A – 7B are photographs of wild type soybean plants (**Fig. 7A**) and transgenic soybean plants expressing FIT1 (**Fig. 7B**).

Figure 7C is a bar graph of the height of the plants shown in Fig. 9A and Fig. 9B 24 days after planting. The error bars indicate the standard deviation of the plant height from the individual plants (n>8).

Figure 8 depicts an alignment between the FIT1 alleles from *Vigna unguiculata* (VuFIT1), *Phaseolus lunatus* (PlFIT1), *Abrus precatorius* (ApFIT1), and the N gene, which gives TMV resistance. The LRR domain is poorly conserved between the FIT1 alleles and the N gene.

DETAILED DESCRIPTION

Definitions

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques and/or substitutions of equivalent techniques that would be apparent to one of skill in the art.

Following long-standing patent law convention, the terms "a," "an," and "the" refer to "one or more" when used in this application, including the claims. For example, the phrase "a cell" refers to one or more cells, and in some embodiments can refer to a tissue and/or an organ. Similarly, the phrase "at least one", when employed herein to refer to an entity, refers to, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, or more of that entity, including but not limited to all whole number values between 1 and 100 as well as whole numbers greater than 100.

Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." The term "about," as used herein when referring to a measurable value such as an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of ±10% from the specified amount, as such variations are appropriate to perform the disclosed methods and/or employ the disclosed compositions, nucleic acids, polypeptides, etc. Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

As used herein, the term "and/or" when used in the context of a list of entities, refers to the entities being present singly or in combination. Thus, for example, the phrase "A, B, C,

and/or D" includes A, B, C, and D individually, but also includes any and all combinations and subcombinations of A, B, C, and D (e.g., AB, AC, AD, BC, BD, CD, ABC, ABD, and BCD). In some embodiments, one or more of the elements to which the "and/or" refers can also individually be present in single or multiple occurrences in the combinations(s) and/or subcombination(s).

As used herein, the term "plant" can refer to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom), to a whole plant, any part thereof, or a cell or tissue culture derived from a plant. Thus, the term "plant" can refer to any of whole plants, plant components or organs (e.g., leaves, stems, roots, etc.), plant tissues, seeds and/or plant cells.

A plant cell is a cell of a plant, taken from a plant, or derived through culture from a cell taken from a plant. Thus, the term "plant cell" includes without limitation cells within seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, shoots, gametophytes, sporophytes, pollen, and microspores.

The phrase "plant part" refers to a part of a plant, including single cells and cell tissues such as plant cells that are intact in plants, cell clumps, and tissue cultures from which plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, and seeds; as well as scions, rootstocks, protoplasts, calli, and the like.

As used herein, the term "resistant", or "resistance", describes a plant, line or variety that shows fewer or reduced symptoms to a biotic pest or pathogen than a susceptible (or more susceptible) plant, line or variety to that biotic pest or pathogen. This term is also applied to plants that show no symptoms, and may also be referred to as "high/standard resistance".

As used herein, the term "tolerant" or "tolerance" describes a plant, line, or variety that that shows some symptoms to a biotic pest or pathogen, but that are still able to produce marketable product with an acceptable yield. These lines may also be referred to as having "moderate/intermediate resistance". Tolerant and moderate/intermediate resistant plant types restrict the growth and development of the specified pest or pathogen, but exhibit a greater range of symptoms or damage compared to plant types with high resistance. Plant types with intermediate resistance will show less severe symptoms than susceptible plant varieties, when grown under similar field conditions and pathogen pressure. A "tolerant" plant may also indicate a phenotype of a plant wherein disease-symptoms remain absent upon exposure of

said plant to an infective dosage of pathogen, whereby the presence of a systemic or local pathogen infection, pathogen multiplication, at least the presence of pathogen genomic sequences in cells of said plant and/or genomic integration thereof can be established. Tolerant plants are therefore resistant for symptom expression but symptomless carriers of the pathogen. Sometimes, pathogen sequences may be present or even multiply in plants without causing disease symptoms. This phenomenon is also known as "latent infection". In latent infections, the pathogen may exist in a truly latent non-infectious occult form, possibly as an integrated genome or an episomal agent (so that pathogen protein cannot be found in the cytoplasm, while PCR protocols may indicate the present of pathogen nucleic acid sequences) or as an infectious and continuously replicating agent. A reactivated pathogen may spread and initiate an epidemic among susceptible contacts. The presence of a "latent infection" is indistinguishable from the presence of a "tolerant" phenotype in a plant.

Methods of evaluating resistance are well known to one skilled in the art. Such evaluation may be performed by visual observation of a plant or a plant part (e.g., leaves, roots, flowers, fruits et. al) in determining the severity of symptoms. For example, when each plant is given a resistance score on a scale of 1 to 5 based on the severity of the reaction or symptoms, with 1 being the resistance score applied to the most resistant plants (e.g., no symptoms, or with the least symptoms), and 5 the score applied to the plants with the most severe symptoms, then a line is rated as being resistant when at least 75% of the plants have a resistance score at a 1, 2, or 3 level, while susceptible lines are those having more than 25% of the plants scoring at a 4 or 5 level. If a more detailed visual evaluation is possible, then one can use a scale from 1 to 10 so as to broaden out the range of scores and thereby hopefully provide a greater scoring spread among the plants being evaluated. Additional methods for evaluating resistance are well known in the art (see for example, jircas.go.jp/sites/default/files/publication/manual_guideline/manual_guideline-_-_73.pdf available on the world wide web).

In addition to such visual evaluations, disease evaluations can be performed by determining the pathogen bio-density in a plant or plant part using electron and/or light microscopy and/or through molecular biological methods, such as protein quantification (e.g., ELISA, measuring pathogen protein density) and/or nucleic acid quantification (e.g., RT-PCR, measuring pathogen RNA density). Another method relies on quantifying the spores produced by the pathogen, which can be quantified using a hemacytometer and evaluated per uredinium, per leaf area, or per leaf.

As used herein, the term “susceptible” is used herein to refer to a plant that is unable to prevent entry of the pathogen into the plant and/or slow multiplication and systemic spread of the pathogen, resulting in disease symptoms. The term “susceptible” is therefore equivalent to “non-resistant”.

As used herein, the term "homologous" or "homolog" is used as it is known in the art and refers to related sequences that share a common ancestor. The term “homolog” is sometimes used to apply to the relationship between genes separated by the event of speciation (“ortholog”) or to the relationship between genes separated by the event of genetic duplication within the same species (“paralog”). Homology can be determined using software programs readily available in the art, such as those discussed in *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.718, Table 7.71.

As used herein, the term “allele” is used both as it is known in the art as one of two or more versions of a gene or peptide, and also to refer to synthetic variants of a gene or peptide containing one or more changes from the native sequence.

As used herein, the term “functional” used in the context of a homolog means that the homolog has the same or very similar function. For example, a functional homolog of FIT1 would recognize an AvrFIT1 effector protein. A “nonfunctional FIT1 homolog” would not recognize AvrFIT1, though it may still be functional in that it is able recognize other effector proteins.

As used herein, the term “sequence identity” refers to the presence of identical nucleotides or amino acids at corresponding positions of two sequences. Readily available sequence comparison and multiple sequence alignment algorithms are, respectively, the Basic Local Alignment Search Tool (BLAST®) and ClustalW/ClustalW2/Clustal Omega programs available on the Internet (e.g., the website of the EMBL-EBI). Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.) and ALIGN Plus (Scientific and Educational Software, Pennsylvania). Other non-limiting alignment programs include Sequencher (Gene Codes, Ann Arbor, Michigan), AlignX, and Vector NTI (Invitrogen, Carlsbad, CA). Other suitable programs include, but are not limited to, GAP, BestFit, Plot Similarity, and FASTA, which are part of the Accelrys GCG Package available from Accelrys, Inc. of San Diego, Calif., United States of America. See also Smith & Waterman, 1981; Needleman & Wunsch, 1970; Pearson & Lipman, 1988; Ausubel et al., 1988; and

Sambrook & Russell, 2001. Unless otherwise noted, alignments disclosed herein utilized Clustal Omega.

As used herein, the phrases "DNA construct", "expression cassette", "chimeric construct", "construct", and "recombinant DNA construct" are used interchangeably herein. A recombinant DNA construct comprises an artificial combination of nucleic acid fragments, *e.g.*, regulatory and coding sequences that are not found together in nature. For example, a construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. Such construct may be used by itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The vector may be a viral vector that is suitable as a delivery vehicle for delivery of the nucleic acid, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, *Proc. Natl. Acad. Sci.* U.S.A. 94:12744-12746).

As used herein "cisgene" refers to a gene from the same species, or a species closely related enough to be conventionally bred. "Transgene" refers to a gene from a different species, and may also be referred to as "heterologous" (an amino acid or a nucleic acid sequence which is not naturally found in the particular organism). Both transgenes and heterologous sequences would be considered "exogenous" as referring to a substance coming from some source other than its native source.

The term "operably linked" refers to the juxtaposition of two or more components (such as sequence elements) having a functional relationship. For example, the sequential arrangement of the promoter polynucleotide with a further oligo- or polynucleotide, resulting in transcription of the further polynucleotide.

As used herein, "promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In some embodiments, the promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue specificity of a promoter.

As used herein, "selectable marker" is a nucleic acid segment that allows one to select for a molecule (e.g., a plasmid) or a cell that contains it, often under particular conditions. These markers can encode an activity, such as, but not limited to, production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like.

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

Overview

The present disclosure provides an isolated, recombinant, or synthetic polynucleotide comprising a FIT1 protein, and homologs, fragments, and variations thereof. The disclosure further relates to plants, plant parts, and plant cells that have been transformed with these polynucleotides, and exhibit resistance or tolerance to a plant pathogen, such as *Phakopsora pachyrhizi.* The disclosure further relates to methods of identifying pathogen resistant genes, and methods of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising targeted gene editing of a homolog (such as FIT1), and plants produced therefrom.

Plant pathogens

There are more than 6000 species of rust fungi, including for example, *Phakopsora pachyrhizi, Puccinia* spp., *Uromyces appendiculatus,* and *Hemileia vastatrix,* that infect a wide range of important crops and ornamental varieties. Some examples of varieties that may be infected include, but are not limited to, *Avena sativa* (oats), *Vicia faba* (broad beans), *Coffea arabica* (coffee), Chrysanthemum, *Cydonia* (quince), *Fuchsia spp.* (garlic), *Hordeum vulgare* (barley), *Juniperus virginiana* (red cedar), *Juniperus communis* (juniper), *Allium ampeloprasum* (leek), *Malus* spp. (apple), *Mentha piperita* (peppermint), *Mespilus* (medlar), Onion, *Pelargonium*, *Primula vulgaris* (primrose), *Pyrus* (pear), *Rosa spp.* (roses), *Triticum spp.* (wheat), *Secale cereale* (rye), *Vitis vinifera* (grape), *Saccharum spp.* (sugarcane) and *Glycine max* (soybean).

In soybean, rust pathogen infections have been reported in South America, Africa, Australia, and Asia, and were first reported on soybean crops in the southern United States in 2004. Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, spreads quickly and can lead to significant yield loss. Initial symptoms of ASR include yellow discoloration on the

upper surfaces of foliage, followed by tan or reddish-brown lesions on the undersides of the leaves and sometimes on petioles, stems or pods. Blisters develop within the lesions, which break open and release spores. Soybean plants infected with ASR will exhibit reduced pod production and can result in a yield loss of greater than 50%. Another disease, New World soybean rust, caused by *Phakopsora meibomiae*, is generally not as harmful as ASR. *P. meibomiae* has not yet been reported in the U.S.

Successful infection of rust fungi relies on the secretion of effector proteins with functions that facilitate host colonization. The effector proteins suppress plant immunity and manipulate the host metabolism to benefit the pathogen (Jones and Dangl, 2006). AvrFIT1 is one such effector protein secreted by *Phakopsora pachyrhizi* that causes ASR. AvrFIT1 is present in several sequenced *Phakopsora pachyrhizi* strains isolated from various locations across the world, including Brazil and North America. A phylogenetic tree of close homologs of AvrFIT1 reveals that there are two similar copies of AvrFIT1 in *Phakopsora pachyrhizi* (Figure 1), (PpAvrFIT1a and PpAvrFIT1b), both of which are recognized by FIT1 in transient assays. AvrFIT1 is also conserved in many other fungal species. Thai1, LA04-1 and MG2006 are three strains of *Phakopsora pachyrhizi* that all contain recognized alleles of AvrFIT1 (Elmore et al., 2020; Link et al., 2014, and information on *Phakopsora pachyrhizi* from the genome portal of the Department of Energy Joint Genome Institute available on the worldwide web.). *Phakopsora pachyrhizi* BR is an unspecified Brazilian population which also contains recognized alleles of AvrFIT1 (de Carvalho et al., 2017). AvrFIT1 is present in species of rust pathogens that cause disease on poplar (*Melampsora larici-populina*), cereals (*Puccinia spp.*) and other plants. Putative orthologs of AvrFIT1 are also present in more distantly related fungal species including both pathogenic and non-pathogenic fungi. For example, AvrFIT1 is present in *Rhizoctonia solani,* a non-rust pathogen that can be problematic for herbaceous plants, causing diseases such as collar rot, root rot, damping off, and wire stem.

AvrFIT1 is a putative peptidyl prolyl isomerase and is predicted to disrupt or perturb the function of one or more plant host proteins. While not wishing to be bound by any particular theory, it is possible that AvrFIT1 could be modifying an unknown plant protein, possibly a conserved component of the plant immune system, and FIT1 could be "guarding" the unknown protein and be activated if the protein is modified by AvrFIT1. There are many examples of NLR receptors acting this way in the literature. Pathogen effectors often convergently evolve to target the same or similar plant host proteins to promote virulence.

There are many examples of two different pathogen effector proteins evolving to target the same plant protein independently. Thus, it is possible that another pathogen might have an effector protein that is unrelated to AvrFIT1 but acts on the same protein as AvrFIT1 and therefore is also capable of being perceived by FIT1. By guarding against proteins with a similar activity or molecular target as AvrFIT1, FIT1 can mediate resistance against pathogens which don't have a close homolog of AvrFIT1 but do have a protein which has evolved to have a similar activity or molecular target as FIT1. Such proteins may be present in a diverse range of plant pathogens including but not limited to fungi, oomycetes, bacteria, nematodes, or viruses.

Plant resistance

Plants have, in some cases, evolved immunity in which resistance gene products recognize the activity of specific effectors resulting in effector-trigger immunity (ETI) (Jones and Dangl, 2006). ETI leads to robust defenses, such as the hypersensitive response (HR), which is a form of programmed cell death that results in the formation of a localized lesion that inhibits pathogen growth at the initial infection site (Dodds and Rathjen, 2010). If the plant has an immune receptor capable of recognizing the pathogen effector protein, the effector protein activates a strong immune response conferring immunity. The perception of intracellular pathogen effector proteins in plants is frequently mediated by proteins from a large gene family known as the nucleotide binding, leucine-rich repeat (NLR) proteins (Jones et al., 2016).

Plant disease resistance traits are often encoded by NLR genes. NLR genes can be incorporated into a susceptible crop variety to confer resistance through a variety of methods including introgression breeding, transformation or genome editing. A typical plant has hundreds of NLR immune receptor genes (Jones et al., 2016). These genes are typically expressed at relatively low levels with the NLR proteins passively surveilling for the presence of cognate effector proteins from invading pathogens. Prior to activation, the NLR proteins have essentially no impact on plant metabolism or growth. Upon activation by a cognate ligand, typically a pathogen effector protein or a protein substrate of an effector, the NLR protein initiates a signalling cascade that activates endogenous plant defense pathways to inhibit pathogen growth. Using NLRs is a natural, safe, and environmentally sustainable mechanism to develop disease-resistant crop varieties to improve plant yields and reduce the need for chemical controls.

FIT1

FIT1 is a plant Toll-like interleukin-1 receptor (TIR) nucleotide binding leucine rich repeat (NLR) immune receptor protein discovered by Applicants. It was identified from *Vigna unguiculata* (cowpea) and is responsible for AvrFIT1 recognition. As shown in Figure 2, expression of FIT1 correlates with resistance to ASR. Accessions of *Vigna radiata*, (PI 378026), *Phaseolus lunatus* (PI 180324), and *Vigna unguiculata* (LG104, Baker Creek Heirloom Seeds) which contain functional copies of FIT1 genes show strong resistance to *Phakopsora pachyrhizi*. In contrast, accessions of *Glycine max* (Williams 82), *Pachyrhizus erosus* (AB105, Baker Creek Heirloom Seeds), and *Glycine peratosa* (PI 583964), which all lack functional FIT1, are highly susceptible to *Phakopsora pachyrhizi* as observed by large disease lesions and spore production. These results indicate a correlation between the presence of FIT1 and resistance to *Phakopsora pachyrhizi* and suggest that expression of FIT1 in plants can confer disease resistance. Additionally, the widespread distribution of AvrFIT1 discussed above suggests that FIT1 can confer disease resistance against a broad range of pathogenic species.

Thus, an embodiment of the present disclosure provides an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, functional homologs, and/or fragments and variations thereof. In some cases, the functional FIT1 homolog shares at least about 70% identity to SEQ ID NO: 2 and recognizes an effector protein secreted by a plant pathogen. In some cases, the functional FIT1 homolog recognizes at least one of AvrFIT1a and AvrFIT1b. In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 2.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about

99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 4.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 6.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 8.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 10.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 12

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about

87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 14.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 16.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 18.

In some cases, the functional FIT1 homolog shares at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 20.

In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 4, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 6, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 8, or an

amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 10, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 12, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 14, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 16, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 18, or an amino acid sequence at least 90% identical thereto. In some aspects, the isolated, recombinant, or synthetic polynucleotide comprises a nucleic acid sequence encoding SEQ ID NO: 20, or an amino acid sequence at least 90% identical thereto.

In another embodiment, the disclosure relates to a transgenic plant, plant part, or cell having resistance or tolerance to at least one plant pathogen, wherein the resistance or tolerance is conferred by a polynucleotide encoding at least one of the functional FIT1 homologs disclosed herein.

In another embodiment, the present disclosure provides an isolated, recombinant, or synthetic polynucleotide, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto, wherein said sequences encode a functional FIT1 protein. In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 1. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%,

about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 3. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 5. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 7. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 9. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about

95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 11. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 13. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 15. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 17. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

In some cases, the polynucleotide shares at least about 70%, at least about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about

99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to SEQ ID NO: 19. In some embodiments, the disclosure relates to genetic constructs comprising these sequences.

The disclosure also encompasses variants and fragments of proteins of an amino acid sequence encoded by the nucleic acid sequences of FIT1, orthologs of FIT1 and/or paralogs of FIT1. The variants may contain alterations in the amino acid sequences of the constituent proteins. The term "variant" with respect to a polypeptide refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence. The variant can have "conservative" changes, or "nonconservative" changes, *e.g.*, analogous minor variations can also include amino acid deletions or insertions, or both.

Functional fragments and variants of a polypeptide include those fragments and variants that maintain one or more functions or domains of the parent polypeptide. As used herein, a protein domain is a distinct functional and/or structural unit in a protein, and are usually responsible for a particular function or interaction. It is recognized that the gene or cDNA encoding a polypeptide can be considerably mutated without materially altering one or more of the polypeptide's functions and/or domains. First, the genetic code is well-known to be degenerate, and thus different codons encode the same amino acids. Second, even where an amino acid substitution is introduced, the mutation can be conservative and have no material impact on the essential function(s) of a protein. See, e.g., *Stryer Biochemistry* 3rd Ed., 1988. Third, part of a polypeptide chain can be deleted without impairing or eliminating all of its functions. Fourth, insertions or additions can be made in the polypeptide chain for example, adding epitope tags, without impairing or eliminating its functions (Ausubel *et al. J. Immunol.* 159(5): 2502-12, 1997). Other modifications that can be made without materially impairing one or more functions of a polypeptide can include, for example, *in vivo* or *in vitro* chemical and biochemical modifications or the incorporation of unusual amino acids. Such modifications include, but are not limited to, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquination, labelling, *e.g.*, with radionucleotides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labelling polypeptides, and labels useful for such purposes, are well known in the art, and include radioactive isotopes such as ^{32}P, ligands which bind to or are bound by labelled specific binding partners (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and anti-ligands. Functional fragments and variants can be of varying length. For example, some fragments have at least 10, 25, 50, 75, 100, 200, or

even more amino acid residues. These mutations can be natural or purposely changed. In some embodiments, mutations containing alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the proteins or how the proteins are made are an embodiment of the present disclosure.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Further information about conservative substitutions can be found, for instance, in Ben Bassat *et al.* (*J. Bacteriol.*, 169:751-757, 1987), O'Regan *et al.* (*Gene*, 77:237-251, 1989), Sahin-Toth *et al.* (*Protein Sci.*, 3:240-247, 1994), Hochuli *et a*l. (*Bio/Technology*, 6:1321-1325, 1988) and in widely used textbooks of genetics and molecular biology. The Blosum matrices are commonly used for determining the relatedness of polypeptide sequences. The Blosum matrices were created using a large database of trusted alignments (the BLOCKS database), in which pairwise sequence alignments related by less than some threshold percentage identity were counted (Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10915−10919, 1992). A threshold of 90% identity was used for the highly conserved target frequencies of the BLOSUM90 matrix. A threshold of 65% identity was used for the BLOSUM65 matrix. Scores of zero and above in the Blosum matrices are considered "conservative substitutions" at the percentage identity selected. The following table 1 shows exemplary conservative amino acid substitutions.

Table 1

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr
Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val

Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

Codon Optimization

In some cases, variants may differ from the disclosed sequences by alteration of the coding region to fit the codon usage bias of the particular organism into which the molecule is to be introduced. In other cases, the coding region may be altered by taking advantage of the degeneracy of the genetic code to alter the coding sequence such that, while the nucleotide sequence is substantially altered, it nevertheless encodes a protein having an amino acid sequence substantially similar to the disclosed amino acid sequences of FIT1, orthologs of FIT1 and/or paralogs of FIT1, and/or fragments and variations thereof.

Protein expression is governed by a host of factors including those that affect transcription, mRNA processing, and stability and initiation of translation. Optimization can thus address any of a number of sequence features of any particular gene. Translation may be paused due to the presence of codons in the polynucleotide of interest that are rarely used in the host organism, and this may have a negative effect on protein translation due to their scarcity in the available tRNA pool. Specifically, it can result in reduced protein expression.

Alternate translational initiation also can result in reduced heterologous protein expression. Alternate translational initiation can include a synthetic polynucleotide sequence inadvertently containing motifs capable of functioning as a ribosome binding site (RBS). These sites can result in initiating translation of a truncated protein from a gene-internal site. One method of reducing the possibility of producing a truncated protein includes eliminating putative internal RBS sequences from an optimized polynucleotide sequence.

Repeat-induced polymerase slippage can result in reduced heterologous protein expression. Repeat-induced polymerase slippage involves nucleotide sequence repeats that have been shown to cause slippage or stuttering of DNA polymerase which can result in frameshift mutations. Such repeats can also cause slippage of RNA polymerase. In an organism with a high G+C content bias, there can be a higher degree of repeats composed of

G or C nucleotide repeats. Therefore, one method of reducing the possibility of inducing RNA polymerase slippage, includes altering extended repeats of G or C nucleotides.

Interfering secondary structures also can result in reduced heterologous protein expression. Secondary structures can sequester the RBS sequence or initiation codon and have been correlated to a reduction in protein expression. Stemloop structures can also be involved in transcriptional pausing and attenuation. An optimized polynucleotide sequence can contain minimal secondary structures in the RBS and gene coding regions of the nucleotide sequence to allow for improved transcription and translation.

The optimization process can begin, for example, by identifying the desired amino acid sequence to be expressed by the host. From the amino acid sequence, a candidate polynucleotide or DNA sequence can be designed. During the design of the synthetic DNA sequence, the frequency of codon usage can be compared to the codon usage of the host expression organism and rare host codons can be removed from the synthetic sequence. Additionally, the synthetic candidate DNA sequence can be modified in order to remove undesirable enzyme restriction sites and add or remove any desired signal sequences, linkers or untranslated regions. The synthetic DNA sequence can be analyzed for the presence of secondary structure that may interfere with the translation process, such as G/C repeats and stem-loop structures.

Optimized coding sequences containing codons preferred by a particular host can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence.

Functional fragments, chimeric, and synthetic polypeptides

In some cases, functional fragments derived from FIT1 orthologs of the present disclosure can still confer resistance to pathogens when expressed in a plant. In some cases, the functional fragments contain one or more conserved regions shared by two or more FIT1 orthologs.

In some cases, functional chimeric or synthetic polypeptides derived from the FIT1 orthologs of the present disclosure are provided. The functional chimeric or synthetic polypeptides can still confer resistance to pathogens when expressed in a plant. In some cases, the functional chimeric or synthetic polypeptides contain one or more conserved regions shared by two or more FIT1 orthologs.

DNA constructs

In some embodiments, the disclosure relates to a DNA construct comprising at least one FIT1 sequence disclosed herein. In some cases, the FIT1 sequence is a polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, functional homologs, and/or fragments and variations thereof. In some cases, the FIT1 protein shares at least about 70% identity to SEQ ID NO: 2. In some cases, the FIT1 sequence is selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto. In some cases, two or more FIT1 sequences are stacked to increase pathogen resistance in a plant. In some cases, at least one FIT1 sequence is stacked with another pathogen resistance gene.

The expression control elements used to regulate the expression of a given protein can either be the expression control element that is normally found associated with the coding sequence (homologous expression element) or can be a heterologous expression control element. A variety of homologous and heterologous expression control elements are known in the art and can readily be used to make DNA constructs for use in the present disclosure. Transcription initiation regions, for example, can include any of the various opine initiation regions, such as octopine, mannopine, nopaline and the like that are found in the Ti plasmids of *Agrobacterium tumefaciens*. Alternatively, plant viral promoters can also be used, such as the cauliflower mosaic virus 19S and 35S promoters (CaMV 19S and CaMV 35S promoters, respectively) to control gene expression in a plant (U.S. Patent Nos. 5,352,605; 5,530,196 and 5,858,742 for example). Enhancer sequences derived from the CaMV can also be utilized (U.S. Patent Nos. 5,164,316; 5,196,525; 5,322,938; 5,530,196; 5,352,605; 5,359,142; and 5,858,742 for example). Lastly, plant promoters such as prolifera promoter, fruit specific promoters, Ap3 promoter, heat shock promoters, seed specific promoters, etc. can also be used.

Either a gamete-specific promoter, a constitutive promoter (such as the CaMV or Nos promoter), an organ-specific promoter (such as the E8 promoter from tomato), or an inducible promoter is typically ligated to the protein or antisense encoding region using standard techniques known in the art. The DNA construct may be further optimized by employing supplemental elements such as transcription terminators and/or enhancer elements.

Thus, for expression in plants, the DNA construct will typically contain, in addition to the protein sequence, a plant promoter region, a transcription initiation site and a transcription termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the expression unit are typically included to allow for easy insertion into a pre-existing vector.

In the construction of heterologous promoter/gene of interest or antisense combinations, the promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. If the mRNA encoded by the gene is to be efficiently processed, DNA sequences which direct polyadenylation of the RNA are also commonly added to the vector construct. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen *et al.*, EMBO *J* 3:835-846 (1984)) or the nopaline synthase signal (Depicker *et al.*, Mol. and Appl. Genet. 1:561-573 (1982)). The resulting expression unit is ligated into or otherwise constructed to be included in a vector that is appropriate for higher plant transformation. One or more expression units may be included in the same vector.

Selection

A DNA construct will typically contain a selectable marker gene expression unit by which transformed plant cells can be identified in culture. Usually, the marker gene will encode resistance to an antibiotic, such as G418, hygromycin, bleomycin, kanamycin, or gentamicin or to an herbicide, such as glyphosate (Round-Up) or glufosinate (BASTA) or atrazine. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host; preferably a broad host range for prokaryotic origin of replication is included. A selectable marker for bacteria may also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers include resistance to antibiotics such as ampicillin, kanamycin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

For positive selection, for example, a foreign gene is supplied to a plant cell that allows it to utilize a substrate present in the medium that it otherwise could not use, such as mannose or xylose (for example, refer US 5767378; US 5994629). More typically, however, negative selection is used because it is more efficient, utilizing selective agents such as herbicides or antibiotics that either kill or inhibit the growth of non-transformed plant cells and reducing the possibility of chimeras. Resistance genes that are effective against negative selective agents are provided on the introduced foreign DNA used for the plant transformation. For example, one of the most popular selective agents used is the antibiotic kanamycin, together with the resistance gene neomycin phosphotransferase (nptII), which confers resistance to kanamycin and related antibiotics (see, for example, Messing & Vierra, *Gene* 19: 259-268 (1982); Bevan et al., *Nature* 304:184-187 (1983)). However, many different antibiotics and antibiotic resistance genes can be used for transformation purposes (refer US 5034322, US 6174724 and US 6255560). In addition, several herbicides and herbicide resistance genes have been used for transformation purposes, including the bar gene, which confers resistance to the herbicide phosphinothricin (White et al., *Nucl Acids Res* 18: 1062 (1990), Spencer et al., *Theor Appl Genet* 79: 625-631(1990), US 4795855, US 5378824 and US 6107549). In addition, the dhfr gene, which confers resistance to the anticancer agent methotrexate, has been used for selection (Bourouis et al., EMBO J. 2(7): 1099-1104 (1983).

Transgenic Plants Comprising Sequences Disclosed Herein

In one embodiment, the present disclosure relates to a transgenic plant, plant part, or plant cell, wherein the transgene comprises at least one polynucleotide coding for FIT1, orthologs of FIT1 and/or paralogs of FIT1, and/or fragments and variations thereof, and exhibit resistance or tolerance to a pathogen. In some cases, the polynucleotide encodes a protein selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and proteins at least 90% identical thereto functional homologs thereof. In some cases, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto. In some cases, the pathogen is a fungus. In some cases, the fungus is from the order Cantharellales or Pucciniales. In some cases, the fungal pathogen is *Rhizoctonia solani*, *Melampsora* spp., *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora* spp., *Puccinia* spp., *Uromyces* spp., *Austropuccinia* spp., *Cronartium* spp. or *Hemileia vastatrix*.

In some cases, the plant, plant part, or plant cell is in the subfamily Papilionoideae. In some cases, the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., *Strophostyles* spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

In some cases, the plant, plant part, or plant cell is *Glycine max*, and the plant, plant part, or plant cell is resistant to Asian Soybean Rust caused by *Phakopsora pachyrhizi*.

Methods of producing transgenic plants are well known to those of ordinary skill in the art. Transgenic plants can now be produced by a variety of different transformation methods including, but not limited to, electroporation; microinjection; microprojectile bombardment, also known as particle acceleration or biolistic bombardment; viral-mediated transformation; and *Agrobacterium*-mediated transformation. See, for example, U.S. Patent Nos. 5,405,765; 5,472,869; 5,538,877; 5,538,880; 5,550,318; 5,641,664; 5,736,369 and 5,736,369; International Patent Application Publication Nos. WO2002/038779 and WO/2009/117555; Lu et al., (Plant Cell Reports, 2008, 27:273-278); Watson et al., Recombinant DNA, Scientific American Books (1992); Hinchee et al., Bio/Tech. 6:915-922 (1988); McCabe et al., Bio/Tech. 6:923-926 (1988); Toriyama et al., Bio/Tech. 6:1072-1074 (1988); Fromm et al., Bio/Tech. 8:833-839 (1990); Mullins et al., Bio/Tech. 8:833-839 (1990); Hiei et al., Plant Molecular Biology 35:205-218 (1997); Ishida et al., Nature Biotechnology 14:745-750 (1996); Zhang et al., Molecular Biotechnology 8:223-231 (1997); Ku et al., Nature Biotechnology 17:76-80 (1999); and, Raineri et al., Bio/Tech. 8:33-38 (1990)), each of which is expressly incorporated herein by reference in their entirety.

Microprojectile bombardment is also known as particle acceleration, biolistic bombardment, and the gene gun (Biolistic® Gene Gun). The gene gun is used to shoot pellets that are coated with genes (e.g., for desired traits) into plant seeds or plant tissues in order to get the plant cells to then express the new genes. The gene gun uses an actual explosive (.22 caliber blank) to propel the material. Compressed air or steam may also be used as the propellant. The Biolistic® Gene Gun was invented in 1983-1984 at Cornell University by John Sanford, Edward Wolf, and Nelson Allen. It and its registered trademark

are now owned by E. I. du Pont de Nemours and Company. Most species of plants can be been transformed using this method.

The most common method for the introduction of new genetic material into a plant genome involves the use of living cells of the bacterial pathogen *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is a naturally occurring bacterium that is capable of inserting its DNA (genetic information) into plants, resulting in a type of injury to the plant known as crown gall. Most species of plants can now be transformed using this method. There are numerous patents governing *Agrobacterium* mediated transformation and particular DNA delivery plasmids designed specifically for use with *Agrobacterium*---for example, US4536475, EP0265556, EP0270822, WO8504899, WO8603516, US5591616, EP0604662, EP0672752, WO8603776, WO9209696, WO9419930, WO9967357, US4399216, WO8303259, US5731179, EP068730, WO9516031, US5693512, US6051757 and EP904362A1. Methods of *Agrobacterium*-mediated plant transformation that involve using vectors with no T-DNA are also well known to those skilled in the art and can be used with the methods of the present disclosure. See, for example, U.S. Patent No. 7,250,554, which utilizes P-DNA instead of T-DNA in the transformation vector. A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome, although multiple copies are possible. Such transgenic plants can be referred to as being hemizygous for the added gene, or may be referred to as an independent segregant, because each transformed plant represents a unique T-DNA integration event (U.S. Patent No. 6,156,953).

Direct plant transformation methods using DNA have also been reported. The first of these to be reported historically is electroporation, which utilizes an electrical current applied to a solution containing plant cells (M. E. Fromm et al., Nature, 319, 791 (1986); H. Jones et al., Plant Mol. Biol., 13, 501 (1989) and H. Yang et al., Plant Cell Reports, 7, 421 (1988).

Another direct method, called "biolistic bombardment", uses ultrafine particles, usually tungsten or gold, that are coated with DNA and then sprayed onto the surface of a plant tissue with sufficient force to cause the particles to penetrate plant cells, including the thick cell wall, membrane and nuclear envelope (US 5,204,253, US 5,015,580).

A third direct method uses fibrous forms of metal or ceramic consisting of sharp, porous or hollow needle-like projections that impale the cells, and also the nuclear envelope of cells. Both silicon carbide and aluminium borate whiskers have been used for plant transformation (Mizuno et al., 2004; Petolino et al., 2000; US5302523 US Application

20040197909) and also for bacterial and animal transformation (Kaepler et al., 1992; Raloff, 1990; Wang, 1995).

Examples of viral vectors include, but are not limited to, recombinant plant viruses. Non-limiting examples of plant viruses include, TMV-mediated (transient) transfection into tobacco (Tuipe, T-H et al (1993), J. Virology Meth, 42: 227-239), ssDNA genomes viruses (e.g., family *Geminiviridae*), reverse transcribing viruses (e.g., families *Caulimoviridae*, *Pseudoviridae*, and *Metaviridae*), dsNRA viruses (e.g., families *Reoviridae* and *Partitiviridae*), (-) ssRNA viruses (e.g., families *Rhabdoviridae* and *Bunyaviridae*), (+) ssRNA viruses (e.g., families *Bromoviridae, Closteroviridae, Comoviridae, Luteoviridae, Potyviridae, Sequiviridae* and *Tombusviridae*) and viroids (e.g., families *Pospiviroldae* and *Avsunviroidae*). Detailed classification information of plant viruses can be found in Fauquet et al (2008, "Geminivirus strain demarcation and nomenclature". *Archives of Virology* 153:783–821, incorporated herein by reference in its entirety), and Khan et al. (Plant viruses as molecular pathogens; Publisher Routledge, 2002, ISBN 1560228954, 9781560228950). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

Non-limiting examples of binary vectors suitable for soybean species transformation and transformation methods are described by Yi et al. 2006 (Transformation of multiple soybean cultivars by infecting cotyledonary-node with *Agrobacterium tumefaciens*, *African Journal of Biotechnology* Vol. 5 (20), pp. 1989-1993, 16 October 2006), Paz et al., 2004 (Assessment of conditions affecting *Agrobacterium*-mediated soybean transformation using the cotyledonary node explant, Euphytica 136: 167–179, 2004), U.S. Patent Nos. 5,376,543, 5,416,011, 5,968,830, and 5,569,834, or by similar experimental procedures well known to those skilled in the art.

Genes can also be introduced in a site directed fashion using homologous recombination. Homologous recombination permits site-specific modifications in endogenous genes and thus inherited or acquired mutations may be corrected, and/or novel alterations may be engineered into the genome. Homologous recombination and site-directed integration in plants are discussed in, for example, U.S. Patent Nos. 5,451,513; 5,501,967 and 5,527,695.

Genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell

An embodiment of the present disclosure teaches a method of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising: providing a plant species that is susceptible to a pathogen; identifying within the genome of the plant species a homolog of FIT1, wherein said homolog is nonfunctional (does not mediate AvrFIT1 recognition), and genetically modifying a plant, plant part, or plant cell from the susceptible plant species with targeted gene editing, wherein said targeted gene editing is directed towards the nonfunctional FIT1 homolog, and wherein said targeted gene editing restores the function of FIT1 (enables the FIT1 homolog to recognize AvrFIT1) and confers resistance or tolerance to a pathogen. In some embodiments, the pathogen is a fungus.

As used herein, a "nonfunctional" FIT1 homolog is a homolog that does not recognize a pathogen effector protein homolog of AvrFIT1, such as AvrFIT1a and/or AvrFIT1b. FIT1 homologous may identified by any number of means known in the art. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (*Adv. Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet *et al.* (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang *et al.* (*Comp. Appls Biosci.*, 8:155-65, 1992); and Pearson *et al.* (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul *et al.* (*Nature Genet.*, 6:119-29, 1994) presents a detailed consideration of sequence alignment methods and homology calculations.

Restoring the function of a FIT1 homolog as used herein relates to modifying the allele such that it restores the recognition of a pathogen effector protein such as AvrFIT1a and/or AvrFIT1b, and confers resistance or tolerance to a pathogen. Restoring the function of a homologous gene by way of genetic engineering has been done and is well known in the art (see for example, Ivics Z, et al., (1997), "Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells", *Cell.* 91 (4): 501–510, and recently, Suh S, et al., Restoration of visual function in adult mice with an inherited retinal disease via adenine base editing, *Nat Biomed Eng* (2020) and Sedeek K, et al., Plant genome engineering for targeted improvement of crop traits, *Front. Plant Sci.*, 12 Feb 2019).

In some embodiments, the targeted gene editing uses an engineered or natural nuclease selected from the group consisting of homing endonucleases/meganucleases (EMNs), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). In some embodiments, the targeted gene editing uses a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas nuclease. In some embodiments, the nuclease is selected from the group consisting of Cas9, Cas12, Cas13, CasX, and CasY. The disclosure also relates to plants, plant parts, and plant cells exhibiting resistance or tolerance to a pathogen produced by genetic modification of a FIT1 homolog.

Gene Editing Using CRISPR

Targeted gene editing can be done using CRISPR technology (Saunders & Joung, *Nature Biotechnology*, 32, 347-355, 2014). CRISPR is a type of genome editing system that stands for Clustered Regularly Interspaced Short Palindromic Repeats. This system and CRISPR-associated (Cas) genes enable organisms, such as select bacteria and archaea, to respond to and eliminate invading genetic material. Ishino, Y., et al. *J. Bacteriol.* 169, 5429–5433 (1987). These repeats were known as early as the 1980s in *E. coli*, but Barrangou and colleagues demonstrated that *S. thermophilus* can acquire resistance against a bacteriophage by integrating a fragment of a genome of an infectious virus into its CRISPR locus. Barrangou, R., et al. *Science* 315, 1709–1712 (2007). Many plants have already been modified using the CRISPR system, including soybean (see for example, Han J, et al., Creation of early flowering germplasm of soybean by CRISPR/Cas9 Technology, *Front. Plant Sci.,* 22 Nov 2019), and many Cas genes have now been characterized and used with the system (see for example, Wang J, et al., The rapidly advancing Class 2 CRISPR-Cas technologies: A customizable toolbox for molecular manipulations. *J Cell Mol Med.* 2020;24(6):3256-3270).

Gene editing can also be done using crRNA-guided surveillance systems for gene editing. Additional information about crRNA-guided surveillance complex systems for gene editing can be found in the following documents, which are incorporated by reference in their entirety: U.S. Application Publication No. 2010/0076057 (Sontheimer et al., Target DNA Interference with crRNA); U.S. Application Publication No. 2014/0179006 (Feng, CRISPR-CAS Component Systems, Methods, and Compositions for Sequence Manipulation); U.S. Application Publication No. 2014/0294773 (Brouns et al., Modified Cascade Ribonucleoproteins and Uses Thereof); Sorek et al., *Annu. Rev. Biochem.* 82:237-266, 2013; and Wang, S. et al., *Plant Cell Rep* (2015) 34: 1473-1476.

Gene editing using TALENs

Transcription activator-like effector nucleases (TALENs) have been successfully used to introduce targeted mutations via repair of double stranded breaks (DSBs) either through non-homologous end joining (NHEJ), or by homology-directed repair (HDR) and homology-independent repair in the presence of a donor template. Thus, TALENs are another mechanism for targeted genome editing in plants. The technique is well known in the art; see for example Malzahn, Aimee et al. "Plant genome editing with TALEN and CRISPR" *Cell & Bioscience* vol. 7 21. 24 Apr. 2017.

Other methods of genome editing

In addition to CRISPR and TALENs, two other types of engineered nucleases can be used for genome editing: engineered homing endonucleases/meganucleases (EMNs), and zinc finger nucleases (ZFNs). These methods are well known in the art. See for example, Petilino, Joseph F. "Genome editing in plants via designed zinc finger nucleases" *In Vitro Cell Dev Biol Plant.* 51(1): pp. 1-8 (2015); and Daboussi, Fayza, et al. "Engineering Meganuclease for Precise Plant Genome Modification" in *Advances in New Technology for Targeted Modification of Plant Genomes*. Springer Science+Business. pp 21-38 (2015).

Breeding Methods

Once a gene has been introduced into a plant, or a gene has been genetically modified, that plant can then be used in conventional plant breeding schemes (e.g., pedigree breeding, single-seed-descent breeding schemes, recurrent selection, backcross breeding) to produce progeny which also contain the gene or modified trait. Thus, another aspect of the present disclosure relates to breeding with, or asexually propagating, plants having been transformed with a FIT1 homolog or an immune receptor gene coding for a protein that recognizes AvrFIT1a and/or AvrFIT1b, or plants wherein a FIT1 nonfunctional homolog was genetically modified to recognize AvrFIT1a and/or AvrFIT1b, wherein the plants exhibit resistance or tolerance to a pathogen. The disclosure further relates to progeny plants produced therefrom.

In some cases, plants or progeny therefrom comprising the gene or modified trait may further comprise one or more additional desired traits. In some cases, the one or more additional desired traits are stacked on the same construct as the gene (for example, the FIT1 genes disclosed herein). In another case, the one or more additional desired traits may be introgressed by conventional breeding.

Backcross Breeding

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. As used herein, backcross breeding is synonymous with introgression. Plants produced therefrom may be referred to a single locus converted or single gene converted plants.

A non-limiting example of a backcross breeding protocol would be the following: a) the first generation F_1 produced by the cross of the recurrent parent A by the donor parent B is backcrossed to parent A, b) selection is practiced for the plants having the desired trait of parent B, c) selected plants are self-pollinated to produce a population of plants where selection is practiced for the plants having the desired trait of parent B and physiological and morphological characteristics of parent A, d) the selected plants are backcrossed one, two, three, four, five, six, seven, eight, nine, or more times to parent A to produce selected backcross progeny plants comprising the desired trait of parent B and the physiological and morphological characteristics of parent A. Step (c) may or may not be repeated and included between the backcrosses of step (d).

Examples of desired traits include, but are not limited to, herbicide resistance (such as bar or pat genes), resistance for bacterial, fungal, or viral disease (such as gene I used for BCMV resistance), insect resistance, enhanced nutritional quality (such as 2s albumin gene), industrial usage, agronomic qualities (such as the "persistent green gene"), yield stability, and yield enhancement.

Pedigree Selection

Pedigree breeding is used commonly for the improvement of self-pollinating crops or inbred lines of cross-pollinating crops. Two parents possessing favorable, complementary traits are crossed to produce an F_1. An F_2 population is produced by selfing one or several F_1s or by intercrossing two F_1s (sib mating). The dihaploid breeding method could also be used. Selection of the best individuals is usually begun in the F_2 population; then, beginning in the F_3, the best individuals in the best families are selected. Replicated testing of families,

or hybrid combinations involving individuals of these families, often follows in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release of new cultivars. Similarly, the development of new cultivars through the dihaploid system requires the selection of the cultivars followed by two to five years of testing in replicated plots.

Open-Pollination

The improvement of open-pollinated populations of such crops as rye, many maizes and sugar beets, herbage grasses, legumes such as alfalfa and clover, and tropical tree crops such as cacao, coconuts, oil palm and some rubber, depends essentially upon changing gene-frequencies towards fixation of favorable alleles while maintaining a high (but far from maximal) degree of heterozygosity. Uniformity in such populations is impossible and trueness-to-type in an open-pollinated variety is a statistical feature of the population as a whole, not a characteristic of individual plants. Thus, the heterogeneity of open-pollinated populations contrasts with the homogeneity (or virtually so) of inbred lines, clones and hybrids.

Population improvement methods fall naturally into two groups, those based on purely phenotypic selection, normally called mass selection, and those based on selection with progeny testing. Interpopulation improvement utilizes the concept of open breeding populations; allowing genes for flow from one population to another. Plants in one population (cultivar, strain, ecotype, or any germplasm source) are crossed either naturally (e.g., by wind) or by hand or by bees (commonly *Apis mellifera* L. or *Megachile rotundata* F.) with plants from other populations. Selection is applied to improve one (or sometimes both) population(s) by isolating plants with desirable traits from both sources.

There are basically two primary methods of open-pollinated population improvement. First, there is the situation in which a population is changed en masse by a chosen selection procedure. The outcome is an improved population that is indefinitely propagable by random-mating within itself in isolation. Second, the synthetic variety attains the same end result as population improvement but is not itself propagable as such; it has to be reconstructed from parental lines or clones. These plant breeding procedures for improving open-pollinated populations are well known to those skilled in the art and comprehensive reviews of breeding procedures routinely used for improving cross-pollinated plants are provided in numerous texts and articles, including: Allard, *Principles of Plant Breeding*, John

Wiley & Sons, Inc. (1960); Simmonds, *Principles of Crop Improvement*, Longman Group Limited (1979); Hallauer and Miranda, *Quantitative Genetics in Maize Breeding*, Iowa State University Press (1981); and, Jensen, *Plant Breeding Methodology*, John Wiley & Sons, Inc. (1988). For population improvement methods specific for soybean *see, e.g.*, J.R. Wilcox, editor (1987) SOYBEANS: Improvement, Production, and Uses, Second Edition, American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., publishers, 888 pages.

Hand-Pollination Method

Hand pollination describes the crossing of plants via the deliberate fertilization of female ovules with pollen from a desired male parent plant. In some cases the donor or recipient female parent and the donor or recipient male parent line are planted in the same field or in the same greenhouse. The inbred male parent can be planted earlier than the female parent to ensure adequate pollen supply at the pollination time. Pollination is started when the female parent flower is ready to be fertilized. Female flower buds that are ready to open in the following days are identified, covered with paper cups or small paper bags that prevent bee or any other insect from visiting the female flowers, and marked with any kind of material that can be easily seen the next morning. The male flowers of the male parent are collected in the early morning before they are open and visited by pollinating insects. The covered female flowers of the female parent, which have opened, are un-covered and pollinated with the collected fresh male flowers of the male parent, starting as soon as the male flower sheds pollen. The pollinated female flowers are again covered after pollination to prevent bees and any other insects visit. The pollinated female flowers are also marked. The marked flowers are harvested. In some cases, the male pollen used for fertilization has been previously collected and stored.

Bee-Pollination Method

Using the bee-pollination method, the parent plants are usually planted within close proximity. More female plants may be planted to allow for a greater production of seed. Insects are placed in the field or greenhouses for transfer of pollen from the male parent to the female flowers of the female parent.

Mass Selection

In mass selection, desirable individual plants are chosen, harvested, and the seed composited without progeny testing to produce the following generation. Since selection is based on the maternal parent only, and there is no control over pollination, mass selection

amounts to a form of random mating with selection. As stated above, the purpose of mass selection is to increase the proportion of superior genotypes in the population.

Synthetics

A synthetic variety is produced by crossing inter se a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination. Parents are selected on general combining ability, sometimes by test crosses or topcrosses, more generally by polycrosses. Parental seed lines may be deliberately inbred (e.g. by selfing or sib crossing). However, even if the parents are not deliberately inbred, selection within lines during line maintenance will ensure that some inbreeding occurs. Clonal parents will, of course, remain unchanged and highly heterozygous.

Hybrids

A hybrid is an individual plant resulting from a cross between parents of differing genotypes. Commercial hybrids are now used extensively in many crops, including corn (maize), sorghum, sugar beet, sunflower and broccoli. Hybrids can be formed in a number of different ways, including by crossing two parents directly (single cross hybrids), by crossing a single cross hybrid with another parent (three-way or triple cross hybrids), or by crossing two different hybrids (four-way or double cross hybrids).

Hybrids may be fertile or sterile depending on qualitative and/or quantitative differences in the genomes of the two parents. Heterosis, or hybrid vigor, is usually associated with increased heterozygosity that results in increased vigor of growth, survival, and fertility of hybrids as compared with the parental lines that were used to form the hybrid. Maximum heterosis is usually achieved by crossing two genetically different, highly inbred lines.

The production of hybrids is a well-developed industry, involving the isolated production of both the parental lines and the hybrids which result from crossing those lines. For a detailed discussion of the hybrid production process, see, e.g., Wright, *Commercial Hybrid Seed Production* 8:161-176, In Hybridization of Crop Plants.

Bulk Segregation Analysis (BSA)

BSA, a.k.a. bulked segregation analysis, or bulk segregant analysis, is a method described by Michelmore et al. (Michelmore et al., 1991, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National*

Academy of Sciences, USA, 99:9828-9832) and Quarrie et al. (Quarrie et al., Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize, 1999, *Journal of Experimental Botany*, 50(337):1299-1306).

For BSA of a trait of interest, parental lines with certain different phenotypes are chosen and crossed to generate F_2, doubled haploid or recombinant inbred populations with QTL analysis. The population is then phenotyped to identify individual plants or lines having high or low expression of the trait. Two DNA bulks are prepared, one from the individuals having one phenotype (e.g., resistant to pathogen), and the other from the individuals having reversed phenotype (e.g., susceptible to pathogen), and analyzed for allele frequency with molecular markers. Only a few individuals are required in each bulk (e.g., 10 plants each) if the markers are dominant (e.g., RAPDs). More individuals are needed when markers are co-dominant (e.g., RFLPs). Markers linked to the phenotype can be identified and used for breeding or QTL mapping.

Gene Pyramiding

The method to combine into a single genotype a series of target genes identified in different parents is usually referred as gene pyramiding. The first part of a gene pyramiding breeding is called a pedigree and is aimed at cumulating one copy of all target genes in a single genotype (called root genotype). The second part is called the fixation steps and is aimed at fixing the target genes into a homozygous state, that is, to derive the ideal genotype (ideotype) from the root genotype. Gene pyramiding can be combined with marker assisted selection (MAS, see Hospital et al., 1992, 1997a, and 1997b, and Moreau et al, 1998) or marker based recurrent selection (MBRS, see Hospital et al., 2000).

Examples of additional desired traits that may be stacked with the pathogen resistance or tolerance traits disclosed herein

In some cases, multiple FIT1 alleles may be combined in a single plant to increase pathogen resistance. In some cases, one or more FIT1 alleles are combined with additional desired traits. These traits may be introduced to a plant through conventional breeding methods, stacked on one or more DNA constructs, and/or generated through targeted mutagenesis. Examples of additional desired traits include, but are not limited to, male sterility, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. Several of these traits are described in, for example, U.S. Pat. Nos. 5,959,185, 5,973,234, and 5,977,445.

Examples of plant species that may be transformed or modified, or serve as a source of functional FIT1

The methods disclosed herein may be applied to a wide range of plants. Non-limiting examples of plants which may be transformed or modified using the methods and sequences disclosed herein include, but are not limited to, corn (*Zea mays*), *Brassica* spp. (e.g., *Brassica napus, Brassica rapa, Brassica juncea*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), broad beans (*Vicia faba*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), quince (*Cydonia*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), apple (*Malus* spp.), medlar (*Mespilus*), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), pear (*Pyrus*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats (for example *Avena sativa*), barley (for example *Hordeum vulgare*), vegetables and herbs (for example onion, leek, garlic peppermint), ornamentals (for example, Chrysanthemum, *Fuchsia* spp., Pelargonium, *Rosa* spp. *Primula vulgaris*), red cedar (*Juniperus virginiana*), and conifers (for example juniper (*Juniperus communis*)).

Examples of plants in the subfamily Papilionoideae include, but are not limited to, *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., *Strophostyles* spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

Examples of legumes include, but are not limited to, the genus *Phaseolus* (e.g., French bean, dwarf bean, climbing bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*),

Tepary bean (*Phaseolus acutifolius*), runner bean (*Phaseolus coccineus*)); the genus *Glycine* (e.g., *Glycine soja*, soybeans (*Glycine max* (L.)); pea (*Pisum*) (e.g., shelling peas (sometime called smooth or round seeded peas; *Pisum sativum*); marrowfat pea (*Pisum sativum*), sugar pea (*Pisum sativum*), also called snow pea, edible-podded pea or mangetout, (*Pisum granda*)); peanut (*Arachis hypogaea*), clover (*Trifolium* spp.), medick (*Medicago*), kudzu vine (*Pueraria lobata*), common lucerne, alfalfa (*Medicago sativa*), chickpea (*Cicer*), lentils (*Lens culinaris*), lupins (*Lupinus*); vetches (*Vicia*), field bean, broad bean (*Vicia faba*), vetchling (*Lathyrus*) (e.g., chickling pea (*Lathyrus sativus*), heath pea (*Lathyrus tuberosus*)); genus *Vigna* (e.g., moth bean (*Vigna aconitifolia*), adzuki bean (*Vigna angu laris*), urd bean (*Vigna mungo*), mung bean (*Vigna radiata*), bambara groundnut (Vigna subterrane), rice bean (*Vigna umbellata*), *Vigna vexillata*, *Vigna unguiculata* (also known as asparagus bean, cowpea)); pigeon pea (*Cajanus cajan*), the genus *Macrotyloma* (e.g., geocarpa groundnut (*Macro tyloma geocarpum*), horse bean (*Macrotyloma uniflorum*; goa bean (*Psophocarpus tetragonolobus*), African yam bean (*Sphenostylis stenocarpa*), Egyptian black bean, lablab bean *(Lablab purpureus*), yam bean (*Pachyrhizus erosus*), guar bean (*Cyamopsis tetragonolobus*); and/or the genus *Canavalia* (e.g., jack bean (*Canavalia ensiformis*)), sword bean (*Canavalia gladiata*).

EXAMPLES

The following examples are provided to illustrate further the various applications and are not intended to limit the disclosure beyond the limitations set forth in the appended claims.

Example 1: FIT1 homologs

As will be understood by one skilled in the art, homologs of FIT1 may be found in any number of species by methods described herein and methods well known in the art. Examples of homologs of FIT1 identified are shown in **Figures 3A-3C**. **Figure 3A** shows a phylogenetic tree of homologs of *Vigna unguiculata* FIT1 identified by performing a BLAST® search and constructing a protein alignment and phylogenetic tree of the resulting sequences. This figure shows putative FIT1 orthologs in *Vigna radiata*, *Vigna angularis*, *Phaseolus acutifolius, Phaseolus lunatus*, *Phaseolus vulgaris*, *Lablab purpureus, Mucuna pruriens*, *Cajanus cajan*, and *Abrus precatorius*. The phylogenetic tree was rooted using paralogs of FIT1 that do not function in AvrFIT1 perception.

Figure 3B shows a protein alignment of the amino acid sequences listed in **Figure 3A**, specifically of the *Vigna unguiculata* allele of FIT1 (VuFIT1) (SEQ ID NO: 2), the

Vigna unguiculata close paralog of FIT1 (VuFIT1b) (SEQ ID NO: 22), *Vigna angularis* allele of FIT1 (VaFIT1) (SEQ ID NO: 12), the *Vigna radiata* allele of FIT1 (VrFIT1) (SEQ ID NO: 4), the *Phaseolus acutifolius* allele of FIT1 (PaFIT1) (SEQ ID NO: 10), the *Phaseolus vulgaris* allele of FIT1 (PvFIT1) (SEQ ID NO: 8), the *Phaseolus lunatus* allele of FIT1 (PlFIT1) (SEQ ID NO: 6), the *Lablab purpureus* allele of FIT1 (LpFIT1) (SEQ ID NO: 14), the *Cajanus cajun* allele of FIT1 (CcFIT1) 1 (SEQ ID NO: 18), the *Mucuna pruriens* allele of FIT1 (MpFIT1) (SEQ ID NO: 16), and the *Abrus precatorius* allele of FIT1 (ApFIT1) (SEQ ID NO: 20).

Figure 3C shows a phylogenetic tree of FIT1 homologs in *Glycine max*. *G. max* (soybean) lacks an ortholog of VuFIT1 but contains many homologs of VuFIT1 paralogs, which can be identified by BLAST® search. Protein homologs of VuFIT1 from soybean were obtained from NCBI and Phytozome, curated for completeness and to remove duplicates, and used to generate the phylogenetic tree shown in **Figure 3C**. The tree was rooted using an outgroup of distantly related TIR-NLR proteins from non-legumes.

Sequence alignments of FIT1 homologs with functional FIT1 genes (such as VuFIT1) can show what genetic changes could be induced to restore recognition of AvrFIT, and confer resistance or tolerance to a pathogen. Such targeted genetic editing is well known in the art and also described herein.

Example 2: Transient expression of FIT1 alleles in leaf tissue

Leaf tissue from a plant lacking an endogenous FIT1 was transformed with constructs containing various FIT1 sequences, AvrFIT1a, AvrFIT1b, and/or Bs3 using standard transformation technology (an example construct comprising VuFIT1 is shown in Figure 4). Suspensions containing the desired expression constructs were infiltrated into the leaf tissue (OD_{600} = 0.4 total) using needleless syringe and imaged four days post infiltration. The site of each infiltration is visible by a small punch through the leaf.

As shown in **Figures 5A** and **5B,** co-expression of AvrFIT1a (SEQ ID NO: 23) with either *Vigna unguiculata* (VuFIT1) (SEQ ID NO: 27), *Phaseolus lunatus* (PlFIT1) (SEQ ID NO: 28), *Vigna radiata* (VrFIT1) (SEQ ID NO: 29), *Vigna angularis* (VaFIT1) (SEQ ID NO: 31), *Phaseolus vulgaris* (PvFIT1) (SEQ ID NO: 32), *Lablab purpureus* (LpFIT1) (SEQ ID NO: 33), or *Abrus precatoris* (ApFIT1) (SEQ ID NO: 34) resulted in a strong cell death response, indicative of immune activation, but no response was observed when the proteins were expressed individually. No immune response was observed when the non-functional paralog VuFIT1b (SEQ ID NO: 30) was expressed individually or with AvrFIT1a.

As shown in Figures 5C and 5D, co-expression of AvrFIT1b (SEQ ID NO: 25) with either *Phaseolus lunatus* (PlFIT1), *Vigna radiata* (VrFIT1), *Vigna angularis* (VaFIT1), *Phaseolus vulgaris* (PvFIT1), or *Lablab purpureus* (LpFIT1) resulted in a cell death response, indicative of immune activation, but no response was observed when the proteins were expressed individually.

Expression of the executor Bs3, a positive control for cell death response, triggers a similar response. This demonstrates that FIT1 mediates the perception of AvrFIT1, and thus can confer resistance or tolerance to pathogens that secrete the effector protein AvrFIT1.

Example 3: Stable expression of *Vigna unguiculata* FIT1 in *Glycine max*

Soybean plants stably expressing VuFIT1 (SEQ ID NO: 1, example construct shown in Figure 4) were generated and tested for resistance to ASR (*Phakopsora pachyrhizi*). **Figures 6A-6D** depicts wild type soybean leaves lacking functional FIT1 (top row) and **Figures 6E-6H** show leaves from soybean plants expressing VuFIT1 (bottom row). Plants were inoculated with *Phakopsora pachyrhizi* and the leaves were photographed at 13-days (**Figures 6A-6B** and **6E-6F**) and 44-days (**Figures 6C-6D** and **6G-6H**) post inoculation. The wild type soybean leaves showed susceptibility to *Phakopsora* as seen by the development of large lesions and many fungal spores. However, soybean expressing VuFIT1 showed strong resistance to the pathogen (**Figures 6E-6H**).

Transgenic expression of VuFIT1 in soybean did not affect plant growth or morphology. Photographs of wild type soybean plants (**Figure 7A**) and transgenic soybean plants expressing VuFIT1 (**Figure 7B**) had no obvious growth abnormalities. The height of the plants was measured at 24 days after planting and no significant difference was observed between the wild type plants and the plants containing FIT1 (**Figure 7C**). The error bars indicate the standard deviation of the plant height from the individual plants (n>8).

Example 4: Introducing FIT1 homologs into other plant species

The FIT1 sequences isolated and described herein can be introduced into other plant species to create a plant having resistance or tolerance to a pathogen.

For example, a sequence encoding any one of the proteins of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof, or sequences of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto can be introduced into a plant to confer resistance or tolerance to a pathogen. For example, as described above, *Glycine max*, which does not possess a functional FIT1 gene and is susceptible to ASR, may be transformed with a transgene comprising SEQ ID

NO: 1, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2, to confer resistance to plant pathogens, such as *Phakopsora pachyrhizi*, that secrete the effector protein AvrFIT1, and cause diseases like ASR. Based on the transient assays described herein (**Fig. 5A-5D**), resistance could also be achieved with a transgene comprising any one of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, and/or 19, or a sequence encoding any one of the proteins of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, and/or 20.

Additional plant species susceptible to ASR or pathogens secreting AvrFIT1-like effector proteins, such as *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp. could also be transformed with any of the FIT1 sequences disclosed herein to confer resistance to a pathogen.

Example 5: Methods of identifying pathogen resistant genes

FIT1 orthologs are likely present in additional species and genera within the Fabaceae family. FIT1 orthologs may identified by any number of means known in the art. This includes sequencing the genome or transcriptome of a plant species, identifying FIT1 homologs using a BLAST search, identifying putative FIT1 orthologs by constructing a phylogenetic tree of the homologous proteins, and then testing the identified putative FIT1 genes for AvrFIT1 recognition activity using a transient assay such as that shown in **Figure 5A-5D**. Alternatively, synthetic alleles of FIT1 may be designed by combining fragments of naturally occurring FIT1 alleles or by introducing amino acid substitutions at positions shown to be variable in an alignment of functional FIT1 proteins and similarly tested for functionality by transient assay. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (*Adv. Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet *et al.* (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang *et al.* (*Comp. Appls Biosci.*, 8:155-65, 1992); and Pearson *et al.* (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul *et al.* (*Nature Genet.*, 6:119-29,

1994) presents a detailed consideration of sequence alignment methods and homology calculations.

While previous reports have listed some FIT1 homologs as predicted TMV resistance proteins, this is unlikely. **Figure 8** depicts an alignment between the FIT1 alleles from *Vigna unguiculata* (VuFIT1), *Phaseolus lunatus* (PlFIT1), *Abrus precatorius* (ApFIT1), and the N gene, which gives TMV resistance. The LRR domain is poorly conserved between the FIT1 alleles and the N gene. Therefore, FIT1 is not expected to have the same activity as the N gene (which recognizes the P50 protein and confers resistance to Tobacco Mosaic Virus). This prediction can be confirmed by transient expression of the proteins, which demonstrates that the N gene can recognize P50 but not AvrFIT1a or AvrFITb and that FIT1 is not able to recognize P50.

The ability of other potential FIT1 homologs or synthetic genes to function for AvrFIT1 perception can be easily and quickly tested using the transient expression assays shown and described herein or similar methods well known in the art. For example, once a FIT1 ortholog is identified in a plant species that is resistant to ASR, the gene can be cloned and tested for effector protein recognition using transient expression assays and the AvrFIT1a (SEQ ID NO: 23) and AvrFIT1b sequences (SEQ ID NO: 25) described herein (see for example **Figures 5A-D**). Examples of leaf tissue suitable for use in a FIT1-AvrFIT1 assay include, but are not limited to, species or accessions of *Nicotiana, Solanum, Physalis, Capsicum, Lactuca, Alysicarpus, Astragalus, Baptisia, Cajanus, Calopogonium, Caragana, Centrosema, Cologania, Crotalaria, Desmodium, Genista, Glycine, Glycyrrhiza, Indigofera, Kummerowia, Lablab, Lathyrus, Lespedeza, Lotus, Lupinus, Macroptilium, Macrotyloma, Medicago, Neonotonia, Pachyrhizus, Pisum, Phaseolus, Pseudovigna, Psoralea, Robinia, Senna, Sesbania, Strophostyles, Tephrosia, Teramnus, Trifolium, Vicia, Vigna*, and *Voandzeia* that lack a functional native FIT1 gene. Similarly, resistance genes for other pathogens may be identified using this same method, wherein a potential gene is identified, cloned, and tested in transient assays with pathogen effector proteins.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

SEQ ID NO: 1 shows the nucleic acid sequence of *Vigna unguiculat*a FIT1.

SEQ ID NO: 2 shows the corresponding amino acid sequence of SEQ ID NO: 1.

SEQ ID NO: 3 shows the nucleic acid sequence of *Vigna radiata* FIT1.

SEQ ID NO: 4 shows the corresponding amino acid sequence of SEQ ID NO: 3.

SEQ ID NO: 5 shows the nucleic acid sequence of *Phaseolus lunatus* FIT1.

SEQ ID NO: 6 shows the corresponding amino acid sequence of SEQ ID NO: 5.

SEQ ID NO: 7 shows the nucleic acid sequence of *Phaseolus vulgaris* FIT1.

SEQ ID NO: 8 shows the corresponding amino acid sequence of SEQ ID NO: 7.

SEQ ID NO: 9 shows the nucleic acid sequence of *Phaseolus acutifolius* FIT1.

SEQ ID NO: 10 shows the corresponding amino acid sequence of SEQ ID NO: 9.

SEQ ID NO: 11 shows the nucleic acid sequence of *Vigna angularis* FIT1.

SEQ ID NO: 12 shows the corresponding amino acid sequence of SEQ ID NO: 11.

SEQ ID NO: 13 shows the nucleic acid sequence of *Lablab purpureus* FIT1

SEQ ID NO: 14 shows the corresponding amino acid sequence of SEQ ID NO: 13.

SEQ ID NO: 15 shows the nucleic acid sequence of *Mucuna pruriens* FIT1.

SEQ ID NO: 16 shows the corresponding amino acid sequence of SEQ ID NO: 15.

SEQ ID NO: 17 shows the nucleic acid sequence of *Cajanus cajun* FIT1.

SEQ ID NO: 18 shows the corresponding amino acid sequence of SEQ ID NO: 17.

SEQ ID NO: 19 shows the nucleic acid sequence of *Abrus precatorius* FIT1.

SEQ ID NO: 20 shows the corresponding amino acid sequence of SEQ ID NO: 19.

SEQ ID NO: 21 shows the nucleic acid sequence of *Vigna unguiculat*a FIT1b.

SEQ ID NO: 22 shows the corresponding amino acid sequence of SEQ ID NO: 21.

SEQ ID NO: 23 shows the nucleic acid sequence of *Phakopsora pachyrhizi* ALL40704.1 (AvrFIT1a).

SEQ ID NO: 24 shows the corresponding amino acid sequence of SEQ ID NO: 23.

SEQ ID NO: 25 shows the nucleic acid sequence of *Phakopsora pachyrhizi* ALL41167.1 (AvrFIT1b).

SEQ ID NO: 26 shows the corresponding amino acid sequence of SEQ ID NO: 25.

SEQ ID NO: 27 shows the nucleic acid sequence of the transient expression vector with *Vigna unguiculata* FIT1.

SEQ ID NO: 28 shows the nucleic acid sequence of the transient expression vector with *Phaseolus lunatus* FIT1.

SEQ ID NO: 29 shows the nucleic acid sequence of the transient expression vector with *Vigna radiata* FIT1.

SEQ ID NO: 30 shows the nucleic acid sequence of the transient expression vector with *Vigna unguiculata* FIT1b.

SEQ ID NO: 31 shows the nucleic acid sequence of the transient expression vector with *Vigna angularis* FIT1.

SEQ ID NO: 32 shows the nucleic acid sequence of the transient expression vector with *Phaseolus vulgaris* FIT1.

SEQ ID NO: 33 shows the nucleic acid sequence of the transient expression vector with *Lablab purpureus* FIT1.

SEQ ID NO: 34 shows the nucleic acid sequence of the transient expression vector with *Abrus precatorius* FIT1.

All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Numbered Embodiments

1. An isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a functional FIT1 protein homologous to SEQ ID NO: 2.
2. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 70% identity to SEQ ID NO: 2.
3. The isolated, recombinant, or synthetic polynucleotide of embodiment 2, wherein the polynucleotide encodes a FIT1 protein from *Abrus precatorius*.
4. The isolated, recombinant, or synthetic polynucleotide of embodiment 3, wherein the polynucleotide comprises SEQ ID NO: 19, a polynucleotide encoding SEQ ID NO: 20, complements thereof, or fragments thereof.
5. The isolated, recombinant, or synthetic polynucleotide of embodiment 2, wherein the polynucleotide encodes a FIT1 protein from *Cajanus cajan*.
6. The isolated, recombinant, or synthetic polynucleotide of embodiment 5, wherein the polynucleotide comprises SEQ ID NO: 17, or a polynucleotide encoding SEQ ID NO: 18.

7. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 75% identity to SEQ ID NO: 2.

8. The isolated, recombinant, or synthetic polynucleotide of embodiment 7, wherein the polynucleotide encodes a FIT1 protein from *Mucuna pruriens*.

9. The isolated, recombinant, or synthetic polynucleotide of embodiment 8, wherein the polynucleotide comprises SEQ ID NO: 15, a polynucleotide encoding SEQ ID NO: 16, complements thereof, or fragments thereof.

10. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 80% identity to SEQ ID NO: 2.

11. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 85% identity to SEQ ID NO: 2.

12. The isolated, recombinant, or synthetic polynucleotide of embodiment 11, wherein the polynucleotide encodes a FIT1 protein from *Lablab purpureus*.

13. The isolated, recombinant, or synthetic polynucleotide of embodiment 12, wherein the polynucleotide comprises SEQ ID NO: 13, a polynucleotide encoding SEQ ID NO: 14, complements thereof, or fragments thereof.

14. The isolated, recombinant, or synthetic polynucleotide of embodiment 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus lunatus*.

15. The isolated, recombinant, or synthetic polynucleotide of embodiment 14, wherein the polynucleotide comprises SEQ ID NO: 5, a polynucleotide encoding SEQ ID NO: 6, complements thereof, or fragments thereof.

16. The isolated, recombinant, or synthetic polynucleotide of embodiment 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus vulgaris*.

17. The isolated, recombinant, or synthetic polynucleotide of embodiment 16, wherein the polynucleotide comprises SEQ ID NO: 7, a polynucleotide encoding SEQ ID NO: 8, complements thereof, or fragments thereof.

18. The isolated, recombinant, or synthetic polynucleotide of embodiment 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus acutifolius*.

19. The isolated, recombinant, or synthetic polynucleotide of embodiment 18, wherein the polynucleotide comprises SEQ ID NO: 9, a polynucleotide encoding SEQ ID NO: 10, complements thereof, or fragments thereof.

20. The isolated, recombinant, or synthetic polynucleotide of embodiment 11, wherein the polynucleotide encodes a FIT1 protein from *Vigna radiata*.

21. The isolated, recombinant, or synthetic polynucleotide of embodiment 20, wherein the polynucleotide comprises SEQ ID NO: 3, a polynucleotide encoding SEQ ID NO: 4, complements thereof, or fragments thereof.

22. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 90% identity to SEQ ID NO: 2.

23. The isolated, recombinant, or synthetic polynucleotide of embodiment 22, wherein the polynucleotide encodes a FIT1 protein from *Vigna angularis*.

24. The isolated, recombinant, or synthetic polynucleotide of embodiment 23, wherein the polynucleotide comprises SEQ ID NO: 11, a polynucleotide encoding SEQ ID NO: 12, complements thereof, or fragments thereof.

25. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 95% identity to SEQ ID NO: 2.

26. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 96% identity to SEQ ID NO: 2.

27. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 97% identity to SEQ ID NO: 2.

28. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 98% identity to SEQ ID NO: 2.

29. The isolated, recombinant, or synthetic polynucleotide of embodiment 1, wherein the polynucleotide encodes a protein having at least 99% identity to SEQ ID NO: 2.

30. An isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof.

31. The isolated, recombinant, or synthetic polynucleotide of embodiment 30, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto.

32. A genetic construct comprising at least one of the nucleic acid sequences of any one of embodiments 1-31.

33. A plant, plant part, or plant cell transformed with at least one of the nucleic acid sequences of any one of embodiments 1-31 or the genetic construct of embodiment 32, wherein said plant, plant part or plant cell is resistant or tolerant to a pathogen.

34. The plant, plant part, or plant cell of embodiment 33, wherein the pathogen is a fungus from the order Cantharellales or Pucciniales.

35. The plant, plant part, or plant cell of embodiment 33 or 34, wherein the fungal pathogen is *Rhizoctonia solani*, *Melampsora spp.*, *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora spp.*, *Puccinia* spp., *Uromyces spp.*, *Austropuccinia spp.*, *Cronartium spp.* or *Hemileia vastatrix*.

36. The plant, plant part, or plant cell of any one of embodiments 33-35, wherein the plant, plant part, or plant cell is in the subfamily Papilionoideae.

37. The plant, plant part, or plant cell of any one of embodiments 33-36, wherein the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

38. The plant, plant part, or plant cell of any one of embodiments 33-37, wherein the plant, plant part, or plant cell is *Glycine max,* and wherein the plant, plant part, or plant cell is resistant to Asian Soybean Rust caused by *Phakopsora pachyrhizi*.

39. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 1, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2.

40. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 3, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 4.

41. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 5, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 6.

42. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 7, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8.

43. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 9, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 10.

44. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 11, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 12.

45. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 13, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 14.

46. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 15, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 16.

47. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 17, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18.

48. The plant, plant part, or plant cell of embodiment 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 19, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 20.

49. A method of producing a plant, plant part, or plant cell having resistance or tolerance to a pathogen, wherein the method comprises:

transforming a plant, plant part, or plant cell with a nucleotide sequence encoding a Toll-like Interleukin-1 Receptor (TIR) Nucleotide binding, Leucine-rich Repeat (NLR) immune receptor protein, wherein said immune receptor protein mediates the perception of pathogen effector protein AvrFIT1 or homologs thereof; and

wherein expression of the immune receptor protein prevents the pathogen from colonizing the plant, or prevents the pathogen from affecting plant growth or yield.

50. The method of embodiment 49, wherein the pathogen effector protein comprises SEQ ID NO: 24, SEQ ID: 26, or sequences at least 90% identical thereto.

51. The method of embodiment 49 or 50, wherein the nucleotide sequence encoding the immune receptor protein has been codon optimized.

52. The method of any one of embodiments 49-51, wherein the immune receptor protein is selected from the group consisting of:

an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof, or

an isolated, recombinant, or synthetic polynucleotide encoding a FIT1 protein, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto.

53. The method of any one of embodiments 49-52, wherein the plant, plant part, or plant cell is transformed with one or more additional desired traits.

54. The method of embodiment 49, wherein the one or more additional desired traits are stacked together with the immune receptor protein on the same DNA construct.

55. The method of any one of embodiments 49-23, further comprising introgressing one or more additional desired traits.

56. The method of any one of embodiments 49-55, wherein the one or more additional desired traits are resistance traits to a disease, pest, or abiotic stress.

57. A plant, plant part, or plant cell produced by the method of any one of embodiments 49-56, wherein the plant, plant part, or plant cell is resistant to a pathogen.

58. A plant, plant part, or plant cell produced by the method of any one of embodiments 49-56, wherein the plant, plant part, or plant cell is tolerant to a pathogen.

59. The plant, plant part, or plant cell of embodiment 57 or 58, wherein the immune receptor protein is transiently expressed.

60. The plant, plant part, or plant cell of embodiment 57 or 58, wherein the immune receptor protein is stably expressed.

61. The plant, plant part, or plant cell of any one of embodiments 49-60, wherein the plant, plant part, or plant cell is in the subfamily Papilionoideae.

62. The plant, plant part, or plant cell of embodiment 61, wherein the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus*

spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

63. The plant, plant part, or plant cell of any one of embodiments 57-62, wherein the plant is *Glycine max,* wherein the plant, plant part, or plant cell is resistant to Asian Soybean Rust caused by *Phakopsora pachyrhizi.*

64. A method of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising:

providing a plant species that is susceptible to a pathogen;

identifying within the genome of the plant species a homolog of FIT1, wherein said homolog does not mediate AvrFIT1 recognition; and

genetically modifying a plant, plant part, or plant cell from the susceptible plant species with targeted gene editing, wherein said targeted gene editing is directed at the FIT1 homolog, and wherein said targeted gene editing enables the FIT1 homolog to recognize AvrFIT1 and confers resistance or tolerance to a pathogen.

65. The method of embodiment 64, wherein the targeted gene editing uses an engineered or natural nuclease selected from the group consisting of homing endonucleases/meganucleases (EMNs), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs).

66. The method of embodiment 64 or 65, wherein targeted gene editing uses a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas nuclease.

67. The method of embodiment 66, wherein the nuclease is selected from the group consisting of Cas9, Cas12, Cas13, CasX, and CasY.

68. The method of any one of embodiments 64-67, further comprising breeding with, or asexually propagating the plant.

69. A genetically modified plant, plant part, or plant cell produced by the method of any one of embodiments 64-68, wherein said plant, plant part, or plant cell exhibits resistance or tolerance to a pathogen.

70. The genetically modified plant, plant part, or plant cell of embodiment 69, wherein the pathogen is a fungus from the order Cantharellales or Pucciniales.

71. The genetically modified plant, plant part, or plant cell of embodiment 70, wherein the fungal pathogen is *Rhizoctonia solani*, *Melampsora spp.*, *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora spp.*, *Puccinia* spp., *Uromyces spp.*, *Austropuccinia spp.*, *Cronartium spp.* or *Hemileia vastatrix*.

72. The genetically modified plant, plant part, or plant cell of any one of embodiments 69-71, wherein the fungal pathogen is *Phakopsora pachyrhizi* and the plant, plant part, or plant cell is *Glycine max*.

73. A method for identifying a functional FIT1 gene and/or allele thereof comprising:

isolating a FIT1 homolog or allele thereof;

expressing all or a substantial fragment of said FIT1 homolog or allele thereof in combination with a homolog of AvrFIT1 in a plant, plant part, or plant cell; and

assaying said plant, plant part, or plant cell for an immune response.

74. The method of embodiment 73, wherein the effector protein comprises SEQ ID NO: 24, SEQ ID: 26, or sequences at least 90% identical thereto.

75. The method of embodiment 73 or 74, wherein the FIT1 allele is a synthetic variant.

76. The method of any one of embodiments 73-75, wherein the plant, plant part, or plant cell is a species of *Nicotiana*, *Solanum*, *Physalis*, *Capsicum*, *Lactuca*, *Alysicarpus*, *Astragalus*, *Baptisia*, *Cajanus*, *Calopogonium*, *Caragana*, *Centrosema*, *Cologania*, *Crotalaria*, *Desmodium*, *Genista*, *Glycine*, *Glycyrrhiza*, *Indigofera*, *Kummerowia*, *Lablab*, *Lathyrus*, *Lespedeza*, *Lotus*, *Lupinus*, *Macroptilium*, *Macrotyloma*, *Medicago*, *Neonotonia*, *Pachyrhizus*, *Pisum*, *Phaseolus*, *Pseudovigna*, *Psoralea*, *Robinia*, *Senna*, *Sesbania*, *Strophostyles*, *Tephrosia*, *Teramnus*, *Trifolium*, *Vicia*, *Vigna*, and *Voandzeia* that lacks a functional native FIT1 gene.

77. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 70% identical thereto.

78. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 80% identical thereto.

79. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 90% identical thereto.

80. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto.

81. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 70% identical thereto.

82. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 80% identical thereto.

83. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 90% identical thereto.

84. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence at least 90% identical thereto.

85. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 70% identical thereto.

86. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 80% identical thereto.

87. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 90% identical thereto.

88. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence at least 90% identical thereto.

89. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 70% identical thereto.

90. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 80% identical thereto.

91. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 90% identical thereto.

92. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence at least 90% identical thereto.

93. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 70% identical thereto.

94. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 80% identical thereto.

95. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 90% identical thereto.

96. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence at least 90% identical thereto.

97. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 70% identical thereto.

98. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 80% identical thereto.

99. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 90% identical thereto.

100. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 12, or an amino acid sequence at least 90% identical thereto.

101. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 70% identical thereto.

102. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 80% identical thereto.

103. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 90% identical thereto.

104. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence at least 90% identical thereto.

105. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 70% identical thereto.

106. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 80% identical thereto.

107. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 90% identical thereto.

108. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 16, or an amino acid sequence at least 90% identical thereto.

109. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 70% identical thereto.

110. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 80% identical thereto.

111. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 90% identical thereto.

112. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence at least 90% identical thereto.

113. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 70% identical thereto.

114. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 80% identical thereto.

115. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 90% identical thereto.

116. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 20, or an amino acid sequence at least 90% identical thereto.

CLAIMS:

1. An isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a functional FIT1 protein homologous to SEQ ID NO: 2.
2. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 70% identity to SEQ ID NO: 2.
3. The isolated, recombinant, or synthetic polynucleotide of claim 2, wherein the polynucleotide encodes a FIT1 protein from *Abrus precatorius*.
4. The isolated, recombinant, or synthetic polynucleotide of claim 3, wherein the polynucleotide comprises SEQ ID NO: 19, a polynucleotide encoding SEQ ID NO: 20, complements thereof, or fragments thereof.
5. The isolated, recombinant, or synthetic polynucleotide of claim 2, wherein the polynucleotide encodes a FIT1 protein from *Cajanus cajan*.
6. The isolated, recombinant, or synthetic polynucleotide of claim 5, wherein the polynucleotide comprises SEQ ID NO: 17, or a polynucleotide encoding SEQ ID NO: 18.
7. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 75% identity to SEQ ID NO: 2.
8. The isolated, recombinant, or synthetic polynucleotide of claim 7, wherein the polynucleotide encodes a FIT1 protein from *Mucuna pruriens*.
9. The isolated, recombinant, or synthetic polynucleotide of claim 8, wherein the polynucleotide comprises SEQ ID NO: 15, a polynucleotide encoding SEQ ID NO: 16, complements thereof, or fragments thereof.
10. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 80% identity to SEQ ID NO: 2.
11. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 85% identity to SEQ ID NO: 2.
12. The isolated, recombinant, or synthetic polynucleotide of claim 11, wherein the polynucleotide encodes a FIT1 protein from *Lablab purpureus*.
13. The isolated, recombinant, or synthetic polynucleotide of claim 12, wherein the polynucleotide comprises SEQ ID NO: 13, a polynucleotide encoding SEQ ID NO: 14, complements thereof, or fragments thereof.

14. The isolated, recombinant, or synthetic polynucleotide of claim 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus lunatus*.

15. The isolated, recombinant, or synthetic polynucleotide of claim 14, wherein the polynucleotide comprises SEQ ID NO: 5, a polynucleotide encoding SEQ ID NO: 6, complements thereof, or fragments thereof.

16. The isolated, recombinant, or synthetic polynucleotide of claim 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus vulgaris*.

17. The isolated, recombinant, or synthetic polynucleotide of claim 16, wherein the polynucleotide comprises SEQ ID NO: 7, a polynucleotide encoding SEQ ID NO: 8, complements thereof, or fragments thereof.

18. The isolated, recombinant, or synthetic polynucleotide of claim 11, wherein the polynucleotide encodes a FIT1 protein from *Phaseolus acutifolius*.

19. The isolated, recombinant, or synthetic polynucleotide of claim 18, wherein the polynucleotide comprises SEQ ID NO: 9, a polynucleotide encoding SEQ ID NO: 10, complements thereof, or fragments thereof.

20. The isolated, recombinant, or synthetic polynucleotide of claim 11, wherein the polynucleotide encodes a FIT1 protein from *Vigna radiata*.

21. The isolated, recombinant, or synthetic polynucleotide of claim 20, wherein the polynucleotide comprises SEQ ID NO: 3, a polynucleotide encoding SEQ ID NO: 4, complements thereof, or fragments thereof.

22. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 90% identity to SEQ ID NO: 2.

23. The isolated, recombinant, or synthetic polynucleotide of claim 22, wherein the polynucleotide encodes a FIT1 protein from *Vigna angularis*.

24. The isolated, recombinant, or synthetic polynucleotide of claim 23, wherein the polynucleotide comprises SEQ ID NO: 11, a polynucleotide encoding SEQ ID NO: 12, complements thereof, or fragments thereof.

25. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 95% identity to SEQ ID NO: 2.

26. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 96% identity to SEQ ID NO: 2.

27. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 97% identity to SEQ ID NO: 2.

28. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 98% identity to SEQ ID NO: 2.

29. The isolated, recombinant, or synthetic polynucleotide of claim 1, wherein the polynucleotide encodes a protein having at least 99% identity to SEQ ID NO: 2.

30. An isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof.

31. The isolated, recombinant, or synthetic polynucleotide of claim 30, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto.

32. A genetic construct comprising at least one of the nucleic acid sequences of claim 1.

33. A plant, plant part, or plant cell transformed with at least one of the nucleic acid sequences of claim 1, wherein said plant, plant part or plant cell is resistant or tolerant to a pathogen.

34. The plant, plant part, or plant cell of claim 33, wherein the pathogen is a fungus from the order Cantharellales or Pucciniales.

35. The plant, plant part, or plant cell of claim 34, wherein the fungal pathogen is *Rhizoctonia solani*, *Melampsora spp.*, *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora spp.*, *Puccinia* spp., *Uromyces spp.*, *Austropuccinia spp.*, *Cronartium spp.* or *Hemileia vastatrix*.

36. The plant, plant part, or plant cell of claim 34, wherein the plant, plant part, or plant cell is in the subfamily Papilionoideae.

37. The plant, plant part, or plant cell of claim 33, wherein the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum*

spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

38. The plant, plant part, or plant cell of claim 33, wherein the plant, plant part, or plant cell is *Glycine max,* and wherein the plant, plant part, or plant cell is resistant to Asian Soybean Rust caused by *Phakopsora pachyrhizi.*

39. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 1, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2.

40. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 3, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 4.

41. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 5, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 6.

42. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 7, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8.

43. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 9, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 10.

44. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 11, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 12.

45. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 13, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 14.

46. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 15, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 16.

47. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 17, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18.

48. The plant, plant part, or plant cell of claim 38, wherein the resistance to Asian Soybean Rust is conferred by a transgene comprising SEQ ID NO: 19, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 20.

49. A method of producing a plant, plant part, or plant cell having resistance or tolerance to a pathogen, wherein the method comprises:

transforming a plant, plant part, or plant cell with a nucleotide sequence encoding a Toll-like Interleukin-1 Receptor (TIR) Nucleotide binding, Leucine-rich Repeat (NLR) immune receptor protein, wherein said immune receptor protein mediates the perception of pathogen effector protein AvrFIT1 or homologs thereof; and

wherein expression of the immune receptor protein prevents the pathogen from colonizing the plant, or prevents the pathogen from affecting plant growth or yield.

50. The method of claim 49, wherein the pathogen effector protein comprises SEQ ID NO: 24, SEQ ID: 26, or sequences at least 90% identical thereto.

51. The method of claim 49, wherein the nucleotide sequence encoding the immune receptor protein has been codon optimized.

52. The method of claim 49, wherein the immune receptor protein is selected from the group consisting of:

an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a FIT1 protein, wherein the protein is selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and functional homologs thereof, or

an isolated, recombinant, or synthetic polynucleotide encoding a FIT1 protein, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 complements thereof, fragments thereof, and sequences at least 70% identical thereto.

53. The method of claim 49, wherein the plant, plant part, or plant cell is transformed with one or more additional desired traits.

54. The method of claim 49, wherein the one or more additional desired traits are stacked together with the immune receptor protein on the same DNA construct.

55. The method of claim 49, further comprising introgressing one or more additional desired traits.

56. The method of claim 49, wherein the one or more additional desired traits are resistance traits to a disease, pest, or abiotic stress.

57. A plant, plant part, or plant cell produced by the method of claim 49, wherein the plant, plant part, or plant cell is resistant to a pathogen.

58. A plant, plant part, or plant cell produced by the method of claim 49, wherein the plant, plant part, or plant cell is tolerant to a pathogen.

59. The plant, plant part, or plant cell of claim 57 or 58, wherein the immune receptor protein is transiently expressed.

60. The plant, plant part, or plant cell of claim 57 or 58, wherein the immune receptor protein is stably expressed.

61. The plant, plant part, or plant cell of claim 57, wherein the plant, plant part, or plant cell is in the subfamily Papilionoideae.

62. The plant, plant part, or plant cell of claim 61, wherein the plant, plant part, or plant cell is *Alysicarpus* spp., *Astragalus* spp., *Baptisia* spp., *Cajanus* spp., *Calopogonium* spp., *Caragana* spp., *Centrosema* spp., *Cologania* spp., *Crotalaria* spp., *Desmodium* spp., *Genista* spp., *Glycine* spp., *Glycyrrhiza* spp., *Indigofera* spp., *Kummerowia* spp., *Lablab* spp., *Lathyrus* spp., *Lespedeza* spp., *Lotus* spp., *Lupinus* spp., *Macroptilium* spp., *Macrotyloma* spp., *Medicago* spp., *Neonotonia* spp., *Pachyrhizus* spp., *Pisum* spp., *Phaseolus* spp., *Pseudovigna* spp., *Psoralea* spp., *Robinia* spp., *Senna* spp., *Sesbania* spp., Strophostyles spp., *Tephrosia* spp., *Teramnus* spp., *Trifolium* spp., *Vicia* spp., *Vigna* spp., or *Voandzeia* spp.

63. The plant, plant part, or plant cell of claim 57, wherein the plant is *Glycine max,* wherein the plant, plant part, or plant cell is resistant to Asian Soybean Rust caused by *Phakopsora pachyrhizi.*

64. A method of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising:

providing a plant species that is susceptible to a pathogen;

identifying within the genome of the plant species a homolog of FIT1, wherein said homolog does not mediate AvrFIT1 recognition; and

genetically modifying a plant, plant part, or plant cell from the susceptible plant species with targeted gene editing, wherein said targeted gene editing is directed at the FIT1 homolog, and wherein said targeted gene editing enables the FIT1 homolog to recognize AvrFIT1 and confers resistance or tolerance to a pathogen.

65. The method of claim 64, wherein the targeted gene editing uses an engineered or natural nuclease selected from the group consisting of homing endonucleases/meganucleases (EMNs), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs).

66. The method of claim 64, wherein targeted gene editing uses a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas nuclease.

67. The method of claim 66, wherein the nuclease is selected from the group consisting of Cas9, Cas12, Cas13, CasX, and CasY.

68. The method of claim 64, further comprising breeding with, or asexually propagating the plant.

69. A genetically modified plant, plant part, or plant cell produced by the method of claim 64, wherein said plant, plant part, or plant cell exhibits resistance or tolerance to a pathogen.

70. The genetically modified plant, plant part, or plant cell of claim 69, wherein the pathogen is a fungus from the order Cantharellales or Pucciniales.

71. The genetically modified plant, plant part, or plant cell of claim 70, wherein the fungal pathogen is *Rhizoctonia solani*, *Melampsora spp.*, *Phakopsora pachyrhizi*, *Phakopsora meibomiae*, *Phakopsora euvitis*, *Phakopsora spp.*, *Puccinia* spp., *Uromyces spp.*, *Austropuccinia spp.*, *Cronartium spp.* or *Hemileia vastatrix*.

72. The genetically modified plant, plant part, or plant cell of claim 69, wherein the fungal pathogen is *Phakopsora pachyrhizi* and the plant, plant part, or plant cell is *Glycine max*.

73. A method for identifying a functional FIT1 gene and/or allele thereof comprising:

isolating a FIT1 homolog or allele thereof;

expressing all or a substantial fragment of said FIT1 homolog or allele thereof in combination with a homolog of AvrFIT1 in a plant, plant part, or plant cell; and

assaying said plant, plant part, or plant cell for an immune response.

74. The method of claim 73, wherein the effector protein comprises SEQ ID NO: 24, SEQ ID: 26, or sequences at least 90% identical thereto.

75. The method of claim 73, wherein the FIT1 allele is a synthetic variant.

76. The method of claim 73, wherein the plant, plant part, or plant cell is a species of *Nicotiana, Solanum, Physalis, Capsicum, Lactuca, Alysicarpus, Astragalus, Baptisia, Cajanus, Calopogonium, Caragana, Centrosema, Cologania, Crotalaria, Desmodium, Genista, Glycine, Glycyrrhiza, Indigofera, Kummerowia, Lablab, Lathyrus, Lespedeza, Lotus, Lupinus, Macroptilium, Macrotyloma, Medicago, Neonotonia, Pachyrhizus, Pisum, Phaseolus, Pseudovigna, Psoralea, Robinia, Senna, Sesbania, Strophostyles, Tephrosia, Teramnus, Trifolium, Vicia, Vigna*, and *Voandzeia* that lacks a functional native FIT1 gene.

77. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 70% identical thereto.

78. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 80% identical thereto.

79. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 1, or a sequence at least 90% identical thereto.

80. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto.

81. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 70% identical thereto.

82. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 80% identical thereto.

83. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 3, or a sequence at least 90% identical thereto.

84. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence at least 90% identical thereto.

85. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 70% identical thereto.

86. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 80% identical thereto.

87. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 5, or a sequence at least 90% identical thereto.

88. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence at least 90% identical thereto.

89. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 70% identical thereto.

90. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 80% identical thereto.

91. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 7, or a sequence at least 90% identical thereto.

92. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence at least 90% identical thereto.

93. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 70% identical thereto.

94. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 80% identical thereto.

95. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 9, or a sequence at least 90% identical thereto.

96. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence at least 90% identical thereto.

97. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 70% identical thereto.

98. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 80% identical thereto.

99. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 11, or a sequence at least 90% identical thereto.

100. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 12, or an amino acid sequence at least 90% identical thereto.

101. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 70% identical thereto.

102. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 80% identical thereto.

103. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 13, or a sequence at least 90% identical thereto.

104. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence at least 90% identical thereto.

105. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 70% identical thereto.

106. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 80% identical thereto.

107. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 15, or a sequence at least 90% identical thereto.

108. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 16, or an amino acid sequence at least 90% identical thereto.

109. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 70% identical thereto.

110. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 80% identical thereto.

111. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 17, or a sequence at least 90% identical thereto.

112. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence at least 90% identical thereto.

113. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 70% identical thereto.

114. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 80% identical thereto.

115. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising SEQ ID NO: 19, or a sequence at least 90% identical thereto.

116. A transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 20, or an amino acid sequence at least 90% identical thereto.

Figure 1

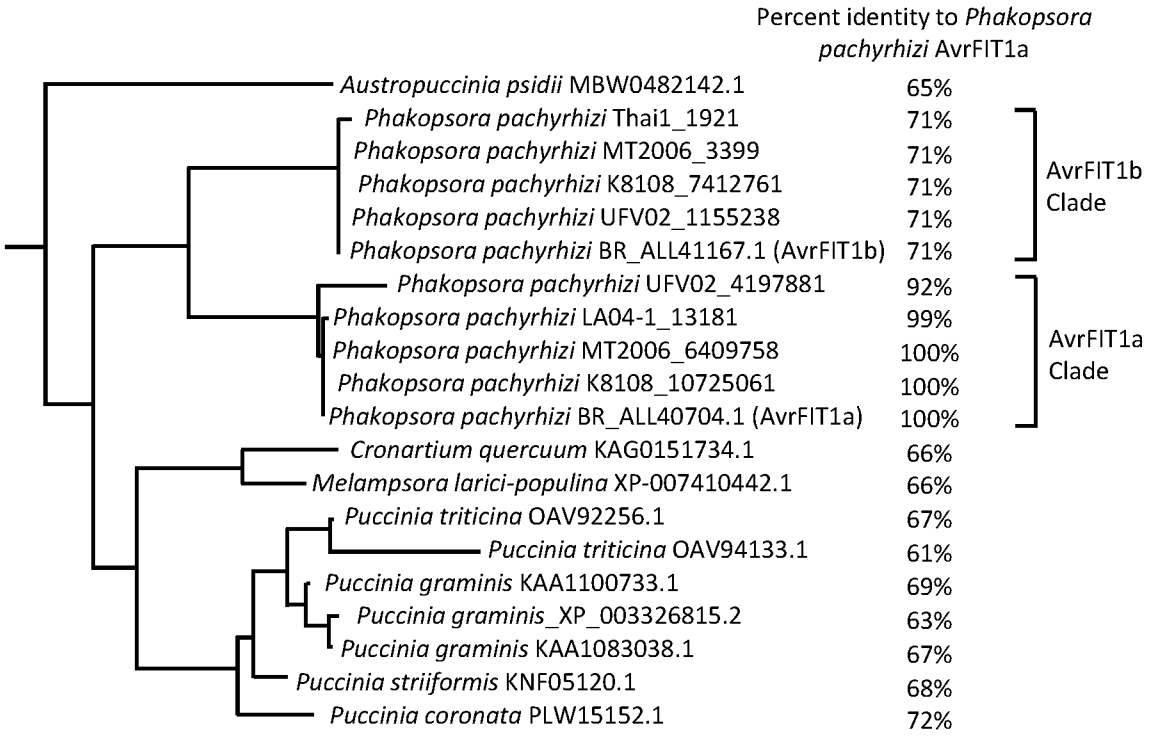

Percent identity to *Phakopsora pachyrhizi* AvrFIT1a
Austropuccinia psidii MBW0482142.1 65%
Phakopsora pachyrhizi Thai1_1921 71%
Phakopsora pachyrhizi MT2006_3399 71%
Phakopsora pachyrhizi K8108_7412761 71%
Phakopsora pachyrhizi UFV02_1155238 71%
Phakopsora pachyrhizi BR_ALL41167.1 (AvrFIT1b) 71%
AvrFIT1b Clade
Phakopsora pachyrhizi UFV02_4197881 92%
Phakopsora pachyrhizi LA04-1_13181 99%
Phakopsora pachyrhizi MT2006_6409758 100%
Phakopsora pachyrhizi K8108_10725061 100%
Phakopsora pachyrhizi BR_ALL40704.1 (AvrFIT1a) 100%
AvrFIT1a Clade
Cronartium quercuum KAG0151734.1 66%
Melampsora larici-populina XP-007410442.1 66%
Puccinia triticina OAV92256.1 67%
Puccinia triticina OAV94133.1 61%
Puccinia graminis KAA1100733.1 69%
*Puccinia graminis*_XP_003326815.2 63%
Puccinia graminis KAA1083038.1 67%
Puccinia striiformis KNF05120.1 68%
Puccinia coronata PLW15152.1 72%

Figure 2

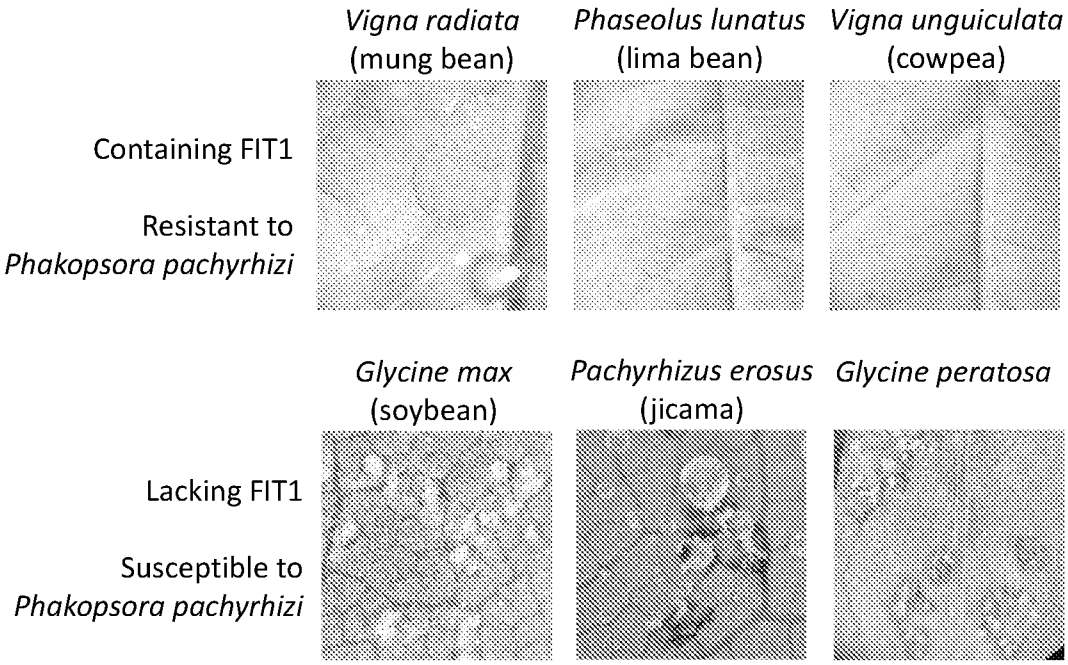
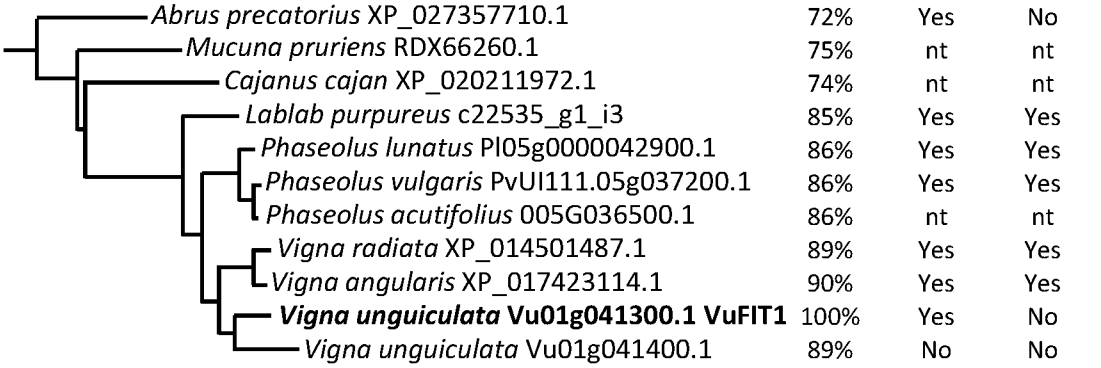


Figure 3A



	% identity to *Vu*FIT1	Recognition activity AvrFIT1a	AvrFIT1b
Abrus precatorius XP_027357710.1	72%	Yes	No
Mucuna pruriens RDX66260.1	75%	nt	nt
Cajanus cajan XP_020211972.1	74%	nt	nt
Lablab purpureus c22535_g1_i3	85%	Yes	Yes
Phaseolus lunatus PI05g0000042900.1	86%	Yes	Yes
Phaseolus vulgaris PvUI111.05g037200.1	86%	Yes	Yes
Phaseolus acutifolius 005G036500.1	86%	nt	nt
Vigna radiata XP_014501487.1	89%	Yes	Yes
Vigna angularis XP_017423114.1	90%	Yes	Yes
***Vigna unguiculata* Vu01g041300.1 VuFIT1**	100%	Yes	No
Vigna unguiculata Vu01g041400.1	89%	No	No

Figure 3B

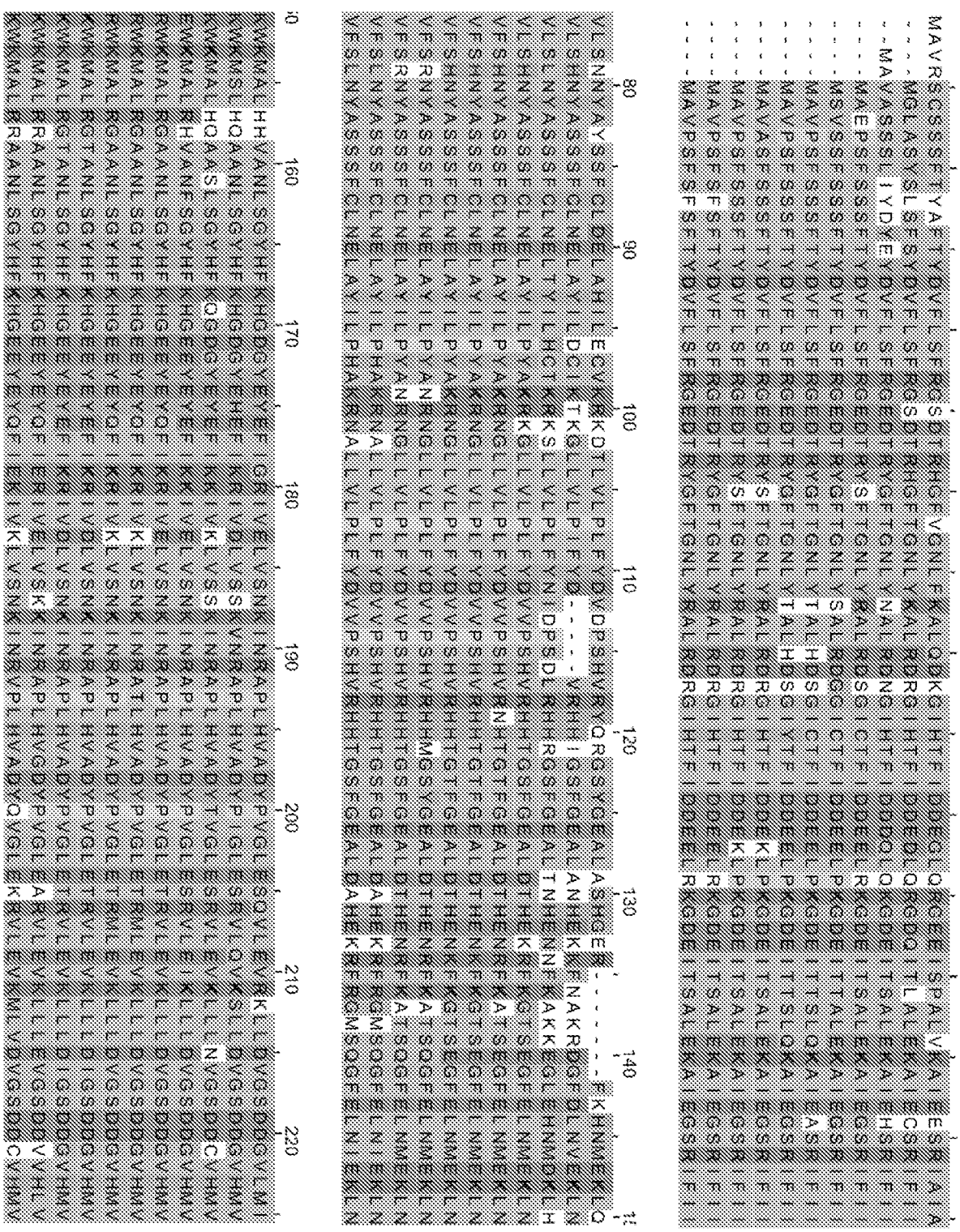

ApFIT1
MpFIT1
CcFIT1
LpFIT1
PlFIT1
PvFIT1
PaFIT1
VrFIT1
VaFIT1
VuFIT1
VuFIT1b

Figure 3B continued

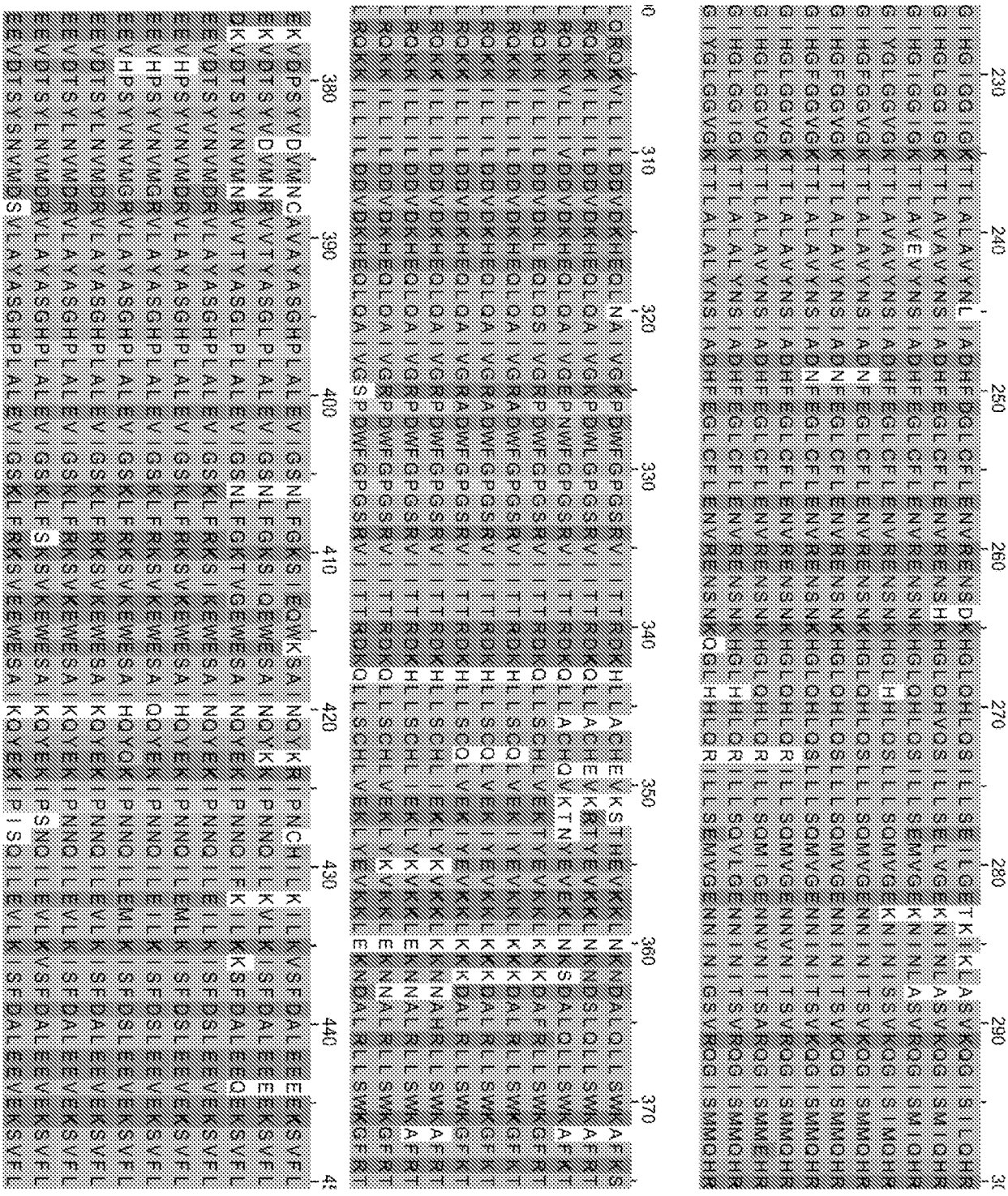


Figure 3B continued

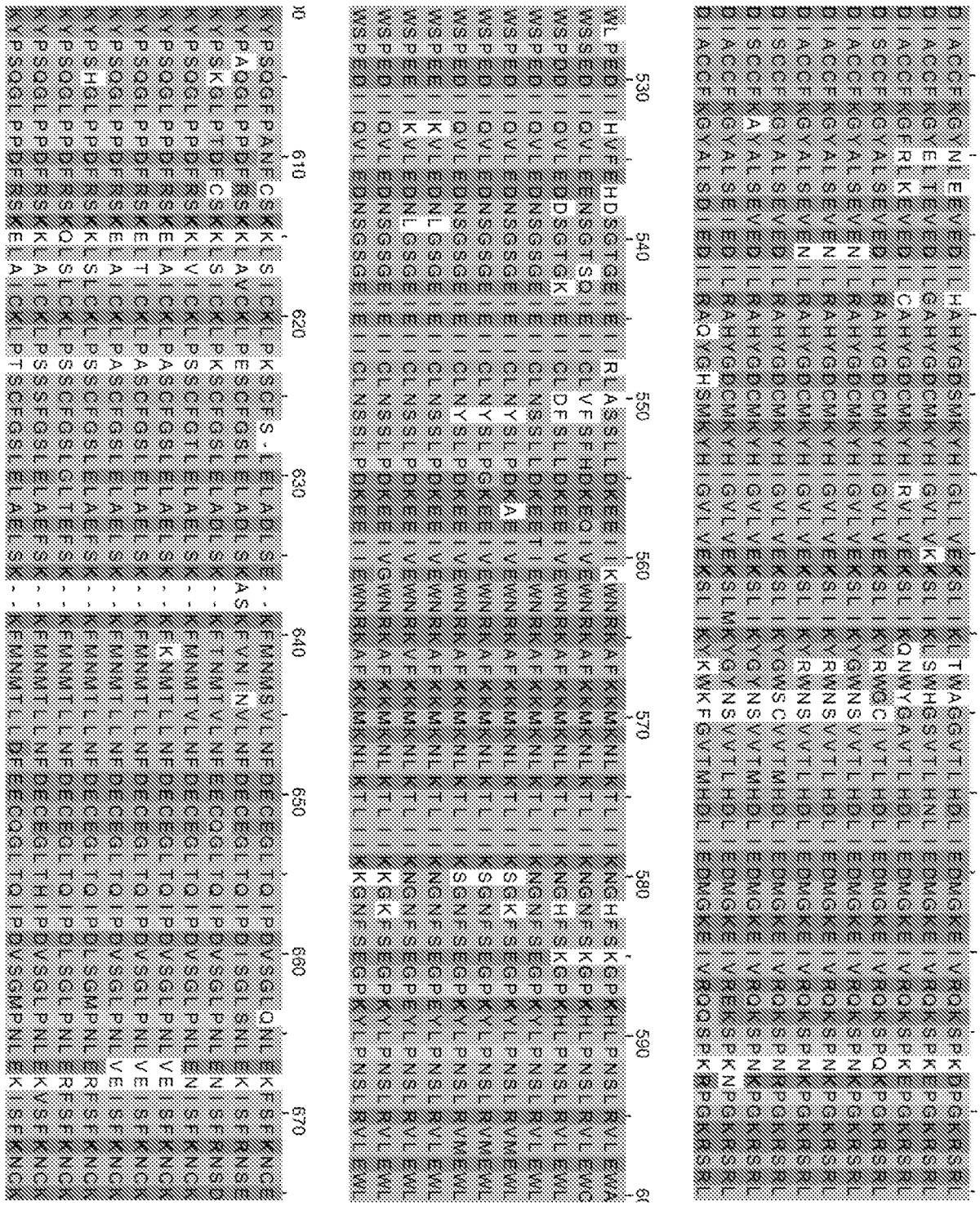


Figure 3B continued

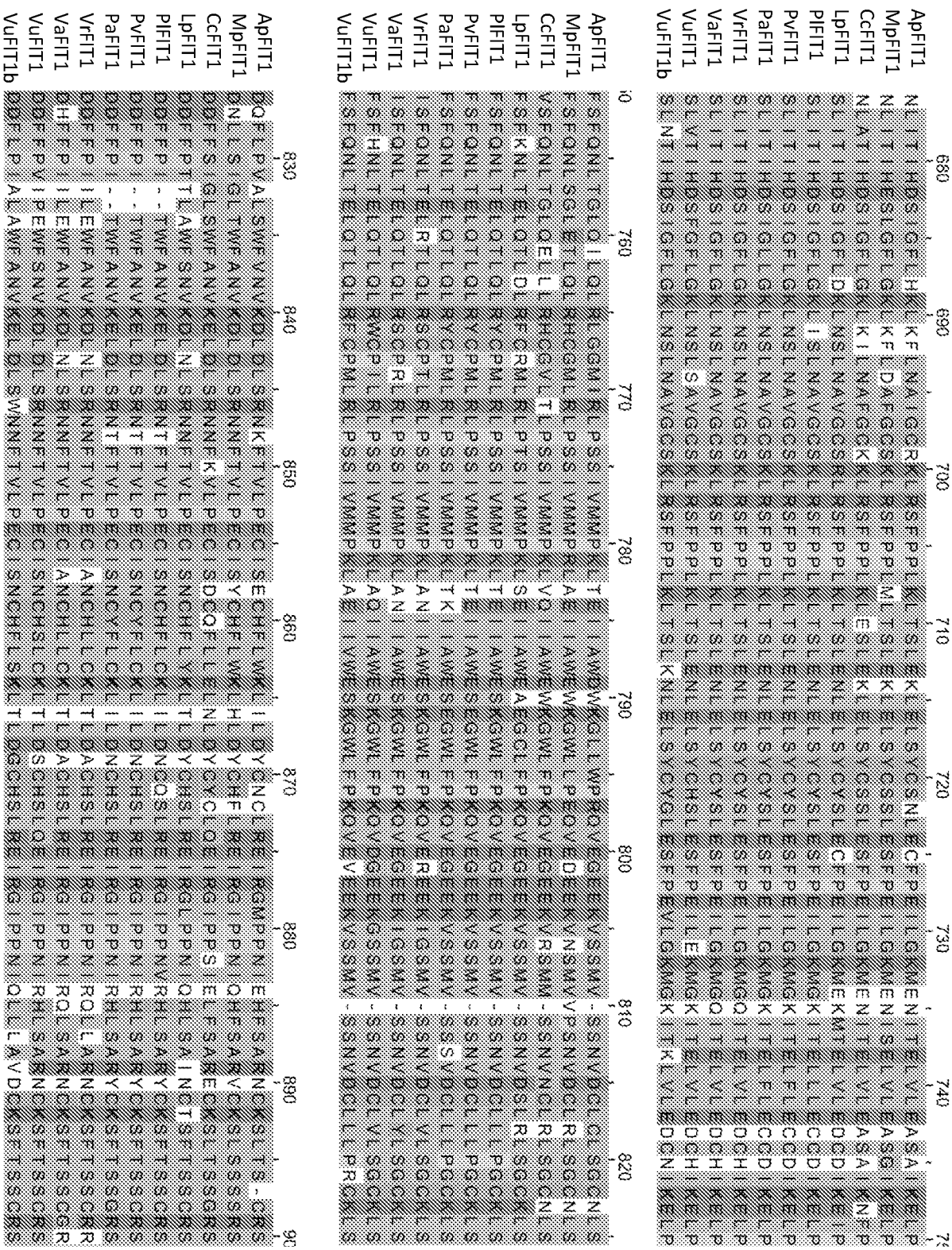


Figure 3B continued

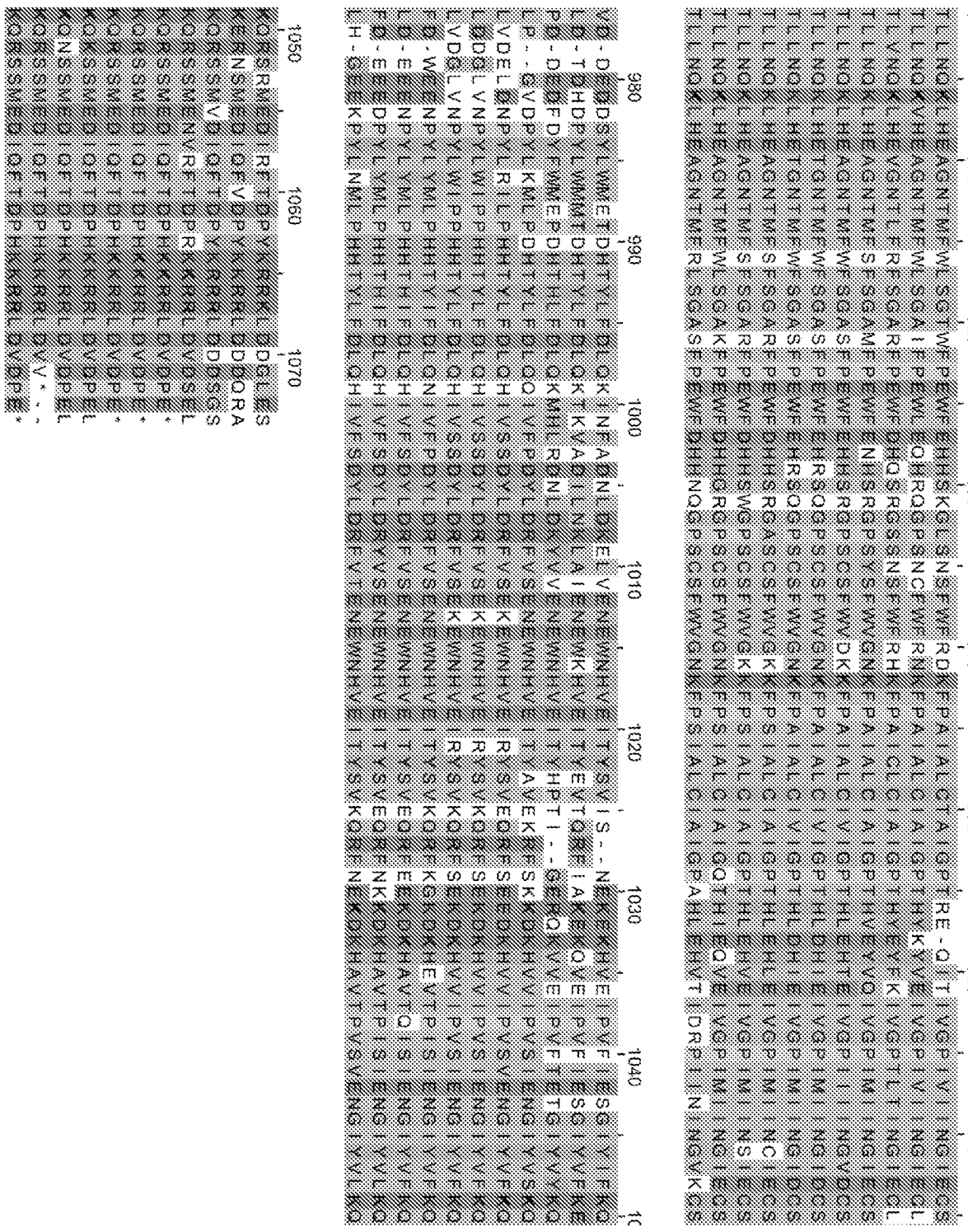


Figure 3C

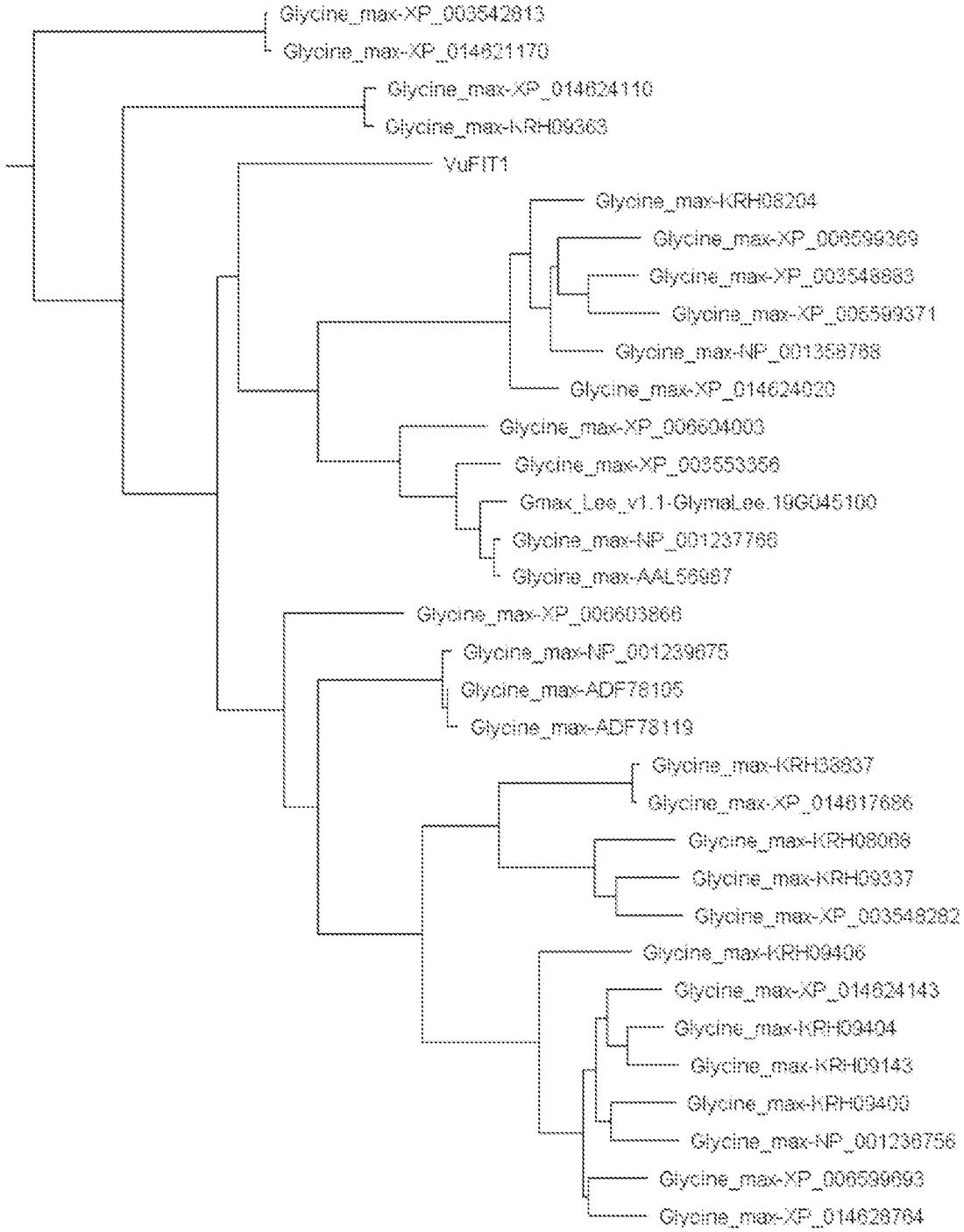
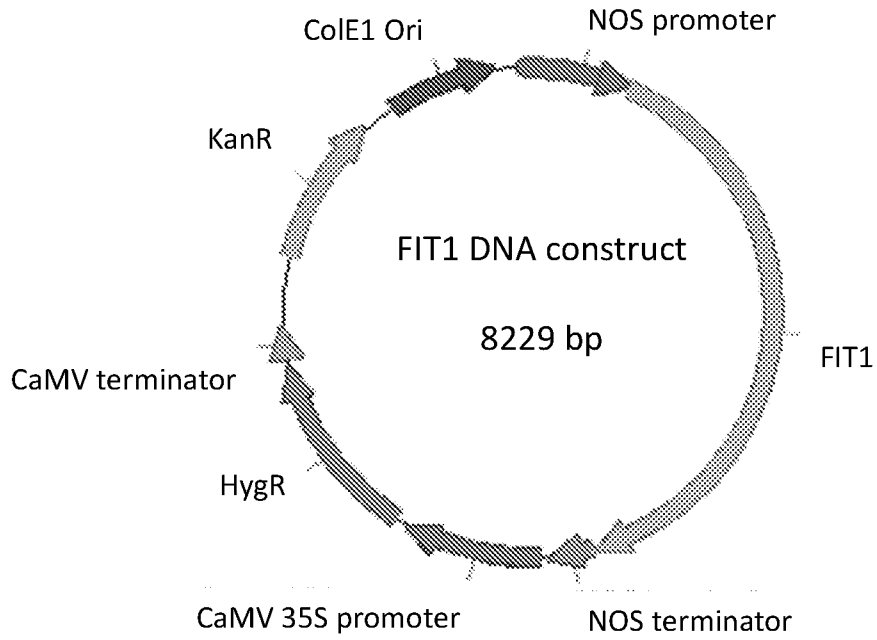

Glycine_max-XP_003542913
Glycine_max-XP_014621170
Glycine_max-XP_014624110
Glycine_max-KRH09363
VuFIT1
Glycine_max-KRH08204
Glycine_max-XP_006599369
Glycine_max-XP_003548983
Glycine_max-XP_006599371
Glycine_max-NP_001358788
Glycine_max-XP_014624020
Glycine_max-XP_006604003
Glycine_max-XP_003553356
Gmax_Lee_v1.1-GlymaLee.19G045100
Glycine_max-NP_001237768
Glycine_max-AAL56987
Glycine_max-XP_006603866
Glycine_max-NP_001239675
Glycine_max-ADF78105
Glycine_max-ADF78119
Glycine_max-KRH33937
Glycine_max-XP_014617686
Glycine_max-KRH08066
Glycine_max-KRH09337
Glycine_max-XP_003549282
Glycine_max-KRH09406
Glycine_max-XP_014624143
Glycine_max-KRH09404
Glycine_max-KRH09143
Glycine_max-KRH09400
Glycine_max-NP_001236756
Glycine_max-XP_006599693
Glycine_max-XP_014629764

Figure 4


ColE1 Ori
NOS promoter
KanR
FIT1 DNA construct
8229 bp
FIT1
CaMV terminator
HygR
CaMV 35S promoter
NOS terminator

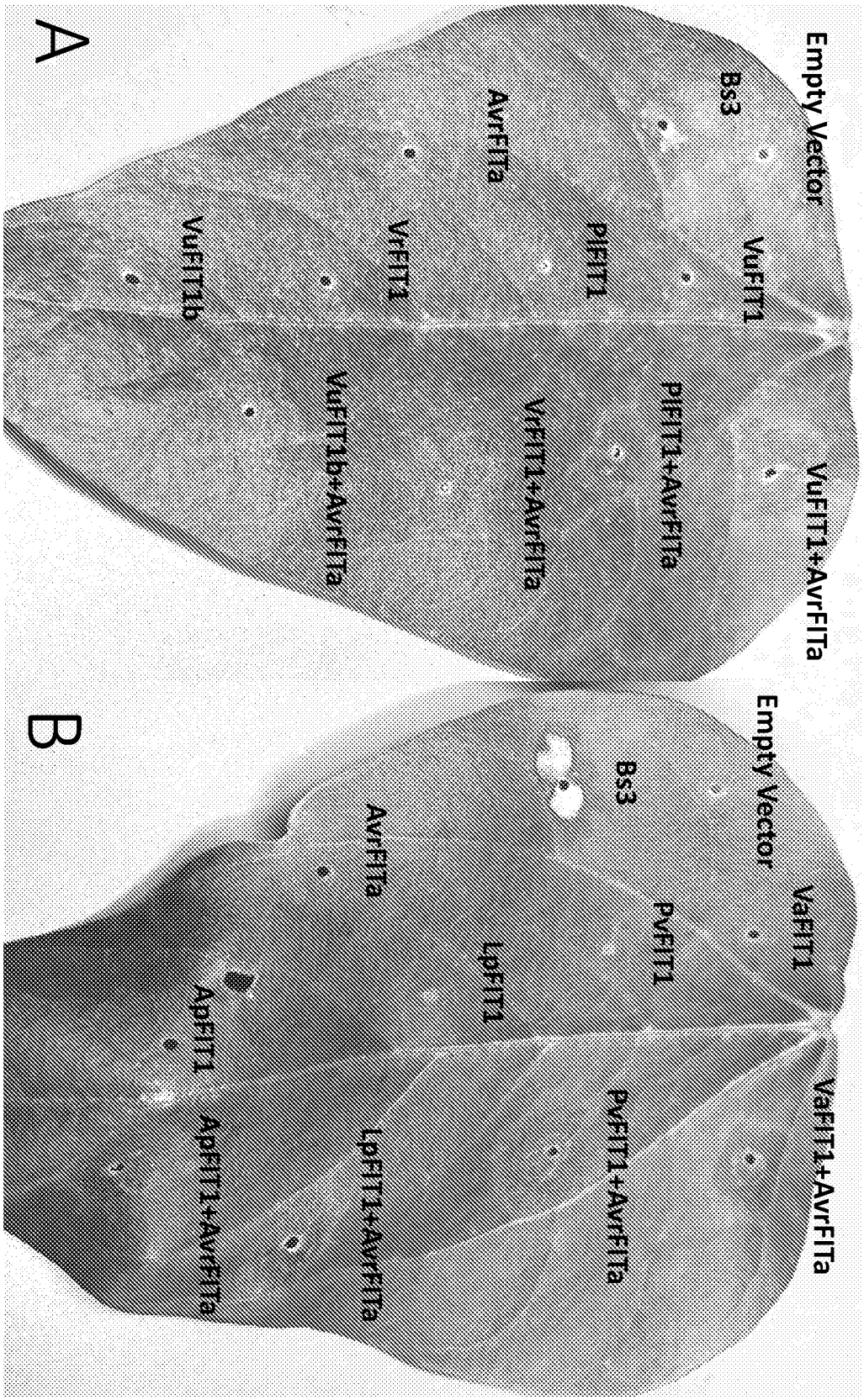

A
Empty Vector
Bs3
VuFIT1
PlFIT1
VrFIT1
VuFIT1b
AvrFlTa
VuFIT1+AvrFlTa
PlFIT1+AvrFlTa
VrFIT1+AvrFlTa
VuFIT1b+AvrFlTa
B
Empty Vector
Bs3
PvFIT1
LpFIT1
ApFIT1
AvrFlTa
VaFIT1
PvFIT1+AvrFlTa
LpFIT1+AvrFlTa
ApFIT1+AvrFlTa
VaFIT1+AvrFlTa

Figure 5

C
Empty Vector
VuFIT1
VuFIT1+AvrFITb
Bs3
PlFIT1
PlFIT1+AvrFITb
AvrFITb
VrFIT1
VrFIT1+AvrFITb
VuFIT1b
VuFIT1b+AvrFITb
D
Empty Vector
VaFIT1
VaFIT1+AvrFITb
Bs3
PvFIT1
PvFIT1+AvrFITb
AvrFITb
LpFIT1
LpFIT1+AvrFITb
ApFIT1
ApFIT1+AvrFITb

Figure 5

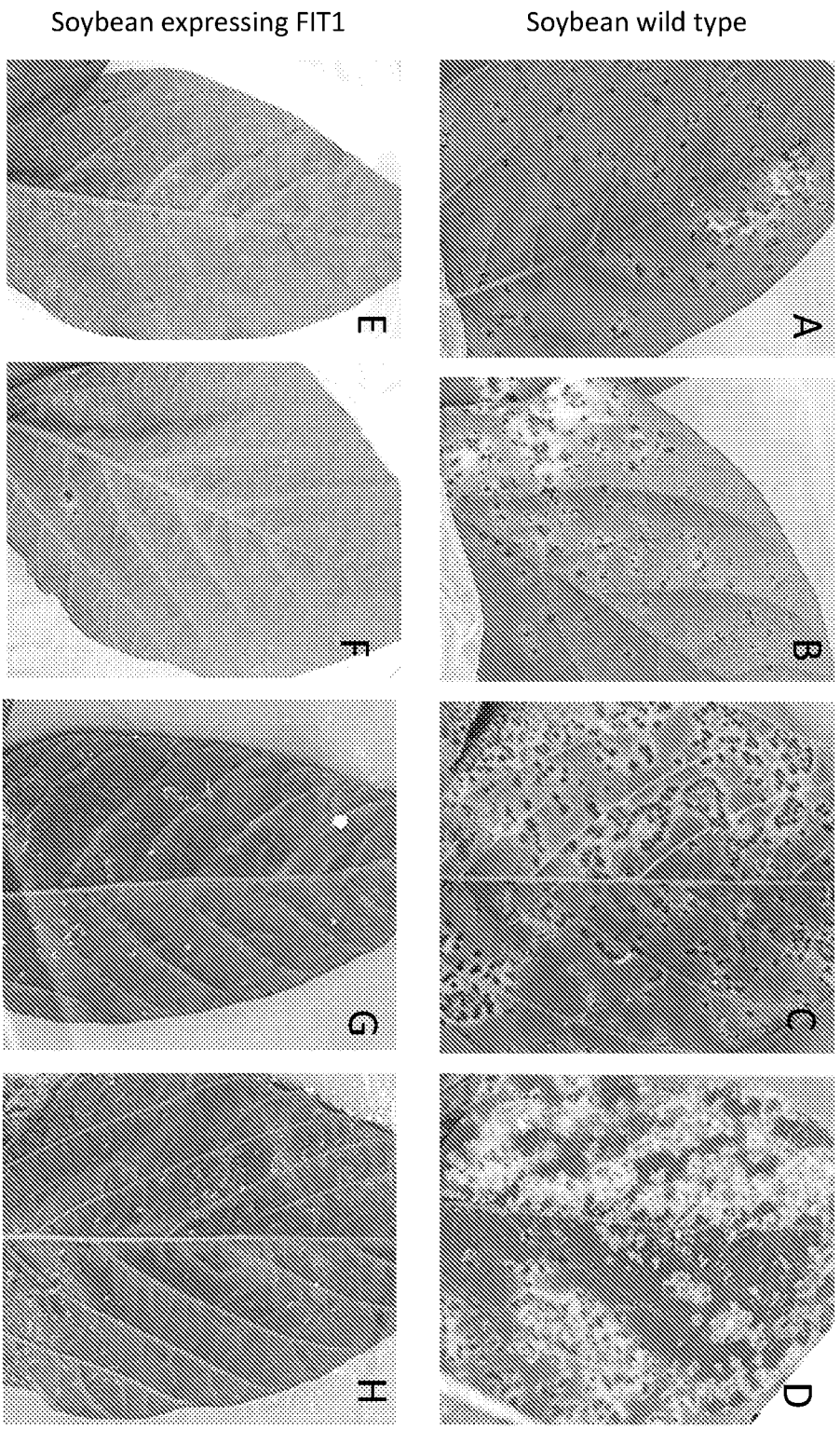

Soybean expressing FIT1
Soybean wild type
13 days post inoculation
44 days post inoculation
A
B
C
D
E
F
G
H

Figure 6

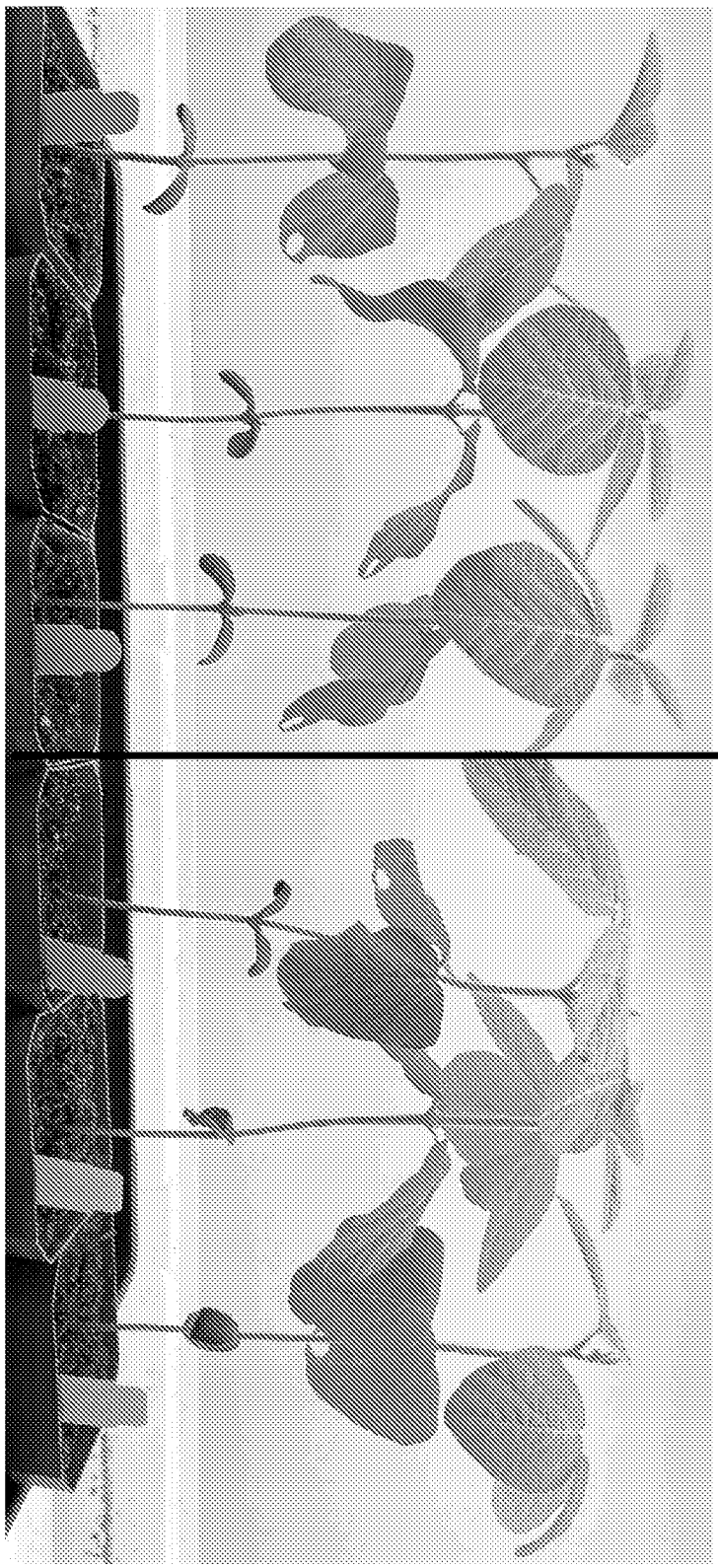


Figure 7A
Expressing FIT1

Figure 7B
Wild type (lacking FIT1)

Figure 7C

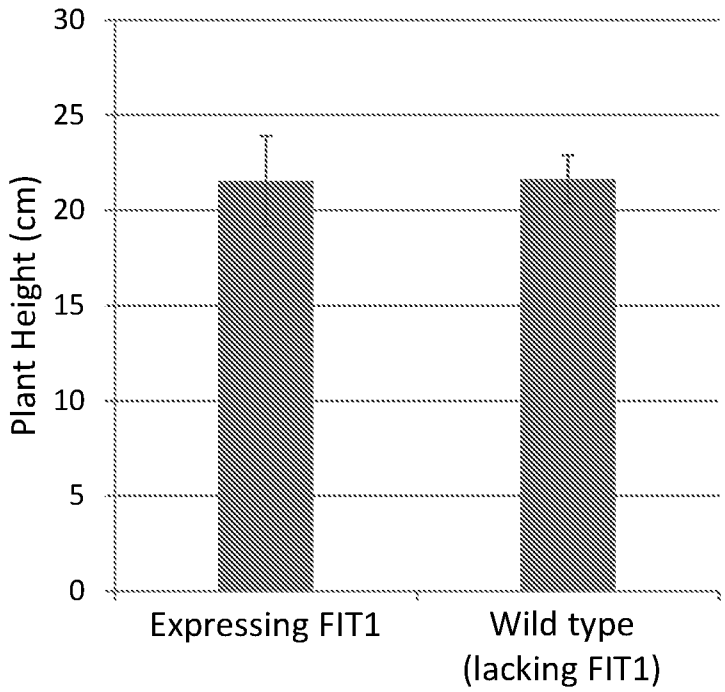
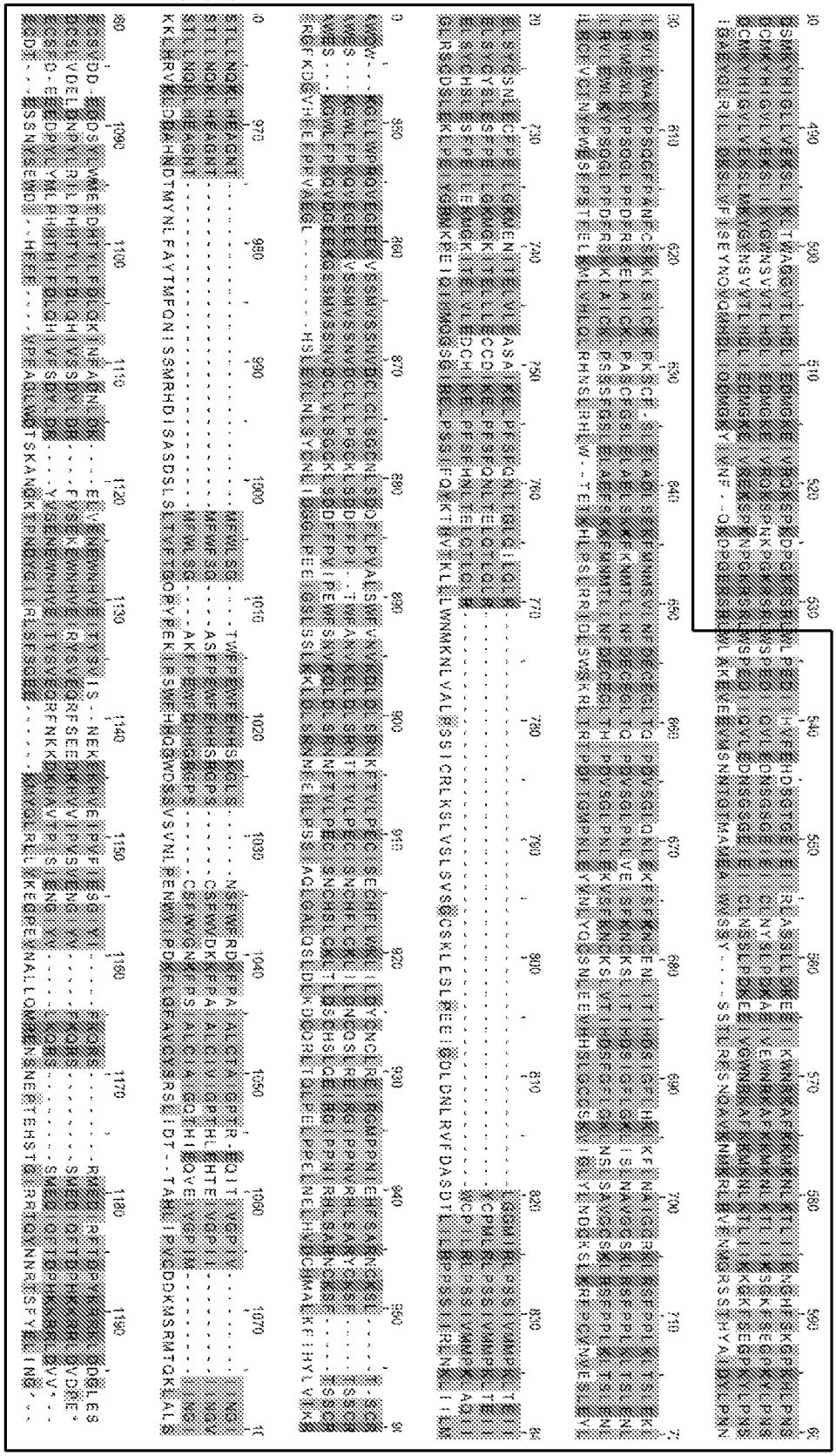

30
25
20
15
10
5
0
Plant Height (cm)
Expressing FIT1
Wild type
(lacking FIT1)

Figure 8

Figure 8 continued



LRR domain

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/056877

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01H 6/54; C12N 15/82 (2022.01)
CPC - A01H 6/54; C12N 15/8282 (2022.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE CARVALHO et al. UniProt Accession A0A0S1MIK1, 12 August 2020 (12.08.2020), Pgs. 1-2 [retrieved on 30 December 2021]. Retrieved from the internet <URL: https://www.uniprot.org/uniprot/A0A0S1MIK1.txt?version=17>. entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	US 2010/0313307 A1 (HERMAN et al) 09 December 2010 (09.12.2010) entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	US 2018/0195082 A1 (MONSANTO TECHNOLOGY LLC) 12 July 2018 (12.07.2018) entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	US 2019/0169629 A1 (MONSANTO TECHNOLOGY LLC) 06 June 2019 (06.06.2019) entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	SAKAI et al., "The power of single molecule real-time sequencing technology in the de novo assembly of a eukaryotic genome," Scientific Reports, 30 November 2015 (30.11.2015), Vol. 5, Art. No. 16780, Pgs. 1-13. entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	SANTOS et al. "QTL Mapping and transcriptome analysis of cowpea reveals candidate genes for root-knot nematode resistance," PLOS One, Vol. 13(1): e0189185, 04 January 2018 (04.01.2018), Pgs. 1-22, [retrieved on 30 December 2021]. Retrieved from the internet: <URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189185>. entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80
A	US 2018/0103600 A1 (TWO BLADES FOUNDATION et al) 19 April 2018 (19.04.2018) entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2022	MAR 11 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/056877

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NCBI Accession XP_014501487, 12 October 2017 (12.10.2017), Pgs. 1-3 [retrieved on 30 December 2021]. Retrieved from the internet: <URL: https://www.ncbi.nlm.nih.gov/protein/950977432>. entire document	1, 2, 7, 10, 11, 25-37, 49-62, 77-80

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/056877

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1, 2, 19, and 24 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/056877

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2, 7, 10, 11, 25-37, 49-62, 77-80

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-63 and 77-116 are drawn to polynucleotides comprising nucleic acid sequences encoding functional FIT1 proteins; genetic constructs comprising the nucleic acid sequences; plants, plant parts, or plant cells resistant or tolerant to a pathogen, wherein the plants, plant parts, or plant cells are transformed with the nucleic acid sequences, and methods of producing the plants, plant parts, or plant cells.

Group II: claims 64-72 are drawn to a method of genetically engineering a pathogen resistance or tolerance trait in a plant, plant part, or plant cell, comprising: genetically modifying a plant, plant part, or plant cell from the susceptible plant species with targeted gene editing.

Group III: claims 73-76 are drawn to a method for identifying a functional FIT1 gene and/or allele thereof.

The first invention of Group I+ is restricted to an Alysicarpus plant, plant part, or plant cell comprising an isolated nucleic acid sequence comprising SEQ ID NO: 1, encoding SEQ ID NO: 2, and a pathogen effector protein comprising SEQ ID NO: 24. It is believed that claims 1, 2, 7, 10, 11, 25-37, 49-62, and 77-80 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional plant specie(s) and/or nucleic acid sequence(s) encoding specified amino acid sequence(s), and/or additional pathogen effector protein sequence(s) for each additional embodiment to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected embodiment requires the selection of a single definition for each plant specie, and/or nucleic acid sequence encoding a specified amino acid sequence, and/or pathogen effector protein sequence. An exemplary election would be an isolated nucleic acid sequence comprising SEQ ID NO: 19, encoding SEQ ID NO: 20. Additional embodiment(s) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+, II, and III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I+, SEQ ID NO: 2, are not present in any of Groups II-III; the special technical features of Group II, gene editing, are not present in any of Groups I+, or III; the special technical features of Group III, a method for identifying a functional FIT1 gene, are not present in any of Groups I+, or II.

The Group I+ formulae do not share a significant structural element, requiring the selection of alternatives for (the election element) and accordingly these groups lack unity a priori.

Additionally, even if Groups I+, II, and III were considered to share the technical features of: an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a functional FIT1 protein homologous to SEQ ID NO: 2; a genetic construct comprising the nucleic acid sequence; a plant, plant part, or plant cell transformed with the nucleic acid sequence, wherein said plant, plant part or plant cell is resistant or tolerant to a pathogen; a method of producing a plant, plant part, or plant cell having resistance or tolerance to a pathogen, wherein the method comprises: transforming a plant, plant part, or plant cell with a nucleotide sequence encoding a Toll-like Interleukin-1 Receptor (TIR) Nucleotide binding, Leucine-rich Repeat (NLR) immune receptor protein, wherein said immune receptor protein mediates the perception of pathogen effector protein AvrFIT1 or homologs thereof; and wherein expression of the immune receptor protein prevents the pathogen from colonizing the plant, or prevents the pathogen from affecting plant growth or yield; a plant, plant part, or plant cell produced by the method, wherein the plant, plant part, or plant cell is resistant or tolerant to a pathogen; a transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust, wherein the resistance is conferred by a transgene; and SEQ ID NO: 24, these shared technical features do not represent a contribution over the prior art, as disclosed by US 2018/0103600 to Two Blades foundation (hereinafter, "Two Blades"), the article "QTL Mapping and transcriptome analysis of cowpea reveals candidate genes for root-knot nematode resistance" by Santos, et al. (hereinafter, "Santos"), NCBI reference sequence XP_014501487 (hereinafter, "XP_014501487"), and UniProt accession A0A0S1MIK1 by de Carvalho et al. (hereinafter, "de Carvalho").

Specifically, Two Blades discloses an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence encoding a gene (genes encoding NB-LRR proteins which confer resistance to Asian soybean rust; abstract); a genetic construct comprising the nuclelc acid sequence (a genetic construct comprising the nucleic acid sequence; Para. [0006]); a plant, plant part, or plant cell transformed with the nucleic acid sequence (a plant transformed with the nucleic acid sequences), wherein said plant, plant part or plant cell is resistant or tolerant to a pathogen (wherein said plant is resistant or tolerant to ASR; Paras. [0005], [0006]); a method of producing a plant, plant part, or plant cell having resistance or tolerance to a pathogen (methods of conferring disease resistance in legume crop species; Para. [0008]), wherein the method comprises: transforming a plant, plant part, or plant cell with a nucleotide sequence encoding a Toll-like Interleukin-1 Receptor (TIR) Nucleotide binding, Leucine-rich Repeat (NLR) immune receptor protein (wherein the method comprises: transforming a plant, plant part, or plant cell with a nucleotide sequence encoding a NB-LRR protein; Para. [0008]); and wherein expression of the immune receptor protein prevents the pathogen from colonizing the plant, or prevents the pathogen from affecting plant growth or yield (wherein expression of the immune receptor protein prevents the pathogen from colonizing the plant, or prevents the pathogen from affecting plant growth or yield; Para. [0035]); a plant, plant part, or plant cell produced by the method (a transgenic legume crop plant stably transformed with a recombinant DNA construct encoding an NB-LRR protein; Para. [0009]), wherein the plant, plant part, or plant cell is resistant or tolerant to a pathogen (wherein the plant, plant part, or plant cell is resistant or tolerant to a pathogen; Para. [0005]); a transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust (a transgenic plant, plant part, or plant cell having resistance to Asian Soybean Rust; Paras. [0005], [0006]), wherein the resistance is conferred by a transgene (wherein the resistance is conferred by a transgene; Paras. [0005], [0006]).

Santos teaches genes encoding NBS-LRR proteins which confer resistance in legumes (genes encoding NBS-LRR proteins which confer resistance in legumes; abstract), including XP_014501487, Vigna radiata TMV resistance protein N (XP_014501487, Vigna radiata TMV resistance protein N; Pg. 15, first Para.).

XP_014501487 teaches a leucine-rich repeat-containing protein from Vigna radiata var. radiata homologous to SEQ ID NO: 2, which recognizes AvrFit1a and AvrFit1b (TMV resistance protein N from Vigna radiata var. radiata; Pg. 1-3, see fig 3A of the instant application for Applicant's indication of binding activity).

de Carvalho teaches a polypeptide comprising SEQ ID NO: 24 (Phakopsora pachyrizi peptidyl-prolyl cis-trans isomerase; Pg. 1-2).

The inventions listed in Groups I+, II, and III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.