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(54) Title: ZAR1 AND JIM2 MEDIATE RESISTANCE AGAINST PLANT PATHOGENS CONTAINING YOPJ-FAMILY EFFECTORS

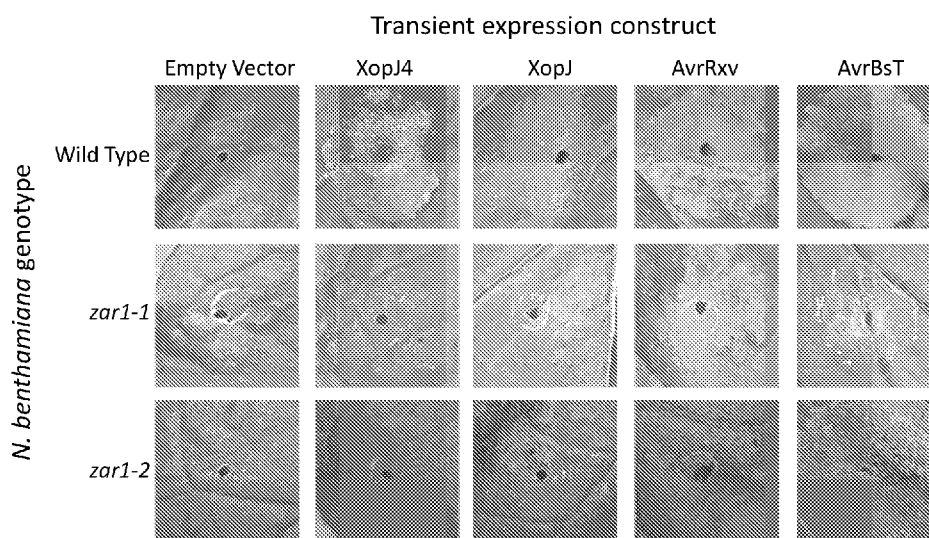


FIG. 1

(57) Abstract: Provided herein is a plant comprising an exogenous polynucleotide encoding a JIM2 polypeptide. In some embodiments, the plants have enhanced resistance to at least one species of *Xanthomonas*.

ZAR1 AND JIM2 MEDIATE RESISTANCE AGAINST PLANT PATHOGENS CONTAINING YOPJ-FAMILY EFFECTORS

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CROSS-REFERENCING

This application claims the benefit of U.S. provisional application serial no. 62/692,537, filed on June 29, 2018, which application is incorporated by reference in its entirety.

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STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

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BACKGROUND

Bacterial phytopathogens in the genus *Xanthomonas* use a Type III Secretion System to deliver effector proteins into the plant cell. Effector proteins can function to inhibit plant immunity or manipulate the metabolism of the host to favor growth of the bacteria (Gürlebeck et al., 2006). If the plant is able to detect the presence of an effector protein, a strong immune response may be induced which prevents pathogen proliferation and restricts host range (Alfano and Collmer, 2004; Castañeda et al., 2005; Wei et al., 2007; Schwartz et al., 2015). This strong immune response often results in localized cell death known as the hypersensitive response. Identifying the pathway responsible for mediating effector protein recognition can enable efforts to engineer disease resistance in susceptible crop species (Wulff et al., 2011).

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). NLR recognition of an effector protein can occur through a physical interaction between the NLR and the cognate effector or by an indirect mechanism in which the NLR protein is “guarding” another component which is modified by the effector (Khan et al., 2016). While plants have additional receptor pathways independent from the NLRs, such as Receptor-Like Kinases (Macho and Zipfel, 2014), NLR proteins have been demonstrated to mediate many resistance responses against a broad range

of pathogens including bacteria, fungi, oomycetes, viruses and nematodes (Dodds and Rathjen, 2010).

Tomato is susceptible to *Xanthomonas perforans* which causes the disease Bacterial Leaf Spot. This disease can result in significant yield losses in commercial tomato production and there is limited genetic resistance available within commercial cultivars (Stall et al., 2009; Kim et al., 2015). The plant *Nicotiana benthamiana* is resistant to *X. perforans* due to the presence of several pathways capable of perceiving effector proteins found in this pathogen including AvrBsT, XopQ and XopJ4/AvrXv4 (Roden et al., 2004; Schwartz et al., 2015). The XopJ4 effector is widely conserved among strains of *X. perforans* and has therefore been proposed as a good target for genetic mechanisms of disease resistance against this pathogen (Timilsina et al., 2016).

XopJ4 is part of the YopJ family of effector proteins. These effectors are distributed across many species of bacterial pathogens of both plants and animals and are thought to be acetyltransferases that disrupt the function of proteins inside the host cell (Ma and Ma, 2016). The YopJ effector protein HopZ1a from *Pseudomonas syringae* is recognized in *Arabidopsis thaliana* and triggers a hypersensitive cell death response (HR) that depends on the NLR protein ZAR1 and the Receptor-Like Cytoplasmic Kinase (RLCK) family XII protein ZED1 (Lewis et al., 2010; Lewis et al., 2013). HopZ1a has been reported to directly acetylate ZED1, which interacts with ZAR1 to initiate an immune response. ZAR1 is also required for the perception of several non-YopJ effectors in *Arabidopsis* including the *Xanthomonas* AvrAC (Wang et al., 2015) and the *Pseudomonas syringae* HopF2a (Seto et al., 2017). In each case, a different RLCK XII family member is required for the immune response and for AvrAC an additional RLCK VII protein is also required.

The YopJ effector PopP2 from *Ralstonia solanacearum* is recognized in *Arabidopsis thaliana* by a ZAR1-independent pathway (Deslandes et al., 2002). PopP2 acetylates a WRKY domain on the NLR protein RRS1 to trigger immune activation (Sarris et al., 2015). The NLR protein RPS4 is required for RRS1-mediated perception of PopP2 and is thought to form a complex with RRS1 (Narusaka et al., 2009; Williams et al., 2014). The widespread distribution of YopJ-family effector proteins and the existence of two evolutionarily independent mechanisms for the perception of YopJ effectors highlights the importance of these proteins in pathogenesis.

Four YopJ effector proteins have been identified in various *Xanthomonas* species, although not all *Xanthomonas* have YopJ effectors. These include XopJ4 / AvrRx4, XopJ, AvrRxv and AvrBsT. AvrRxv, XopJ4 and AvrBsT have all been associated with avirulence

responses in various Solanaceous plant species, although the recognition mechanisms for the perception of these effectors are not well understood (Minsavage et al., 1990; Whalen et al., 1993; Astua-Monge et al., 2000). The AvrBsT effector triggers a strong avirulence response on pepper and *N. benthamiana*. The recognition of AvrBsT in pepper has been reported to be dependent on SGT1 and PIK1 (Kim et al., 2014). AvrRxv triggers an avirulence response on tomato line Hawaii 7998, the basis for which is multi-genic based on segregation analysis (Whalen et al., 1993; Yu et al., 1995). XopJ4 triggers an avirulence response on *Solanum pennellii* accession LA716. While efforts have been made to map the resistance genes for AvrRxv and XopJ4 (Yu et al., 1995; Astua-Monge et al., 2000; Sharlach et al., 2013), the genes involved have not been conclusively identified.

SUMMARY

Provided herein is a plant comprising an exogenous polynucleotide encoding a JIM2 polypeptide, where the exogenous polynucleotide is operably linked to a promoter and the plant expresses the JIM2 polypeptide. In some embodiments, the plants have enhanced resistance to at least one species of *Xanthomonas*.

These and other inventions are described in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

Fig. 1. Response to *Xanthomonas* YopJ effectors in the *zar1* mutants. The indicated YopJ effectors were transiently expressed in wild type or the mutant *N. benthamiana* plants using *Agrobacterium* infiltrated at an OD₆₀₀ of 0.5. The plants were imaged three days post infiltration. XopJ4 and AvrBsT are from *X. perforans* 4B whereas XopJ and AvrRxv are from *X. euvesicatoria* 85-10. The data shown in this figure indicates that the NbZAR1 is required for perception of XopJ4, XopJ and AvrRxv in *N. benthamiana*. The visible cell death response to AvrBsT is weaker but still present in the *zar1* mutant plants, indicating that NbZAR1 mediates perception of AvrBsT but there is also an NbZAR1-independent pathway for perception of this effector.

Fig. 2. *Xanthomonas* effector proteins from the YopJ family were transiently expressed with an empty vector (left) or the codon-altered VIGS-resistant NbJIM2 protein

(NbJIM2_VR, right) in *N. benthamiana* plants silenced for GUS (as a negative control, top) or the native NbJIM2 gene (bottom). *Agrobacterium* was infiltrated at an OD₆₀₀ of 0.5 total and the plants were photographed at three days post infiltration. The data in this figure indicate that the NbJIM2 gene is required for perception of XopJ4, XopJ and AvrRxv in *N. benthamiana*. As observed for NbZAR1, disruption of NbJIM2 reduces but does not eliminate the response to AvrBsT indicating that NbJIM2 mediates perception of AvrBsT but that there may be an NbJIM2-independent recognition pathway.

Fig. 3. PopP1 perception in *zar1* mutants and JIM2-silenced plants. PopP1 wild type and the C229A catalytic mutant were transiently expressed using *Agrobacterium* in wild type, *zar1-1* and *zar1-2* mutants (left) and wild type plants silenced for GUS (as a negative control) or NbJIM2 (right). The *Agrobacterium* was infiltrated at an OD₆₀₀ of 0.5 and plants were imaged at four days post infiltration. The data in this figure indicates that recognition of the *Ralstonia solanacearum* effector protein PopP1 is dependent on NbZAR1 and NbJIM2.

Fig. 4. Phylogenetic tree of YopJ family effector proteins. The protein sequences from various plant pathogen YopJ-family effectors were used to construct a phylogenetic tree. The five YopJ effector protein sequences that were observed to be recognized by NbZAR1 and NbJIM2 are in a separate clade from HopZ1a, which is recognized by AtZAR1 and ZED1. The data in this figure shows that the YopJ effectors perceived by NbZAR1 and NbJIM2 fall within a clade that is distinct from HopZ1a. This suggests that the effector proteins within the top clade can be perceived by NbZAR1 and NbJIM2, whereas those in the lower clade containing HopZ1a may not be. This indicates the functional divergence between the AtZAR1/ZED1 and NbZAR1/NbJIM2 recognition pathways.

Fig. 5. Bacterial growth and visible immune response to *Xanthomonas perforans*. *Nicotiana benthamiana* wild type and *zar1* mutants were infiltrated with the indicated genotype of *X. perforans* at OD₆₀₀ = 0.0001. Bacterial growth was assayed at six days post infiltration and the visible immune response was photographed at seven days post infiltration. Error bars indicated standard deviation from three biological replicates. The data in this figure shows that perception of XopJ4 mediated by NbZAR1 correlates with resistance to the *Xanthomonas* pathogen containing this effector protein. Δ indicates that the following effector gene was knocked out in that strain of *Xanthomonas*.

Fig. 6. AtZAR1 complementation of *zar1-1*. *Agrobacterium* was used to transiently express the indicated genes in leaf tissue of wild type *Nicotiana benthamiana* and the *zar1-1* mutant. The *Agrobacterium* was infiltrated at an OD₆₀₀ of 0.3 for each construct and the plants were imaged at two days post infiltration. The data in this figure shows that the previously known gene AtZAR1 is not able to mediate perception of XopJ4 and therefore has distinct functionality from NbZAR1.

Fig. 7. Functional complementation testing of SIZAR1. The indicated genes were transiently expressed using *Agrobacterium* in *Nicotiana benthamiana* wild type and the *zar1-1* mutant. The plants were infiltrated at an OD₆₀₀ of 0.3 for each construct and imaged at three days post infiltration. The data in this figure shows that SIZAR1 is not able to complement the function of NbZAR1 despite tomato and *N. benthamiana* being closely related.

Fig. 8. Multiple protein align for SIZAR1. Putative orthologs of ZAR1 were identified by BLAST search of the NCBI and 1KP databases. A multiple sequence alignment was performed using ClustalO of the protein sequences, a subset of which is shown. The ZAR1 protein from *Solanum lycopersicum* contains several missense mutations at conserved locations including Q430K indicated above. This figure identifies a mutation in SIZAR1 at a highly conserved position which may explain why SIZAR1 is unable to complement the Nb *zar1* mutant. The sequences are set forth as follows: *Bougainvillea spectabilis* (SEQ ID NO:61); *Boerhavia coccinea* (SEQ ID NO: 62); *Synsepalum dulcificum* (SEQ ID NO: 63); *Manilkara zapota* (SEQ ID NO: 64); *Ardisia humilis* (SEQ ID NO: 65); *Mertensia paniculate* (SEQ ID NO: 66); *Phacelia campanularia* (SEQ ID NO: 67); *Heliotropium mendocinum* (SEQ ID NO: 68); *Ligustrum sinense* (SEQ ID NO: 69); *Nicotiana tabacum* (SEQ ID NO: 70); *Nicotiana sylvestris* (SEQ ID NO: 71); *Nicotiana attenuate* (SEQ ID NO: 72); NbZAR1 (SEQ ID NO: 73); *Capsicum annuum* (SEQ ID NO: 74); *Solanum ptycanthum* (SEQ ID NO: 75); *Solanum tuberosum* (SEQ ID NO: 76); SIZAR1 (SEQ ID NO: 77); *Solanum pennellii* (SEQ ID NO: 78); *Wrightia natalensis* (SEQ ID NO: 79); *Apocynum androsaemifolium* (SEQ ID NO: 80); *Coffea canephora* (SEQ ID NO: 81); *Psychotria ipecacuanha* (SEQ ID NO: 82); *Centella asiatica* (SEQ ID NO: 83); *Hedera helix* (SEQ ID NO: 84); *Exocarpos cupressiformis* (SEQ ID NO: 85); *Hakea prostrata* (SEQ ID NO: 86); *Annona muricata* (SEQ ID NO: 87); *Eupomatia bennettii* (SEQ ID NO: 88); *Hibbertia grossulariifolia* (SEQ ID NO: 89); *Ludovia* sp. (SEQ ID NO: 90); *Anthurium amnicola* (SEQ ID NO: 91); *Pistia stratiotes* (SEQ ID NO: 92).

Fig. 9. SpJIM2 functions in XopJ4 perception. A homolog of JIM2 from *Solanum pennellii* (SpJIM2) was cloned and transiently expressed in a line of *N. benthamiana* deficient for NbJIM2 using *Agrobacterium*. Co-expression of XopJ4 and SpJIM2 resulted in a strong immune response at three days post infiltration. The data in this figure shows that the *Solanum pennellii* JIM2 gene can mediate perception of XopJ4 despite having significant sequence divergence from NbJIM2.

Fig. 10. ZAR1 and JIM2 confer resistance to *Xanthomonas perforans*. The *Solanum pennellii* alleles of ZAR1 and JIM2 were transformed into tomato. To test for disease resistance, a bacterial solution of *Xanthomonas perforans* was infiltrated into leaf tissue at a low inoculum ($OD_{600} = 0.0001$). The infiltrated leaves were photographed at 14 days post infiltration to observed visual disease symptoms (A). The wild type tomato (lacking ZAR1 and JIM2) developed severe yellowing and necrotic lesions in the infiltrated region (A, left) whereas the tomato line expressing ZAR1 and JIM2 appeared healthy (A, right). To measure bacterial proliferation, leaf punches were collected at six days post infiltration, homogenized in water, and plated on agar plates. Approximately twenty-five times more colony forming units were detected from wild type than from tomato expressing ZAR1 and JIM2 (B). These results indicate that ZAR1 and JIM2 confer resistance to *Xanthomonas perforans* in tomato. Error bars indicate standard deviation.

Fig. 11. ZAR1 and JIM2 confer resistance to *Ralstonia solanacearum*. *Ralstonia solanacearum* causes severe wilting of a susceptible tomato variety (left) whereas tomato expressing ZAR1 and JIM2 is resistant (right). The plants were inoculated by placing bacterial solution ($OD_{600} = 0.0005$) onto the exposed surface of a cut petiole. The plants were imaged at 10 days post inoculation.

Fig. 12. Sequence of the codon-altered, VIGS-resistant JIM2 construct. The VIGS construct was designed to target part of the 2nd exon of JIM2. To design a VIGS-resistant version of JIM2, the codon usage was altered so that the VIGS cassette would have limited identity with the nucleotide sequence but the predicted amino acid sequence would not be affected. The sequence is set forth in SEQ ID NO: 60.

DEFINITIONS

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference.

Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

The headings provided herein are not limitations of the various aspects or embodiments of the invention. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, et al., *DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY*, 2D ED., John Wiley and Sons, New York (1994), and Hale & Markham, *THE HARPER COLLINS DICTIONARY OF BIOLOGY*, Harper Perennial, N.Y. (1991) provide one of skill with the general meaning of many of the terms used herein. Still, certain terms are defined below for the sake of clarity and ease of reference.

It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely”, “only” and the like in connection with the recitation of claim elements, or the use of a “negative” limitation.

As used herein, “resistance” is a relative term in that the presence of a polypeptide of the invention (i) reduces the disease symptoms of a plant comprising the gene (R (resistant) gene) that confers resistance, relative to a plant lacking the R gene, and/or (ii) reduces pathogen reproduction or spread on a plant or within a population of plants comprising the R gene. Resistance as used herein is relative to the “susceptible” response of a plant to the same pathogen. Typically, the presence of the R gene improves at least one production trait of a plant comprising the R gene when infected with the pathogen, such as grain yield, when compared to an isogenic plant infected with the pathogen but lacking the R gene. The

isogenic plant may have some level of resistance to the pathogen, or may be classified as susceptible. Thus, the terms “resistance” and “enhanced resistance” are generally used herein interchangeably. Furthermore, a polypeptide of the invention does not necessarily confer complete pathogen resistance, for example when some symptoms still occur or there is some pathogen reproduction on infection but at a reduced amount within a plant or a population of plants. Resistance may occur at only some stages of growth of the plant, for example in adult plants (fully grown in size) and less so, or not at all, in seedlings, or at all stages of plant growth. By using a transgenic strategy to express an polypeptide in a plant, the plant of the invention can be provided with resistance. Enhanced resistance can be determined by a number of methods known in the art such as analysing the plants for the amount of pathogen and/or analysing plant growth or the amount of damage or disease symptoms to a plant in the presence of the pathogen, and comparing one or more of these parameters to an isogenic plant lacking an exogenous gene encoding a polypeptide of the invention.

The term “exogenous” means that the sequence of the polynucleotide is not found in the wild type species of plant in which it is present. A plant that contains an exogenous polynucleotide has a genome that has been modified to contain the polynucleotide. The polynucleotide may be from another plant or it may have a nucleotide sequence that encodes a polypeptide from another plant. An exogenous polynucleotide may encode a variant of a polypeptide from another plant (e.g., a polypeptide that is at least 95% identical to a reference polypeptide). For example, a tomato plant that contains an exogenous polynucleotide has a genome that has been modified to contain a polynucleotide that is not from tomato. An “exogenous” nucleic acid can be introduced into a genome of a cell via a number of different methods. For example, in some cases the plant may be transgenic, in which case the exogenous nucleic acid may be introduced from the outside (e.g., by introducing a coding sequence into the plant). In other cases, an exogenous nucleic acid can be introduced by modifying a sequence that already exists in the genome by genome editing. In other cases, the plant may be cisgenic, in which case the exogenous nucleic acid may be introduced into by plant breeding, i.e., by introgressing a gene from another species into the plant.

Reference to a particular protein, e.g., JIM2, includes wild type proteins from other plant species as well as variants of those proteins that do not have a wild type sequence but are at least 80%, e.g., at least 85%, at least 90% or at least 95% identical to a wild type sequence and remain functional.

Other definitions of terms may appear throughout the specification.

DETAILED DESCRIPTION

Before the present invention is described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular
5 embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between
10 the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also
15 encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention
20 belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure
25 supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" includes a plurality of such nucleic acids
30 and reference to "the compound" includes reference to one or more compounds and equivalents thereof known to those skilled in the art, and so forth.

The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and

immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used.

- 5 Such conventional techniques and descriptions can be found in standard laboratory manuals such as *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV), *Using Antibodies: A Laboratory Manual*, *Cells: A Laboratory Manual*, *PCR Primer: A Laboratory Manual*, and *Molecular Cloning: A Laboratory Manual* (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) *Biochemistry* (4th Ed.) Freeman, New York, Gait, “*Oligonucleotide*
10 *Synthesis: A Practical Approach*” 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, A., *Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) *Biochemistry*, 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

- The publications discussed herein are provided solely for their disclosure prior to the
15 filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

A plant comprising an exogenous polynucleotide encoding a JIM2 polypeptide is provided. In some embodiments, the plant may comprise an exogenous polynucleotide encoding a ZAR1 polypeptide. The plant may have enhanced resistance to bacterial pathogens that contain a YopJ effector. For example, the plant may have enhanced resistance to at least one species of *Xanthomonas* (e.g., *Xanthomonas perforans*) relative to a control plant that is otherwise identical to the plant but does not contain the exogenous polynucleotide. In these embodiments, the plant should not have an endogenous functional JIM2 gene, i.e., a JIM2 gene that is native to the plant and mediates recognition of XopJ4 or a related YopJ effector protein.

In some embodiments, the JIM2 polypeptide may be at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 98%, at least 99% identical, or 100% identical to) to the *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2). The amino acid sequences of these proteins are shown below:

Nicotiana benthamiana JIM2 (SEQ ID NO: 1):

MDCIKKMWSVVKKFRKEEDVANLFLQNGGALLEELISFSSGTYDIPIPSYSA

QQLVNATNNFSGRVHASTYGYICRGTLQGHISIFVKMFINIPGNLASHSEFDILAGAV
 RDISITSLMSGNKNVLKIIGCCLEFRYPALVYEDARFETLANFLDPNCDKLLSWKCRL
 KIAKSIA SAILYLHTAFPTPIIYRILNPHNIILDHHCVPKLFDFS FVISLPPGELKVEDDLI
 WIPGYFDPEYQSSRFVTQKTDVYSFGVLLLVLLNGQGPICRANEDDPEHIVNYVNDH
 IHKDDQFKHIVDPKILNESSVNHQQLQAFIDIALRCVQAKGENRPDMFEIARKILQFE

Solanum pennellii JIM2 (SEQ ID NO: 2):

MQFFREL TIRKKQSLSEWRKKEHDYYLHNGSAVLEELLALCNGNCRIPRY
 FTASEIDDAISYSQNELEIFDGRMVAGSMDKRLVFVRFFPNYFRNFFNIFRDIAITAQ
 MSHLKNVLR LVGCCVEFEKPMVYVEAISLHTLLFEKGNHDDQTRKSLLSWG N
 RLRIANEVASAVVFLHTEFTTPIIYKDLKPSNVIIDQNSGVAKLLNFSLSVSLPPGELQ
 VIKDVTCTGYGYLAPEYAVSGIVTQNTDVYSFGVLLQLLTGKNMGTL DIKDRKYI
 MYDVESDLDPIDIEKIYVMDIADKAILEEYGIEIQQLEDCWDLVKKCTKSKGEERP
 YMIEVAKELRRIYNCFRVLTGQNQLHK

In some embodiments, the ZAR1 polypeptide may be at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 98% or 100%) identical to the *Nicotiana benthamiana* ZAR1 polypeptide (SEQ ID NO: 3). Examples of partial sequences from several ZAR1 polypeptides are shown in Fig. 8. The amino acid sequence of the *Nicotiana benthamiana* ZAR1 polypeptide is shown below:

Nicotiana benthamiana ZAR1 (SEQ ID NO: 3):

MVDAVVTVFLEKLLHVLTEESRFLT KYRQQFEKLKNELLFMQSFLKDAERL
 KRKNNTLKGVMSCLRDLIFEAEIILED CQNQSADSDRATTCFHPKRLSLRHQTGKCL
 AKINDRISEIKQNISTYLGVP LLEEGSMEAHNNLMSRWTSSLYDHTQVVGLEGDTEK
 IKDWLFEARDGLLTIAFVGMGGLGKT TLAQKVFNDKRVEDHLERRIWVSVSQT FTE
 EQVMRSILRSLGDACVGDDQCELLR KINQYLLGKRFLIVMDDVWSWDNAWWQKI
 YTGLPKGNGSTVIVTTRNELVARKMGV TEARIHWPKFLNEHYSWLLFRKIAFAGSA
 GECHFPELEDVGKEIVEKCKGLPLAIKAVGGV MLCKPSYYHEWRRISNHFRDELKE
 NDDSVMASLQLSYDELPPYLKSCFLCFS LFPEDCVIPK DQLIRWWIGEGFIPLRSGRL
 STEVGEDCFSQLSNRCLIEVVDKAYNGV IHTCKMHDMVRDLVIKLAEDDAFFTPAD
 ATCRHLGIKSEM NWKQLLSNQKL RALLTTTKSGEVN KIHSDIAKKLCKSRHLQVLD
 LSKSIFDVPLSSLLEGIGSAKQLTYLS LSNTHPMIGVPASISKLEKLQILDFSYCQNMK
 MLPSCVLTFEELAVLDVN NCGSLEYLPKGLSRLSNLQVLLGFKPAKLSQPGGCRIAE
 LRSLTRLR TSLRLTENEEIGDDEGNALVDLQELQFLTISCFGSQDNGLATKLGRLYP
 PRQLHELILKFYPSKTSPEWLNPNLSPML RYLSIISGDITQMHENFWGDGSTAWKIEG
 LMLESLSDLRLEWSAMHQVMPSLRILK VSWCPELESFPIEDAGFRGGLWKKEEHRN

The plant may be monocotyledonous or dicotyledonous. Target plants include, but are not limited to, the following: cereals (for example, wheat, barley, rye, oats, rice, maize, sorghum and related crops); grapes; beet (sugar beet and fodder beet); pomes, stone fruit and soft fruit (mango, kiwi, apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries and black-berries); leguminous plants (beans, lentils, peas, soybeans); oil plants (rape or other Brassicas, mustard, poppy, olives, sunflowers, safflower, flax, coconut, castor oil plants, cocoa beans, groundnuts); cucumber plants (marrows, cucumbers, melons); fibre plants (cotton, flax, hemp, jute); citrus fruit (oranges, lemons, grapefruit, mandarins); vegetables (spinach, lettuce, asparagus, cabbages, carrots, onions, tomatoes, peppers, potatoes, paprika); lauraceae (avocados, cinnamon, camphor); or plants such as maize, cassava, nuts (walnut), coffee, sugar cane, tea, vines, hops, turf, bananas and natural rubber plants, as well as ornamentals (flowers, shrubs, broad-leaved trees and evergreens, such as conifers). In some embodiments, the plant may be susceptible to infection by one or more species of *Xanthomonas* (e.g., one or more species of *Xanthomonas*) without the exogenous polynucleotide. In some cases the plant may be a hybrid. In some cases, the introduction of the exogenous polynucleotide may provide resistance to infection by other *Xanthomonas* species, e.g., *Xanthomonas gardneri*, *Xanthomonas perforans*, *Xanthomonas euvesicatoria*, *Xanthomonas oryzae pv oryzae*, *Xanthomonas oryzae pv. oryzicola*, *Xanthomonas hortorum*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Xanthomonas citri*, *Xanthomonas arboricola*, *Xanthomonas asicola*, *Xanthomonas fragariae*, and/or *Xanthomonas sacchari*. Other bacterial pathogens that contain a YopJ effector include, but are not limited to: *Ralstonia solanacearum*, *Acidovorax citrulli*, *Acidovorax konjaci*, *Brenneria goodwinii*, *Pseudomonas amygdali*, *Pseudomonas syringae*, *Pseudomonas coronafaciens*, *Pseudomonas coronafaciens*, and *Erwinia mallotivora*.

In some embodiments, the plant may be a tomato, pepper, citrus, strawberry, walnut, onion, melon, potato, eggplant, banana, geranium, rose, soybean, rice, brassica, or cassava. In particular embodiments, the plant may be a tomato plant that comprises an exogenous polynucleotide encoding the *Solanum pennellii* JIM2 polypeptide of SEQ ID NO: 2.

Methods for making transgenic plants are very well known in the art, as are the choices for promoters and other regulatory regions (see, e.g., US20160076050, US20170218386 and US20160208279). As such, the present plants may be readily implemented by adapting any suitable method. In some embodiments, the exogenous polynucleotide is operably linked to a promoter. The promoter can be exogenous to the plant or endogenous to the plant. In some embodiments, the plant may be made by replacing a

coding sequence in the genome of the plant with the exogenous polynucleotide.

Also provided is a tomato (*Solanum lycopersicum*) plant comprising an exogenous polynucleotide encoding a polypeptide that is at least 80% (at least 85%, at least 90%, at least 95%, at least 98% or 100%) identical to the *Nicotiana benthamiana* ZAR1 polypeptide of SEQ ID NO: 3 or the *Solanum pennellii* ZAR1 polypeptide of SEQ ID NO: 4, wherein the tomato plant is resistant to bacteria that have XopJ4-like effector such as *Xanthomonas*. In these embodiments, the tomato plant may have enhanced resistance to at least one species of *Xanthomonas*, relative to a control plant that is otherwise identical to the plant but does not contain the exogenous polynucleotide. The amino acid sequence of the *Solanum pennellii* ZAR1 polypeptide (SEQ ID NO: 4) is shown below:

MVDAVVTVFLEKLLNVLTEESRFLSQHRQQFEKLKNELLFMQSFLKDAERL
 KRKHTTLKTVMACLRDLIFEAEIELEDQCQNQSADSDGSTRFSTRLHPKRLSHRHQTG
 KRLSEINDKITEIKQNISTYLGVPMLKEGSMEAHNLMTRWTSSLYDHTQVVGLEG
 DTEKIKDWLFEASDGLLAVAFVGMGGLGKTTLAQKVFNERSMENHFERRI WVSVS
 QTFTEEQVMRSILKTLGDACIGDDQGELLRKINQYLLGKRFLIVMDDVWSLDNAW
 WQKIYSGLPKGNSSVIVTTRNELVARKMGVTEARTHWP KFLNEHYSWLLFRKIAF
 AATAGECDFPELEDVGKEIVEKCKGLPLAIKAVGGVMLCKPPYYHEWRIADHFRD
 ELKENDNSVMASLQLSYDELPPYLKSCFLCFSLPEDCVILKDQLIRWWIGESFIPLRS
 GRLSTEVGEDCFSQLSNRCLIEVVDKAYNGVIHTCKMHDMVRDLVIKIADDDSFSTP
 SDANCRHLGINSAMNGKQLLSNRKLRALLTTTKSGEVNKIPSDIAKKFCNSRHLQVL
 DLSKSIFDVPLSSLLEGIGSARQLAYLSLSNTHPLIGVPDSISNLEKLQILDFSQCQNM
 KMLPSCVLTFFVELAILDLNHCGSLEYLPKGLSKLSNLQVLLGFKPAKLSQRGGCRISE
 LRSLTRLRRLSLRLTQDEEIGDDEGNALIGLQELQFLTISCFDSQDDGLVT KLGLYP
 PRQLHELILKFYPGKISPEWLNPTSLPMLRYMSIVSGDMKEMHDNFWGDHSTFWKI
 EGLMLEALTDLRLEWSAINRVMPSLRILKASWCPEVEAFPIEDAGFRGGLWKKEEH
 SHRC

The XopJ4 effector or a YopJ family effector that is recognized by ZAR1 and JIM2, can be selected from XopJ4, PopP1, AvrRxx, AvrBST, and XopJ, for example.

In any embodiment, the plant may be a transgenic plant, meaning that the exogenous polynucleotide has been introduced from the outside, or it may have been made by altering the sequence of a pre-existing gene. Alternatively, in any embodiment, the plant may be cisgenic, meaning that the exogenous polynucleotide has been bred into the plant from a closely related species.

Also provided is a seed of a plant described above. These seeds may be made by

selfing the plant or crossing the plant with another plant of the same species to produce, e.g., hybrid seed.

Also provided is a population of at least 100 of the plants, e.g., at least 1,000, or at least 10,000 of the plants, growing in a field.

Also provided is a method for enhancing the resistance of a plant to a bacterial pathogen that contains a YopJ effector, e.g., at least one species of *Xanthomonas* (such as *Xanthomonas perforans*). In some embodiments, this method may comprise: (a) introducing an exogenous polynucleotide encoding JIM2 polypeptide into a plant cell that is from a plant that is susceptible to infection by the pathogen, e.g., *Xanthomonas* and (b) regenerating a transgenic plant from the plant cell. As noted above, in some embodiments, the JIM2 polypeptide may be at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 98% or 100%) identical to the *Nicotiana benthamiana* JIM2 polypeptide (SEQ ID NO: 1) or *Solanum pennellii* JIM2 polypeptide (SEQ ID NO: 2). This method may further comprise introducing an exogenous polynucleotide encoding a ZAR1 polypeptide into the plant. In these embodiments, the ZAR1 polypeptide may be at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 98% or 100%) identical to the *Nicotiana benthamiana* ZAR1 polypeptide (SEQ ID NO: 3).

As noted above, methods for making plants are very well known in the art, as are the choices for promoters and other regulatory regions (see, e.g., US20160076050, US20170218386 and US20160208279). As such, the present plants may be readily implemented by adapting any suitable method.

EXAMPLES

Aspects of the present teachings can be further understood in light of the following examples, which should not be construed as limiting the scope of the present teachings in any way.

In this study, a forward genetic screen was used to identify components of the XopJ4 perception pathway in the model plant *N. benthamiana*. This effort resulted in the identification of an NLR protein, NbZAR1, with homology to the *Arabidopsis thaliana* protein ZAR1 (AtZAR1). A subsequent reverse genetic screen identified an RLCK XII gene also required for the perception of XopJ4 which was named XOPJ4 IMMUNITY 2 (JIM2). These genes mediate recognition of XopJ4 as well as other YopJ-family effector proteins

and can therefore be used to develop crop varieties with resistance against bacterial pathogens containing these effectors.

Materials and Methods

Genetic mapping in Nicotiana benthamiana using high-throughput sequencing

5 The *N. benthamiana* *zar1-1* mutant was backcrossed to the wild type and the F1 progeny were selfed to create an F2 mapping population. F2 plants were phenotyped by transient expression of XopJ4 using *Agrobacterium* and placed into two separate pools, based on the presence or absence of a cell death response, prior to genomic DNA extraction. Illumina DNA sequencing was performed using one HiSeqX lane with 150 bp paired-end
10 reads for each pool. The reads were mapped to the *N. benthamiana* reference genome (Naim et al., 2012) and SNPs were identified using GATK (DePristo et al., 2011). The SNPs were filtered for mapping quality, possibility of being caused by EMS, and having a large difference in frequency between the mutant and wild type pools (>0.25).

Transient expression

15 *Agrobacterium tumefaciens* strain GV3101 was used for transient expression. The binary plasmids pE1776 (with OCS promoter and UAS for strong expression) (Ni et al., 1995) and pORE E4 (Coutu et al., 2007) were used as expression vectors for the desired genes. The primer sequences used for cloning are listed in Table 1 below. To construct the VIGS-resistant version of JIM2, the codon usage of the region targeted by the JIM2 VIGS
20 construct was altered while conserving the predicted amino acid sequence (Fig. 12). This sequence was subsequently fused to the rest of the JIM2 coding sequence and cloned into a vector for transient expression. The plasmids were transformed into *Agrobacterium* and cultures were grown overnight in LB media with appropriate selection (rifampicin 100 µg / mL, gentamycin 25 µg / mL, kanamycin 50 µg / mL). The cultures were centrifuged,
25 suspended in infiltration buffer (10 mM MgCl₂, 10 mM MES pH 5.6), diluted to the appropriate OD₆₀₀ and infiltrated into leaf tissue using a needleless syringe.

Xanthomonas gene knockout and complementation

For the knockout of XopJ4 in *Xanthomonas perforans* 4B, 1046 bp upstream and 1127 bp downstream of XopJ4 was cloned into the pLVC18 plasmid containing a SacB
30 counter-selectable marker (Lindgren et al., 1986). This plasmid was conjugated into *Xanthomonas perforans* already lacking the XopQ and AvrBsT genes (Schwartz et al., 2015) and selected on NYG (0.5% peptone, 0.3% yeast extract, 2% glycerol) plates containing tetracycline (10 µg / mL). Colonies were screened for a single crossover event at the target

- locus by PCR. Positive colonies were grown overnight and plated on NYG plates with 5% sucrose to select for a second crossover event. Colonies were again screened by PCR to obtain XopJ4 deletion strains. For complementation, the XopJ4 gene including the promoter and terminator was cloned onto the plasmid pVSP61 (obtained from William Tucker, DNA Plant Technology, Oakland CA). This plasmid, which can replicate in *Xanthomonas*, was
- 5 conjugated into *Xanthomonas perforans* and selected for with 25 µg / mL kanamycin.

SEQ ID	Sequence	Purpose
5	TGGTCTCCGAGCATGGTGGACGCAGTG	Forward SIZAR1
6	TGGTCTCCTTGGTCAACACCTATGGCTATATTC	Reverse SIZAR1
7	TGGTCTCCGAGCATGGTGGACGCTGTTGTAAAC	Forward AtZar1
8	TGGTCTCCTTGGTCAGGTTCTGTGCAATG	Reverse AtZar1
9	TGGTCTCCGAGCATGAGCAAGAACAATAAGAAG	Forward AtZed1
10	TGGTCTCCTTGGTCAAGAGAGTTTCTCAATCAA	Reverse AtZed1
11	TGGTCTCCGAGCatgggaaatgtatgcgtc	Forward PsHopZ1a
12	TGGTCTCCTTGGTtagcgctgctcttcg	Reverse PsHopz1a
13	AAGCCTCGGTCTCCGAGCATGGATTGCATAAAGAAGATGTG	Forward part1 NbJIM2
14	AAGCCTCGGTCTCCAAGCGCTGGATATCTGAATTCCAAAC	Reverse part1 NbJIM2
15	AAGCCTCGGTCTCCGCTTgtatacgaggacgcg	Reverse part2 NbJIM2_VigsResist
16	AAGCCTCGGTCTCCCGAAgtatcctggtatccagataagatc	Forward part2 NbJIM2_VigsResist
17	AAGCCTCGGTCTCCTTCGATCCAGAGTACCAATCTT	Forward part3 NbJIM2
18	AAGCCTCGGTCTCCTTGGTTACTCAAATTGCAGGATCTTTC	Reverse part3 NbJIM2
19	AAGCCTCGGTCTCCAAAGCGATGGATTCCGGCATAGT	Forward VIGS GUS
20	AAGCCTCGGTCTCCTTGGTAAGCTTGCATGCCTGCA	Reverse VIGS GUS
21	AAGCCTCGGTCTCCGAGCAGCTGAAGAAAGAGCAGTAT	Forward RLCKXII-1

		(Nbv5tr6201919)
22	AAGCCTCGGTCTCCTTGGTAAGCACACTCTTGAGATGA	Reverse RLCKXII-1
23	AAGCCTCGGTCTCCGAGCATAACCTGCAACAGACATA	Forward RLCKXII-2 (Nbv5tr6223390)
24	AAGCCTCGGTCTCCTTGGCGCAATCTATTTTCCCAAGA	Reverse RLCKXII-2
25	AAGCCTCGGTCTCCGAGCGCACTAGTGTATGAAGATGC	Forward RLCKXII-4 (JIM2) (Nbv5tr6220632)
26	AAGCCTCGGTCTCCTTGGATAGCCAGGAATCCAAATCA	Reverse RLCKXII-4 (JIM2)
27	AAGCCTCGGTCTCCGAGCAGAGTTAACCATGAGGGAAA	Forward RLCKXII-3 (Nbv5tr6217417)
28	AAGCCTCGGTCTCCTTGGAGAAGACACTCCATTGTAGG	Reverse RLCKXII-3
29	atactgcaggagctcGGTACCATGGTGGATGCGGTGGTC	Forward NbZAR1, into pORE E4 KpnI, PvuI gibson
30	tgccaaatgtttgaacgatcgTCAGTTCCTATGTTCTTCCTTC	Reverse NbZAR1, into pORE E4 KpnI, PvuI gibson
31	CTGTTTGCAGACTTAATCTTTG	Sequencing NbZAR1
32	GCTCAGAAAGTCTTCAATGACAA	Sequencing NbZAR1
33	TTCTTGGCAATGTCGGAATG	Sequencing NbZAR1
34	GGAAGCAACTATTGAGCAATCA	Sequencing NbZAR1
35	GCTTATGTTTTTCAATCTCTGGAC	Sequencing NbZAR1
36	GTTTCTTTCACCTGCTCCTT	Sequencing NbZAR1
37	TTCTCATGACTTGTTCTCTCA	Sequencing NbZAR1
38	GAATGGCATAACGGGACG	forward genotyping XpΔXopJ4 knockout
39	ATTGCGGAGAGTTATCAGAA	reverse genotyping XpΔXopJ4 knockout
40	CGCGAAAATGTTTCGTCAAG	reverse genotyping XpΔXopJ4 knockout

41	TTTGTACAAAAAAGCAGGCTCCGCGCGGGACGATCTGGGCACT	forward 5' XpXopJ4 (Complementation and KO fusion)
42	GACTCAACGCATGACGAATGGATCCTTCATCGATCAAGTCCGTATAA	reverse for 5' XpXopJ4 KO fusion
43	TACGGACTTGATCGATGAAGGATCCATTCGTCATGCGTTGAGTC	forward for 3' XpXopJ4 KO fusion
44	TACAAGAAAGCTGGGTCGGCGCGCC CAGAAAGCCGACGCTGCT	reverse 3' XpXopJ4 (Complementation and KO fusion)
45	GTGTATAGATTCCCGCTGAA	Forward primer for sequencing NbJIM2
46	TCTTCCATATTTGGCGAGTC	Forward primer for sequencing NbJIM2
47	TCTCCACTGAGTCTGAAAAC	Reverse primer for sequencing NbJIM2
48	ATGGTCTCCTTGGGCCCATCCTTTCTTTTATGAACA	SpZAR1 part 1 forward (BsaI cloning)
49	ATGGTCTCTGAGACCTATCAGTGCATTCC	SpZAR1 part 1 reverse (BsaI cloning)
50	ATGGTCTCCTCTCCAAGAACTTCAATTCT	SpZAR1 part 2 forward (BsaI cloning)
51	ATGGTCTCCGTCAATTTATGTAACGCTCTCT	SpZAR1 part2 reverse (BsaI cloning)
52	AATGTACTGGGGTGGTTTTGGGCCCCACCCAAAATTTAGCTAATCG	SpJIM2 forward (Gibson cloning Apal, HpaI)
53	AGTCAAATTTTCCGTGATAGTTAACAGTGGACAAGTCAACCTATT	SpJIM2 reverse (Gibson cloning Apal, HpaI)

Bacterial growth assays and visible immune responses

Xanthomonas liquid cultures were grown in NYG media with selection overnight.

Cells were collected by centrifugation, washed once and suspended in 10 mM MgCl₂ to an

5 OD₆₀₀ of 0.0001. Plant leaves were infiltrated by needleless syringe. For the growth assay,

punches were collected from infiltrated leaf tissue at 0 and 6 days post infiltration, homogenized in 10 mM MgCl₂ and serially diluted prior to plating on NYG plates with rifampicin (100 µg / mL) and cycloheximide (50 µg / mL). The plates were incubated at 30 °C for two days and colony counts were obtained to determine the colony forming units for each sample. For visual disease symptoms, the same inoculation conditions were used but the disease was allowed to develop for 14 days before the leaves were photographed. For *Ralstonia solanacearum* disease assays a cut petiole inoculation assay was performed as previously described (Khokhani et al., 2018). Briefly, an overnight *Ralstonia solanacearum* culture was spun down, washed, and resuspended in water. The petiole of the first leaf was cut approximately two centimeters from the stem. Bacterial solution (2 µL, OD₆₀₀ of 0.005) was pipetted onto the cut petiole surface and disease symptoms were photographed at ten days post infiltration.

Viral-induced gene silencing

For VIGS, approximately 300 bp of the target gene was cloned into the TRV2 vector (Liu et al., 2002). This vector was transformed into *Agrobacterium tumefaciens* GV3101. The resulting *Agrobacterium* strain was grown overnight and co-infiltrated with another *Agrobacterium* strain harboring the TRV1 vector at an OD₆₀₀ of 0.2 each by needleless syringe. Plants were infiltrated at approximately four weeks old and used for transient assays two to four weeks after infiltration.

Generation of tomato expressing ZAR1 and JIM2

The *Solanum pennellii* alleles of ZAR1 and JIM2 were cloned with native promoters and terminators. The *Solanum pennellii* ZAR1 gene was cloned in two pieces using primers with sequences ATGGTCTCCTTGGGCCCATCCTTTCTTTTATGAACA (SEQ ID NO:54), ATGGTCTCTGAGACCTATCAGTGCATTCC (SEQ ID NO: 55), ATGGTCTCCTCTCCAAGAACTTCAATTCT (SEQ ID NO: 56), and ATGGTCTCCGTCAATTTATGTAACGCTCTCT (SEQ ID NO: 57) into a derivative of the pORE E4 vector. The *Solanum pennellii* JIM2 gene was amplified and used for Gibson cloning into this plasmid with the restriction enzymes ApaI and HpaI using the primers with sequences AATGTACTGGGGTGGTTTTGGGCCCAACCCAAAATTTAGCTAATCG (SEQ ID NO:58) and AGTCAAATTTTCCGTGATAGTTAACAGTGGACAAGTCAACCTATT (SEQ ID NO: 59). The plasmid was transformed into *Agrobacterium tumefaciens* and tomato transgenics

were generated as previously described (McCormick et al., 1986). Tomato transformants were confirmed by genotyping with PCR to test for the presence of the transgene.

5

Results

Identification of two allelic N. benthamiana mutants impaired in XopJ4 recognition

A forward genetic screen of 2,000 M2 plants from an EMS-mutagenized population of *N. benthamiana* for individuals lacking a cell death response to transiently expressed XopJ4. Two allelic mutants were identified that failed to respond to transiently-expressed XopJ4 (Fig. 1). A mapping by sequencing approach was used to identify the genetic basis of these mutants. Both mutants were found to have mutations in the gene *Nbv5tr6207061*, named NbZAR1 after its Arabidopsis homolog. The mutants, named *zar1-1* and *zar1-2*, had single nucleotide polymorphisms resulting in Q195Stop and T191I changes in the predicted amino acid sequence of NbZAR1 respectively. In addition to lacking an immune response to transiently expressed XopJ4, these mutants were deficient for an immune response to other YopJ-family effector proteins including XopJ, AvrRxx, AvrBsT and PopP1 (Fig. 1., Fig. 3).

Identification of JIM2, a Receptor-Like Cytoplasmic Kinase required for XopJ4 perception

The ZAR1 protein from *Arabidopsis thaliana* interacts with several RLCK XII proteins which are required for the recognition of specific bacterial effectors including ZED1 (HopZ1a recognition) (Lewis et al., 2013), RKS1 (AvrAC recognition) (Wang et al., 2015) and ZRK3 (HopF2a recognition) (Seto et al., 2017). This suggested that an RLCK XII protein may be involved in the ZAR1-mediated recognition of XopJ4. Four RLCK XII genes were identified in the genome of *N. benthamiana* and targeted for silencing by Viral Induced Gene Silencing (VIGS). The silencing of one particular RLCK XII, hereafter named XOPJ4 IMMUNITY 2 (JIM2), compromised the ability of the plant to recognize XopJ4, XopJ, AvrRxx, AvrBsT and PopP1 (Fig. 2, Fig. 3). The YopJ-family effector proteins recognized by NbZAR1 and JIM2 form a clade that is distinct from PsHopZ1a, which is recognized by AtZAR1 and ZED1 (Fig. 4).

N. benthamiana zar1-1 and zar1-2 are deficient for resistance against X. perforans expressing XopJ4

To test whether the avirulence activity of XopJ4 was compromised in the *zar1* mutants, the XopJ4 gene was knocked out in an *X. perforans* (Xp) strain already deficient for XopQ and AvrBsT, as these two effectors trigger avirulence responses in *N. benthamiana* (Schwartz et al., 2015). This knockout strain, along with parental and complemented strains, was infiltrated into *N. benthamiana* leaves at a low inoculum and bacterial growth was assayed by measuring colony forming units at six days post infiltration. Growth of Xp Δ AvrBsT Δ XopQ Δ XopJ4 was found to be approximately 100-fold greater in wild type *N. benthamiana* leaf tissue compared to Xp Δ AvrBsT Δ XopQ and the complemented strain Xp Δ AvrBsT Δ XopQ Δ XopJ4 + XopJ4 (Fig. 5). This indicates that XopJ4 triggers an avirulence response on wild type *N. benthamiana*. No avirulence effect of XopJ4 was observed on the *zar1-1* and *zar1-2* mutants as a similar high level of bacterial growth was observed regardless of the presence of XopJ4 (Fig. 5). Consistent with the growth phenotypes, a visible immune response was observed in wild type *N. benthamiana* plants infiltrated with Xp expressing XopJ4 (Fig. 5). This response was not observed in the *zar1-1* and *zar1-2* mutants.

AtZAR1 and SlZAR1 fail to complement the N. benthamiana zar1-1 mutant

To test whether AtZAR1 is functionally equivalent to NbZAR1, AtZAR1 was transiently expressed in the *zar1-1* mutant along with JIM2 and XopJ4. Whereas transient expression of NbZAR1 was sufficient to restore XopJ4 recognition in the *zar1-1* mutant, expression of AtZAR1 was not (Fig. 6). In contrast, transient expression of AtZAR1 in *zar1-1* was able to complement the immune response triggered by co-expression of ZED1 and HopZ1a. The inability of AtZAR1 to complement the XopJ4 perception defect in *zar1-1* plants indicates a partial functional divergence between NbZAR1 and AtZAR1. Tomato (*Solanum lycopersicum*) contains a putative ZAR1 ortholog but is unable to perceive the XopJ4 effector protein (Astua-Monge et al., 2000). SlZAR1 (Soly02g084890) was cloned and transiently expressed in the *zar1-1* mutant to test if this gene can functionally complement NbZAR1 for XopJ4 perception. Transient expression of SlZAR1, JIM2 and XopJ4 failed to trigger a visible immune response in the *zar1-1* mutant (Fig. 7). A multiple sequence alignment of ZAR1 proteins from various plant species revealed several missense mutations at conserved sites in the SlZAR1 protein which may make the protein nonfunctional (Fig. 8).

A JIM2 homolog from Solanum pennellii can complement N. benthamiana plants deficient for NbJIM2

Solanum pennellii was previously known to be able to recognize XopJ4 and be resistant to *Xanthomonas perforans*. A highly conserved ortholog of NbZAR1 was identified in the genome of *S. pennellii* (Fig. 8) but the closest homolog to NbJIM2 has significant sequence divergence. Transient expression of SpJIM2 in an *N. benthamiana* plant deficient for NbJIM2 revealed that SpJIM2 is indeed functional and able to mediate perception of XopJ4 (Fig. 9).

ZAR1 and JIM2 confer resistance to Xanthomonas perforans

Given that ZAR1 and JIM2 are required for recognition of XopJ4 and resistance to the bacterial pathogen *Xanthomonas perforans* in *Nicotiana benthamiana*, we believed that these genes would work in other plant species to confer resistance to this disease. We transformed ZAR1 and JIM2 from *Solanum pennellii* into tomato. The transformed tomato plants were tested for resistance against *Xanthomonas perforans* by infiltrating a low inoculum of bacteria into leaf tissue. At 14 days post infiltration, wild type tomato leaves had severe disease symptoms as observed by yellow and necrotic lesions in the infiltrated area of the leaves whereas tomatoes expressing ZAR1 and JIM2 appeared healthy (Fig. 10A). Bacterial counts obtained at six days post infiltration indicated that the *Xanthomonas perforans* proliferated to a twenty-five-fold lower bacterial titer on the ZAR1+JIM2 plants than on wild type plants (Fig. 10B). These data indicated that the tomato plants expressing ZAR1 and JIM2 are qualitatively and quantitatively resistant to *Xanthomonas perforans*. This is consistent with resistance being mediated by recognition of the effector protein XopJ4, which is present in *Xanthomonas perforans* strain 4B.

ZAR1 and JIM2 confer resistance to Ralstonia solanacearum

Ralstonia solanacearum is a vascular pathogen and that can cause severe wilting in susceptible plants. We hypothesized that expression of ZAR1 and JIM2 would be sufficient to confer resistance to *Ralstonia solanacearum* strains containing PopP1 or similar effectors recognized by ZAR1 and JIM2. To test this, we expressed ZAR1 and JIM2 in a tomato variety that is otherwise susceptible to this pathogen. Wild type tomato and plants expressing ZAR1+JIM2 were infected with *Ralstonia solanacearum* using the cut petiole inoculation method. At ten days post inoculation, wild type plants were severely wilted whereas tomato plants expressing ZAR1+JIM2 appeared healthy (Fig. 11). These results indicate that ZAR1 and JIM2 can be used to confer resistance against *Ralstonia solanacearum*.

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

That which is claimed is:

1. A plant comprising an exogenous polynucleotide encoding a JIM2 polypeptide.
2. The plant of claim 1, further comprising an exogenous polynucleotide encoding a ZAR1 polypeptide.
3. The plant of any prior claim, wherein the plant has enhanced resistance to at least one species of bacterium that has a XopJ4 effector or a YopJ family effector that is recognized by ZAR1 and JIM2, relative to a control plant that is otherwise identical to the plant but does not contain the exogenous polynucleotide.
4. The plant of any prior claim, wherein the plant has enhanced resistance to at least *Xanthomonas perforans*, relative to a control plant that is otherwise identical to the plant but does not contain the exogenous polynucleotide.
5. The plant of any prior claim, wherein the JIM2 polypeptide is at least 80% identical to the *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2).
6. The plant of any prior claim, wherein the JIM2 polypeptide is at least 90% identical to the *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2).
7. The plant of any prior claim, wherein the JIM2 polypeptide is at least 95% identical to the *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2).
8. The plant of any prior claim, wherein the JIM2 polypeptide is identical to *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2).

9. The plant of any claims 2-8, wherein the ZAR1 polypeptide is at least 80% identical to *Nicotiana benthamiana* ZAR1 (SEQ ID NO: 3).
10. The transgenic plant of any prior claim, wherein the plant is a monocot.
11. The transgenic plant of any prior claim, wherein the plant is a dicot.
12. The plant of any prior claim, wherein the plant is a tomato plant.
13. The plant of any prior claim, wherein the plant comprises an exogenous polynucleotide encoding the *Solanum pennellii* JIM2 polypeptide of SEQ ID NO: 2.
14. The plant of any prior claim, wherein the exogenous polynucleotide is operably linked to a promoter.
15. The plant of claim 14, wherein the promoter is exogenous to the plant.
16. The plant of claim 14, wherein the promoter is endogenous to the plant.
17. A tomato plant comprising an exogenous polynucleotide encoding a polypeptide that is at least 80% identical to the *Nicotiana benthamiana* ZAR1 polypeptide of SEQ ID NO: 3 or the *Solanum pennellii* ZAR1 polypeptide of SEQ ID NO: 4, wherein the exogenous polynucleotide provides resistance to bacteria that have XopJ4-like effector.
18. The tomato plant of claim 17, comprising an exogenous polynucleotide encoding a polypeptide that is at least 90% identical to the *Nicotiana benthamiana* ZAR1 polypeptide of SEQ ID NO: 3 or the *Solanum pennellii* ZAR1 polypeptide of SEQ ID NO: 4.
19. The tomato plant of claim 17, comprising an exogenous polynucleotide encoding a polypeptide that is at least 95% identical to the *Nicotiana benthamiana* ZAR1 polypeptide of SEQ ID NO: 3 or the *Solanum pennellii* ZAR1 polypeptide of SEQ ID NO: 4.
20. The tomato plant of claim 17, comprising an exogenous polynucleotide encoding a polypeptide that is identical to the *Nicotiana benthamiana* ZAR1 polypeptide of SEQ ID

NO: 3 or the *Solanum pennellii* ZAR1 polypeptide of SEQ ID NO: 4.

21. A seed of a plant of any prior claim.
22. A population of at least 100 plants of any of claims 1-20, growing in a field.
23. A method for enhancing the resistance of a plant to at least one species of *Xanthomonas*, comprising:
 - (a) introducing an exogenous polynucleotide encoding a JIM2 polypeptide into the plant cell; and
 - (b) regenerating a transgenic plant from the plant cell.
24. The method of claim 23, wherein the JIM2 polypeptide is at least 80% identical to the *Nicotiana benthamiana* JIM2 (SEQ ID NO: 1) or *Solanum pennellii* JIM2 (SEQ ID NO: 2).
25. The method of claims 23 or 24, further comprising introducing an exogenous polynucleotide encoding a ZAR1 polypeptide into the plant.

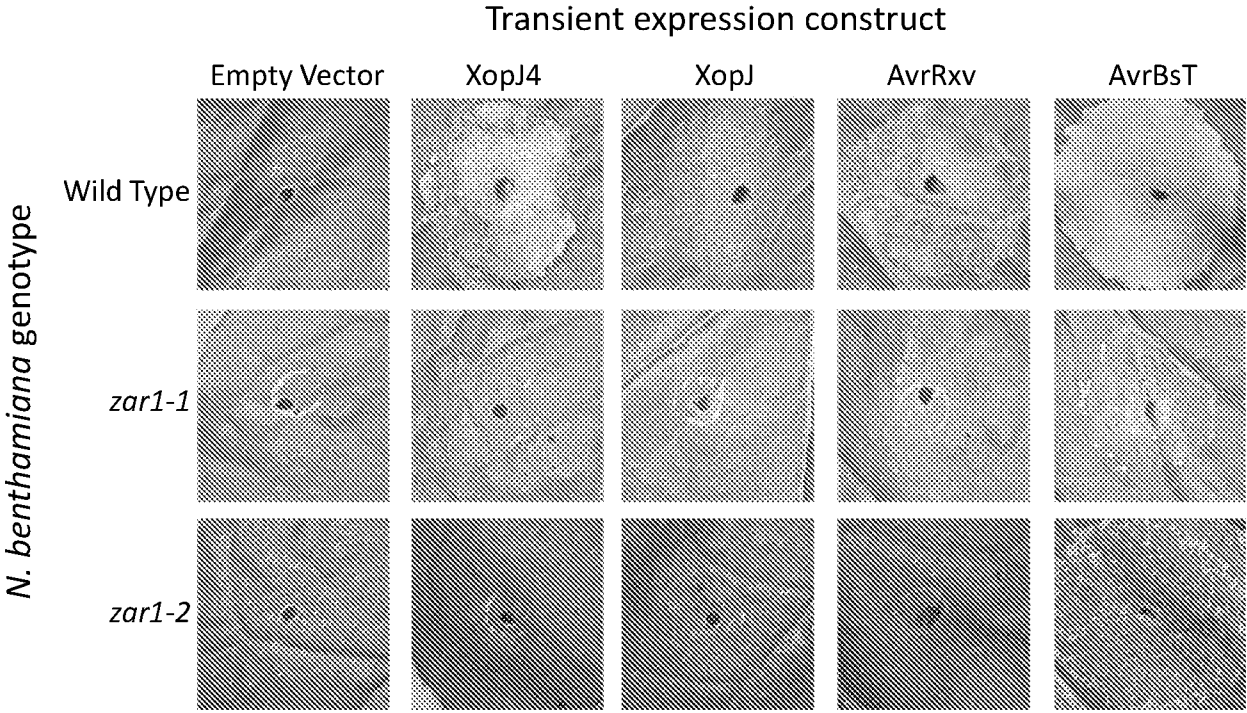


FIG. 1

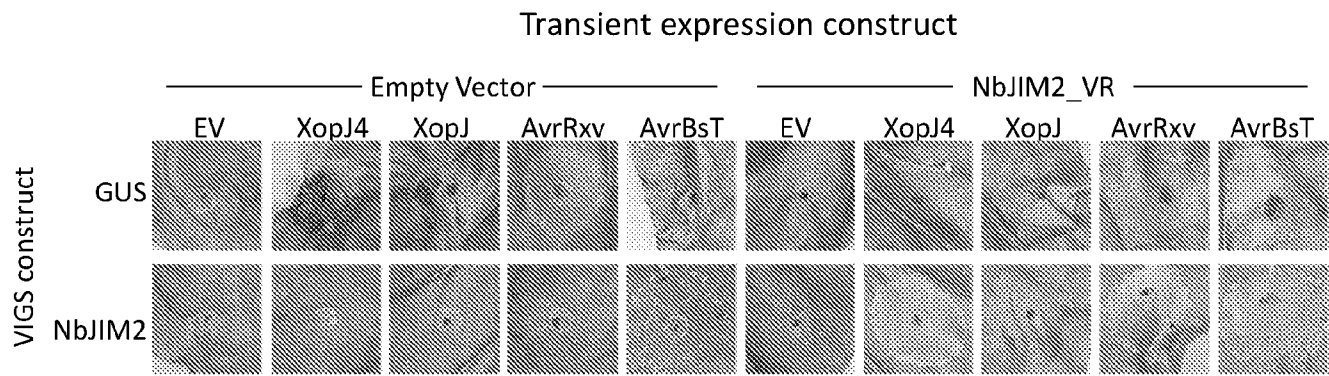


FIG. 2

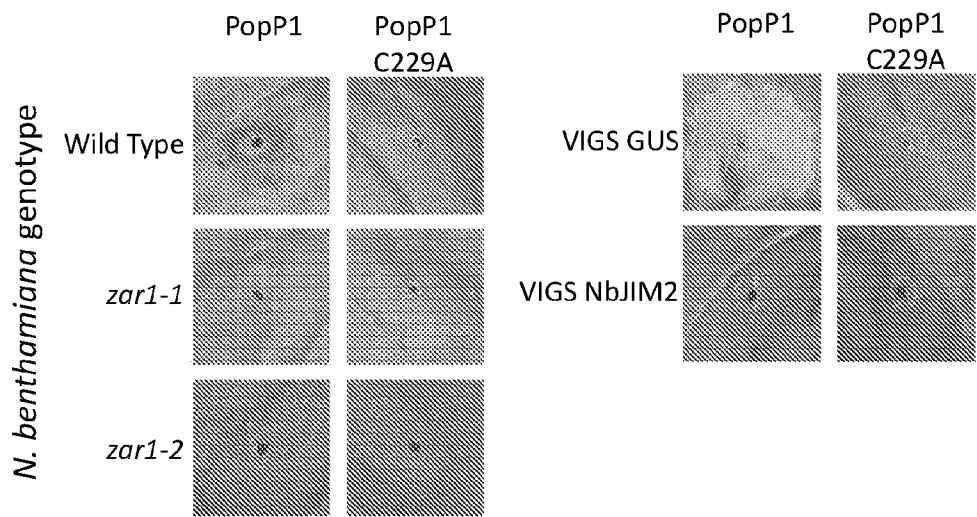


FIG. 3

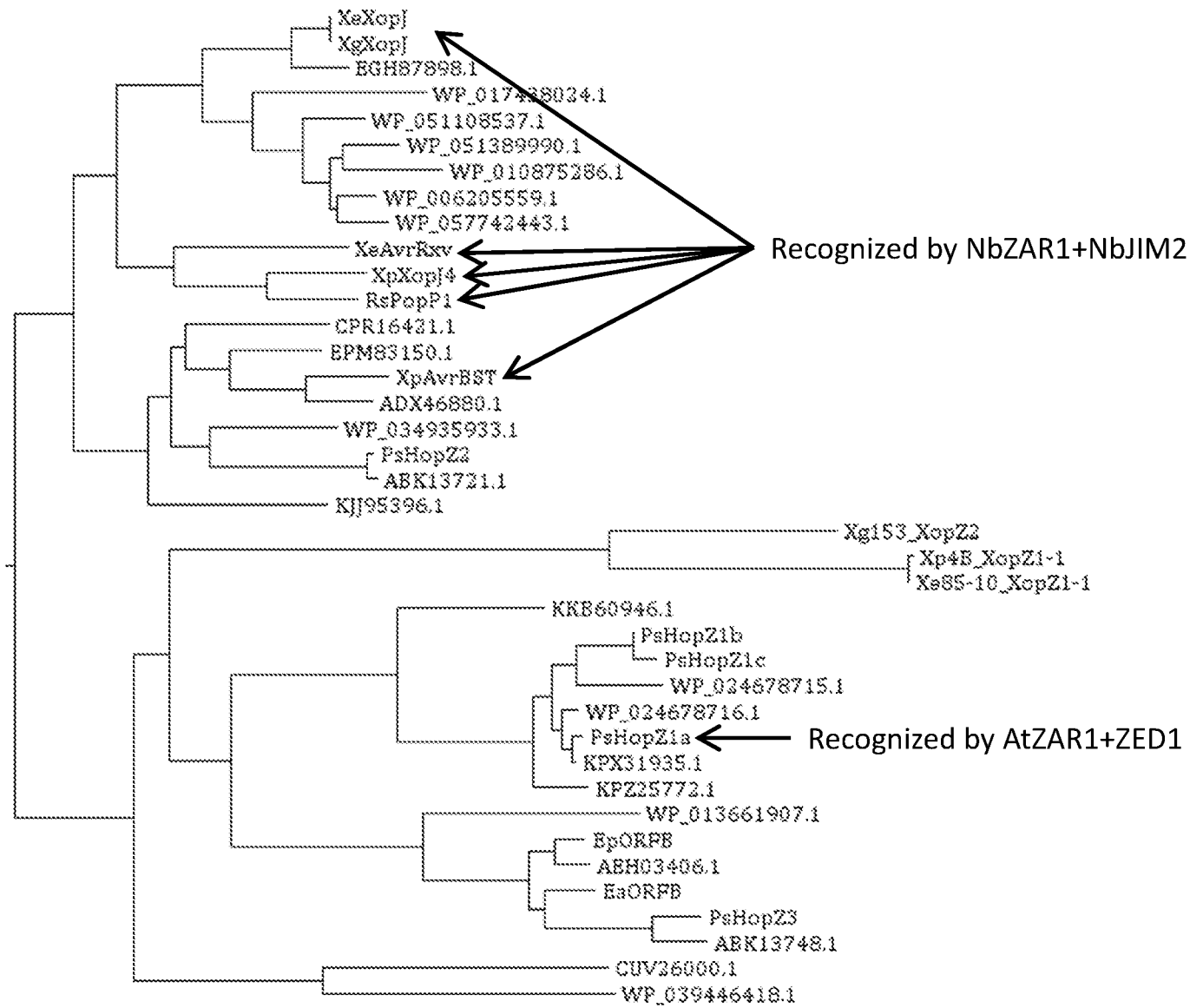


FIG. 4

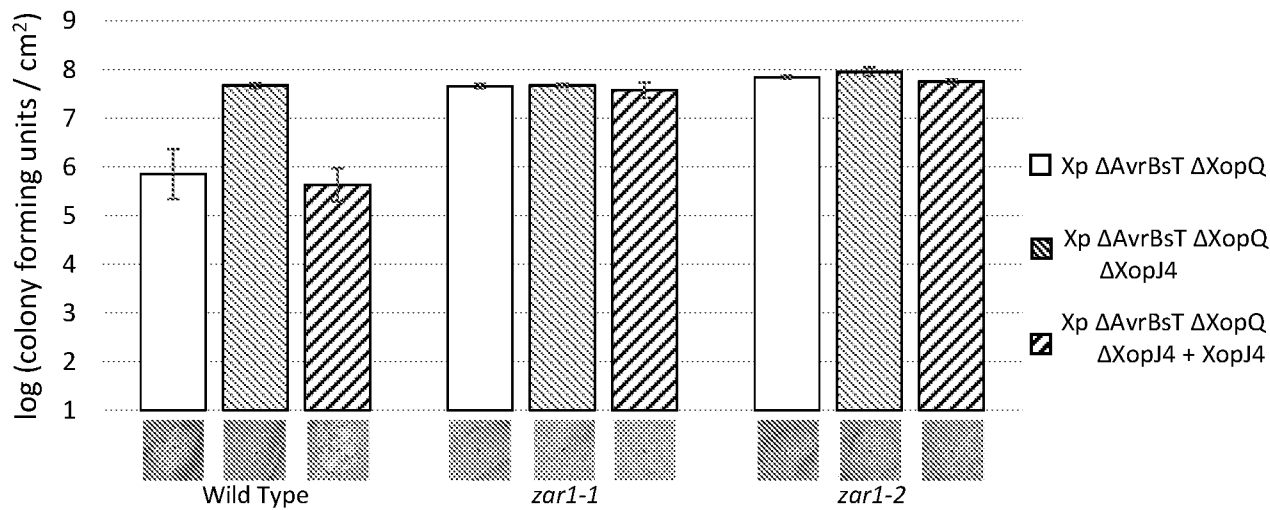


FIG. 5

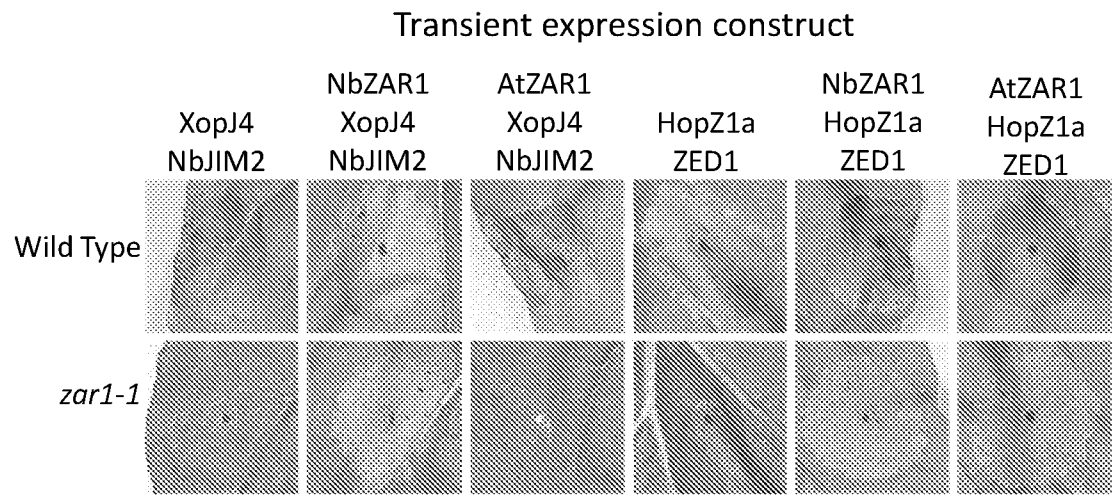


FIG. 6

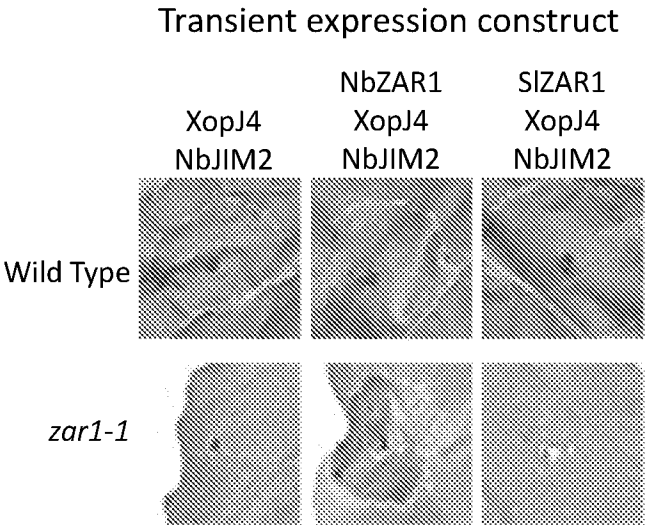
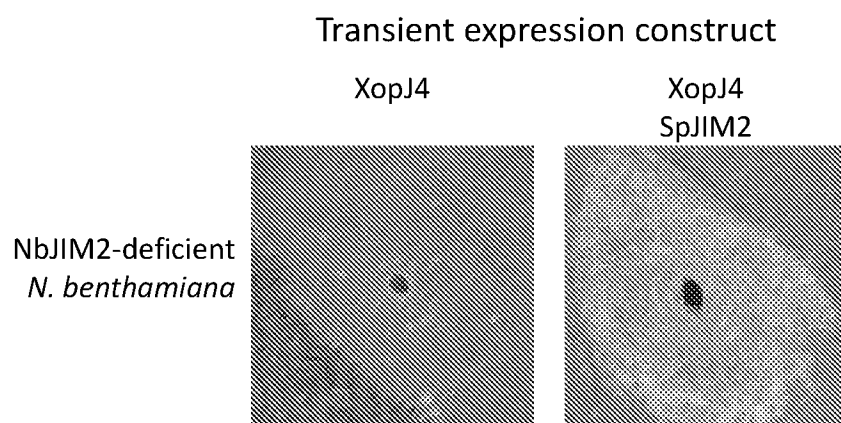


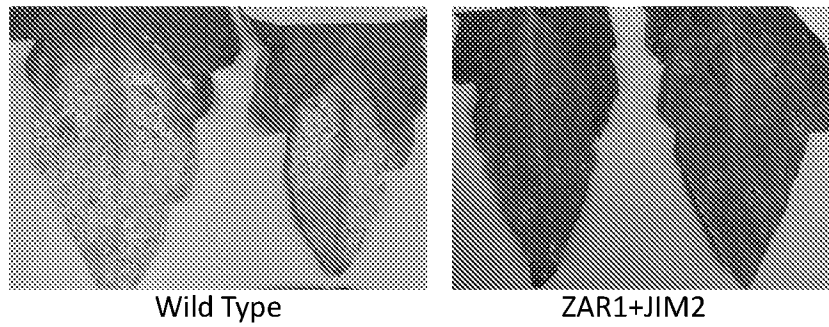
FIG. 7

Bougainvillea-specta
 Echehavia-coccinea_2
 Synssepalum-dulcificou
 Manilkara-zapota_ZET
 Ardisia-humilis_ODDO
 Nerzenea-paniculata
 Phacelia-campanulari
 Heliotropium-mendocini
 Liguistrium-sinense_M2
 Nicotiana-tabacum_X2
 Nicotiana-sylvestris
 Nicotiana-attenuata__
 NBZARI
 Cepaicum-annuum_X2_0
 Solanum-ptycanthum_0
 solanum-tuberosum_X2
 slzari_solyc02g08489
 Solanum-pennellii_X2
 Wrightia-natalensis__
 Apocynum-androsaemif
 Coffea-canephora_CD2
 Psychotria-ipeacacuan
 Centella-asiatice_W2
 Hedera-helix_JEYE-20
 Excoecarpus-cupressifol
 Haekes-prostrata_O307
 Annona-muricata_Y2RI
 Eupomatia-bennettii
 Hibbertia-groenulari__
 Ludovia-ep_vvvv-2008
 Anthurium-annicula_Y
 Platia-estracioides_M

FIG. 8

**FIG. 9**

A. Disease symptoms



B. Bacterial growth

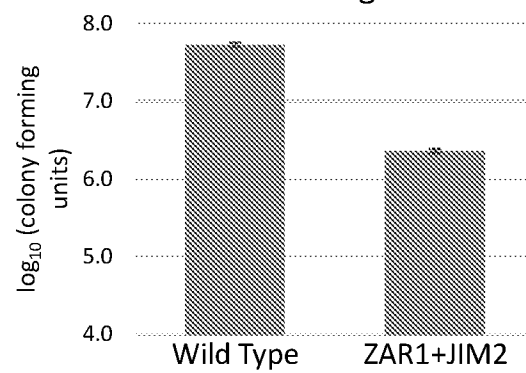


FIG. 10

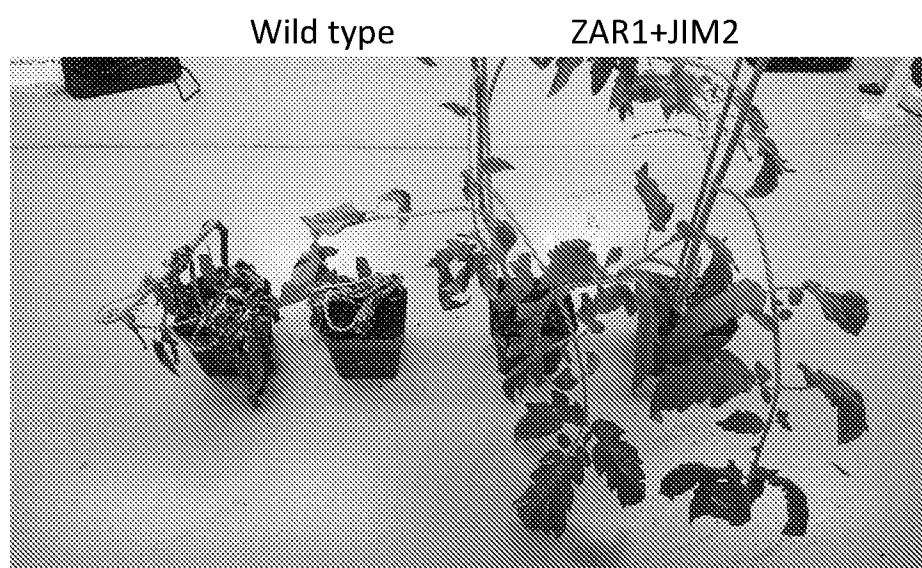


FIG. 11

ATGGATTGCATAAAGAAGATGTGGTCAGTCGTGAAGAAATTTAGGAAGGAAGAAGAAGATGTA
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ACATATGACATTCCAATCCCTAGTTACAGTGCTCAACAACCTTGTTAACGCAACAAACAACCTTC
TCTGGACGTGTCCATGCTAGCACCTACGGTTACATCTGTAGAGGAACTCTGCAAGGCCACTCT
ATCTTCGTCAAAATGTTCAATAATATTCCAGGtaaccaaccatgtgggttctcaaattaattc
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ctttatgggtcaagggttagtgtagcagttgtggttgccctcttcgtgaggttggttctttcgat
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tattcgacttttagtttcgtcataagtttgccgccaggggaactcaagggttgaggacgatctta
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AAGCTTTCATTGATATCGCTTTAAGATGTGTCCAGGCTAAGGGAGAAAATAGACCAGATATGT
TCGAGATTGCAAGAAAGATCCTGCAATTTGAGGATTATAAGGACCATGACGGAGACTATAAGG
ACCATGACCTCGATTATAAGGACGATGACGATAAGTAG

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/033962

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 9/12; C12N 15/82 (2019.01)

CPC - C12N 9/1205; C12N 15/8279; C12N 15/8281 (2019.08)

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

USPC - 435/194; 800/301 (keyword delimited)

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.
X	SHARLACH, M. "Mapping and Identification of the RXopJ4 Resistance Gene and the Search for	1, 3
--	New Sources of Durable Resistance to Bacterial Spot Disease of Tomato," University of	-----
Y	California at Berkeley, Dissertation, 31 December 2013 (31.12.2013), Pgs. 1-89. Retrieved from the Internet: <https://escholarship.org/uc/item/1zx6v1gg> on 08 August 2019 (08.08.2019).	2, 23, 25
Y	RUFIAN et al. "Auto-acetylation on K289 is not essential for HopZ1a-mediated plant defense suppression," Frontiers in Microbiology, 08 July 2015 (08.07.2015), Vol. 6, Art. 684, Pgs. 1-12. entire document	2, 25
Y	US 2004/0016029 A1 (WEI et al) 22 January 2004 (22.01.2004) entire document	23, 25
P, X	SCHULTINK et al. "Using forward genetics in Nicotiana benthamiana to uncover the immune signaling pathway mediating recognition of the Xanthomonas perforans effector XopJ4," New Phytologist, 29 August 2018 (29.08.2018), Vol. 221, No. 2, Pgs. 1001-1009. entire document	1-3, 17-20, 23-25
A	US 2010/0043097 A1 (WANG et al) 18 February 2010 (18.02.2010) entire document	1-3, 17-20, 23-25
A	ASTUA-MONGE et al. "Xv4-vrxv4: A New Gene-for-Gene Interaction Identified Between Xanthomonas campestris pv. Vesicatoria Race T3 and the Wild Tomato Relative Lycopersicon pennellii," Molecular Plant-Microbe Interactions, 19 February 2007 (19.02.2007), Vol. 13, No. 12, Pgs. 1346-1355. entire document	1-3, 17-20, 23-25

☒ 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

"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

08 August 2019

Date of mailing of the international search report

03 SEP 2019

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/033962

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DESLANDES et al. "Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus," Proceedings of the National Academy of Sciences, 24 June 2003 (24.06.2003), Vol. 100, No. 13, Pgs. 8024-8029. entire document	1-3, 17-20, 23-25
A	SHARLACH et al. "Fine genetic mapping of RXopJ4, a bacterial spot disease resistance locus from Solanum pennellii LA716," Theoretical and Applied Genetics, 02 November 2012 (02.11.2012), Vol. 126, No. 3, Pgs. 601-609. entire document	1-3, 17-20, 23-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/033962

Box No. 1 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 13ter.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 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.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: 1-4 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/033962

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.: 4-16, 21, 22
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:

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.:

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.