

1 Evolutionary trade-offs at the *Arabidopsis* *WRR4A* resistance locus 2 underpin alternate *Albugo candida* recognition specificities

3 Baptiste Castel^{1,2}, Sebastian Fairhead^{1,3}, Oliver J. Furzer^{1,4}, Amey Redkar^{1,5},
4 Shanshan Wang¹, Volkan Cevik^{1,6}, Eric B. Holub³, Jonathan D. G. Jones¹

5 1 The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, NR4 7UH, Norwich,
6 United Kingdom

7 2 Department of Biological Sciences, National University of Singapore, Singapore 117558

8 3 Warwick Crop Centre, School of Life Sciences, University of Warwick, CV35 9EF, Wellesbourne,
9 United Kingdom

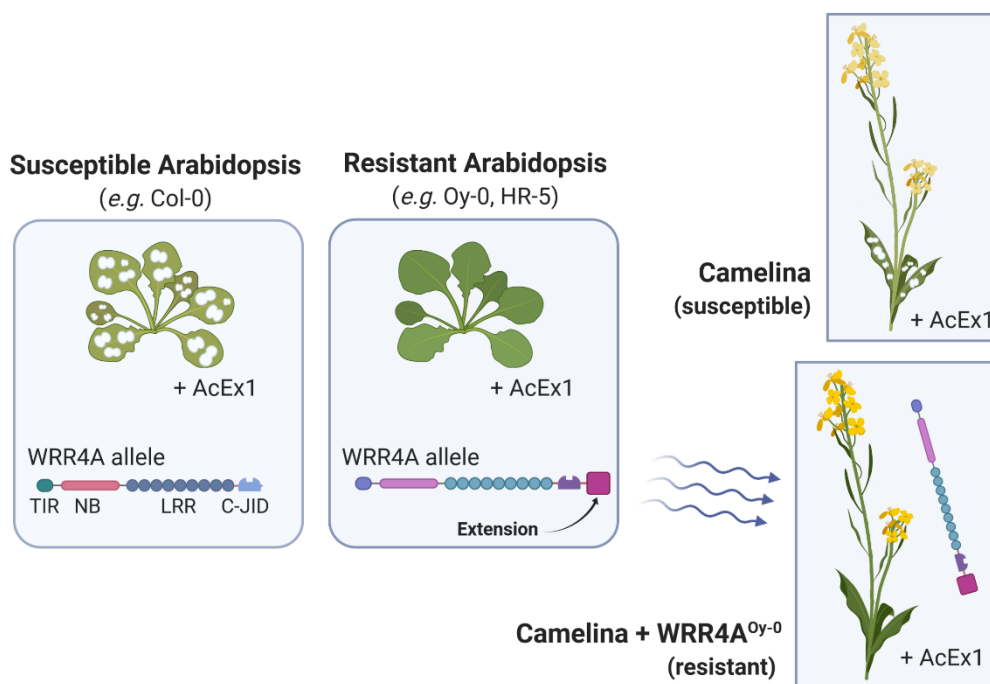
10 4 Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

11 5 Department of Genetics, University of Cordoba, 14071 Cordoba, Spain

12 6 The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, BA2
13 7AY Bath, United Kingdom

14

15 Graphical abstract:



16

17

18 **Summary:**

19 The oomycete *Albugo candida* causes white rust of Brassicaceae, including vegetable and
20 oilseed crops, and wild relatives such as *Arabidopsis thaliana*. Novel *White Rust Resistance*
21 (*WRR*)-genes from *Arabidopsis* enable new insights into plant/parasite co-evolution. *WRR4A*
22 from *Arabidopsis* accession Col-0 provides resistance to many but not all white rust races,
23 and encodes a nucleotide-binding (NB), leucine-rich repeat (LRR) (NLR) immune receptor
24 protein. Col-0 *WRR4A* resistance is broken by a Col-0-virulent isolate of *A. candida* race 4
25 (AcEx1). We identified an allele of *WRR4A* in *Arabidopsis* accession Oy-0 and other
26 accessions that confers full resistance to AcEx1. *WRR4A^{Oy-0}* carries a C-terminal extension
27 required for recognition of AcEx1, but reduces recognition of several effectors recognized by
28 the *WRR4A^{Col-0}* allele. *WRR4A^{Oy-0}* confers full resistance to AcEx1 when expressed as a
29 transgene in the oilseed crop *Camelina sativa*.

30

31 **Key-words:**

32 immunity, resistance gene, NLR, natural variation, evolution, effector recognition, crop
33 protection, *Arabidopsis thaliana*, camelina,

34

35 **Significance:**

36 A C-terminal extension in an allele of the *Arabidopsis* resistance-protein *WRR4A* changes
37 effector recognition specificity, enabling the *WRR4A^{Oy-0}* allele to confer immunity to *Albugo*
38 *candida* races that overcome the *WRR4A^{Col-0}* allele. This resistance can be transferred to the
39 oil-producing crop *Camelina sativa*.

40

41 **Introduction:**

42 Plants have evolved powerful defense mechanisms that can arrest attempted colonization by
43 microbial pathogens. Timely defense activation requires perception of pathogen-derived
44 molecules by cell-surface pattern-recognition receptors (PRRs) and intracellular Nucleotide-
45 binding (NB), Leucine-rich repeat (LRR), or NLR, immune receptors (Jones and Dangl, 2006).
46 Extensive NLR genetic diversity within plant populations is associated with robustness of NLR-

47 mediated immunity (Baggs *et al.*, 2017), and plant NLR sequences reveal diversifying
48 selection on NLR genes compared to other genes (Kuang *et al.*, 2004; Monteiro and
49 Nishimura, 2018; Meyers *et al.*, 1998). To investigate NLR diversity, next-generation
50 sequencing technologies were combined with sequence capture to develop *Resistance (R)-*
51 *gene enrichment sequencing (RenSeq)* (Jupe *et al.*, 2013). This method has shed new light
52 on NLR repertoires in several plant genomes including tomato, potato and wheat (Andolfo *et*
53 *al.*, 2014; Witek *et al.*, 2016; Steuernagel *et al.*, 2016). A comparison of 64 *Arabidopsis*
54 *thaliana* (*Arabidopsis*) accessions using RenSeq documented NLR sequence diversity within
55 a single species, revealing the *Arabidopsis* "pan-NLRome" (Van de Weyer *et al.*, 2019). Each
56 *Arabidopsis* accession contains 150-200 NLR-encoding genes. About 60% are found in
57 clusters (within 200 kb from each other) that show copy number variation (Lee and Chae,
58 2020). From all the NLRs of the 64 accessions, 10% are singletons and the rest are distributed
59 between 464 orthogroups. Each accession contains a unique subset comprising, on average,
60 25% of the orthogroups.

61 NLRs vary in their intramolecular architecture. Plant NLR proteins usually display either a "Toll,
62 Interleukin-1, *R*-gene" (TIR), a "Coiled-Coil" (CC) or "Resistance to Powdery mildew 8"
63 (RPW8) N-terminal domain, a central NB domain and a C-terminal LRR domain. Some NLRs
64 also comprise a post-LRR (PL) domain. For example, RRS1 is an *Arabidopsis* TIR-NLR with
65 PL WRKY domain required to detect the effectors AvrRps4 (from the bacterium *Pseudomonas*
66 *syringae*) and PopP2 (from the bacterium *Ralstonia solanacearum*). The integrated WRKY is
67 called a decoy as it mimics the authentic AvrRps4 and PopP2 effector targets (Sarris *et al.*,
68 2015; Le Roux *et al.*, 2015). Several other integrated decoy domains have been described
69 (Cesari, 2017). RPP1 and Roq1, two TIR-NLRs from *Arabidopsis* and *Nicotiana benthamiana*
70 respectively, form tetrameric resistosomes upon activation. In this structure, a PL C-terminal
71 jelly-roll/Ig-like domain (C-JID) physically binds the cognate effector, along with the LRR
72 domain (Ma *et al.*, 2020; Martin *et al.*, 2020). Analysis of NLR integrated domains can
73 potentially reveal novel effector targets (Kroj *et al.*, 2016).

74 *A. candida* causes white blister rust in Brassicaceae and serious annual yield losses in
75 brassica crops such as oilseed mustard (*Brassica juncea*) in India (Gupta *et al.*, 2018). It
76 comprises several host-specific subclades, which includes race 2 from *B. juncea*, race 7 from
77 *Brassica rapa*, race 9 from *Brassica oleracea* and race 4 from crop relatives (*e.g.*, *Capsella*
78 *bursa-pastoris*, *Arabidopsis spp.* and *Camelina sativa*) (Jouet *et al.*, 2018; Pound and
79 Williams, 1963). These have been proposed to evolve by rare recombination events that
80 occurred between the races, followed by clonal propagation on susceptible hosts (McMullan
81 *et al.*, 2015). The *Arabidopsis* Columbia (Col-0) allele of *WRR4A* can confer resistance to
82 isolates of all four races (Borhan *et al.*, 2010; Borhan *et al.*, 2008). The allele encodes a

83 canonical TIR-NLR and belongs to an orthogroup of three genes in Col-0 at the same locus.
84 The accession Ws-2 (susceptible to *A. candida* race 4) lacks *WRR4A* but contains the two
85 other paralogs, illustrating intra-species copy number variation within clusters. Interestingly,
86 one of these paralogs, *WRR4B*, also confers resistance to the Ac2V isolate of race 2 (Cevik
87 *et al.*, 2019). In addition, the CC-NLR-encoding *BjuWRR1*, which confers resistance to several
88 *A. candida* isolates collected on *B. juncea*, was mapped and cloned from the European
89 accession of *B. juncea* Donskaja-IV (Arora *et al.*, 2019).

90 Several Col-0-virulent isolates of *A. candida* race 4 have been collected from naturally infected
91 Arabidopsis plants. They were used subsequently to identify an alternative source of broad-
92 spectrum white rust resistance. One was found in Arabidopsis accession Oy-0 that mapped
93 to the *WRR4* locus (Fairhead, 2016). Interestingly, one of these isolates (AcEx1), as well as
94 the related white rust pathogen *Albugo laibachii*, were shown to suppress non-host resistance
95 in Arabidopsis to the potato late blight pathogen *Phytophthora infestans* (Prince *et al.*, 2017;
96 Belhaj *et al.*, 2017). Thus, we set out to clone the gene conferring AcEx1 resistance in Oy-0,
97 and characterise the corresponding pathogen effector(s).

98 AcEx1 is also virulent in *Camelina sativa*, which is an emerging oilseed crop and has been
99 engineered to provide an alternative source of fish-oil-derived long chain omega-3
100 polyunsaturated fatty acids (LC-PUFAs). Algal-derived genes were expressed in the seed to
101 produce eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as an improved LC-
102 PUFA source for fish feed or as a human nutritional supplement (Ruiz-Lopez *et al.*, 2014;
103 Petrie *et al.*, 2014). EPA and DHA, recognised for their health benefits, are mainly sourced
104 from oily fish, and are acquired from feeding on algae-consuming plankton. In salmon farming,
105 the major source for of omega-3 oil derives from oceanic fish. Industry collects 750,000 metric
106 tons of fish oil every year, raising sustainability concerns (Napier *et al.*, 2015). Transgenic
107 camelina oil is equivalent to fish oil for salmon feeding and for human health benefits (Betancor
108 *et al.*, 2018; West *et al.*, 2019). Despite challenges to distribute a product derived from a
109 genetically modified crop (Napier *et al.*, 2019), an increase in camelina cultivation can be
110 expected in the near future. Fields of *C. sativa* will be exposed to *A. candida* and early
111 identification of *R*-genes will enable crop protection.

112 In this study we identified two alleles of *WRR4A* conferring full resistance to AcEx1 from
113 Arabidopsis accessions Oy-0 and HR-5. They both encode proteins with a C-terminal
114 extension compared to the Col-0 *WRR4A* allele. This extension enables recognition of at least
115 one effector from AcEx1. We propose that *WRR4A*^{Oy-0} is the ancestral state, and that in the
116 absence of AcEx1 selective pressure, an early stop codon in *WRR4A* generated the Col-0-
117 like allele, enabling more robust recognition of other *A. candida* races while losing recognition

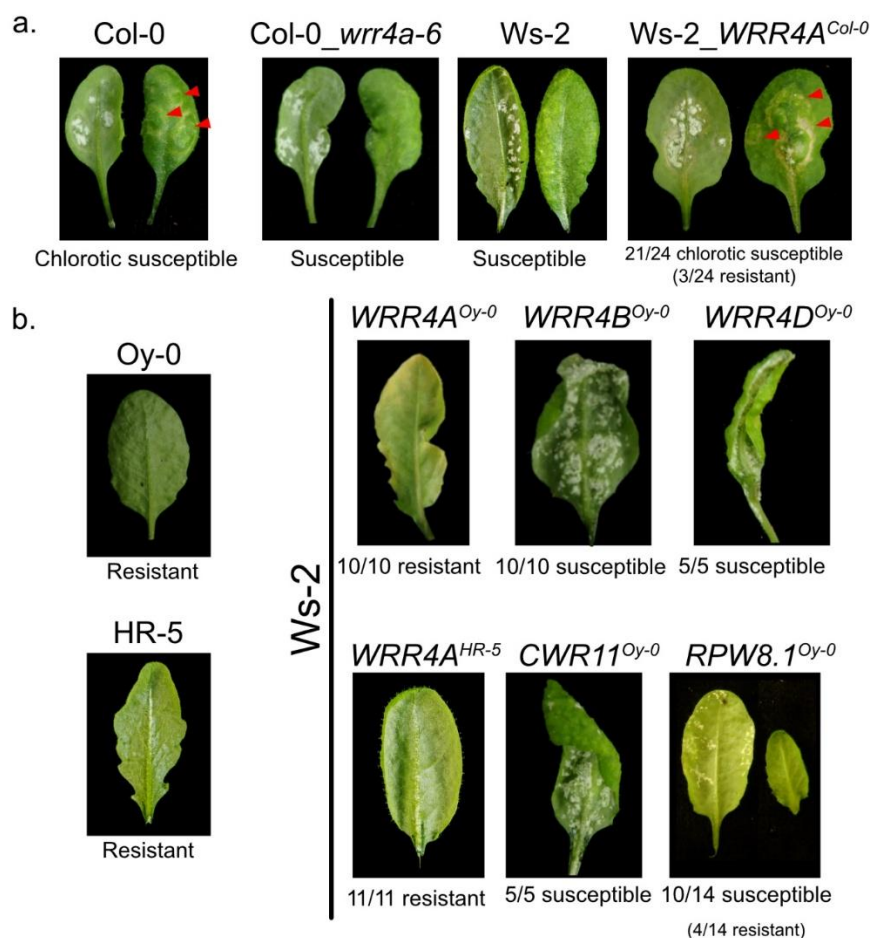
118 of AcEx1. Finally, we successfully transferred *WRR4A*^{Oy-0}-mediated resistance to AcEx1 from
 119 Oy-0 into *Camelina sativa*.

120

121 **Results:**

122 ***Resistance to AcEx1 is explained by WRR4A alleles of HR-5 and Oy-0***

123 AcEx1 growth on Col-0 results in chlorosis that is not seen in the fully susceptible accession
 124 Ws-2 (**Figure 1a**). Since *WRR4A* confers resistance to all other *A. candida* races tested and
 125 Ws-2 lacks *WRR4A*, we tested if the chlorotic response could be explained by *WRR4A*, by
 126 testing a Col-0_*wrr4a-6* mutant, and found that it shows green susceptibility to AcEx1. We
 127 also tested Ws-2 transgenic lines carrying *WRR4A* from Col-0 and observed chlorotic
 128 susceptibility (**Figure 1a**). Thus, *WRR4A* from Col-0 weakly recognises AcEx1 and provides
 129 partial resistance. However, AcEx1 is still able to complete its life cycle on Col-0.



130

131 **Figure 1: Oy-0 and HR-5 alleles of WRR4A confers full resistance to AcEx1**

132 5-week old plants were sprayed inoculated with AcEx1. Plants were phenotyped 14 days after inoculation. **a.** Col-
 133 0 and Ws-2_*WRR4A*^{Col-0} present a chlorotic susceptible, indicated by red arrows; Col-0_*wrr4a-6* and Ws-2 do not.

134 b. Oy-0 and HR-5 are fully resistant, as well as *Ws-2_WRR4A^{Oy-0}* and *Ws-2_WRR4A^{HR-5}*. *Ws-2* expressing
135 *WRR4B^{Oy-0}*, *WRR4D^{Oy-0}* or *CWR11^{Oy-0}* are fully susceptible. *Ws-2* lines expressing *RPW8.1^{Oy-0}* are generally
136 susceptible, 4/14 showed dwarfism and resistance. Numbers indicate the number lines showing similar
137 phenotype out of the number of plants tested.

138

139 In a search for more robust sources of AcEx1 resistance, we tested 283 Arabidopsis
140 accessions (**Table S2**). We identified 57 (20.1%) fully resistant lines, including Oy-0 and HR-
141 5. We phenotyped 278 Recombinant Inbred Lines (RILs) between Oy-0 (resistant) and Col-0
142 (susceptible) and conducted a quantitative trait locus (QTL) analysis that revealed one major
143 QTL on chromosome 1 and two minor QTLs on chromosomes 3 and 5 (**Figure S1a**). All loci
144 contribute to resistance, with a predominant contribution of the QTL on chromosome 1 (see
145 Figure 3.7 of Fairhead, 2016). We did not investigate the minor QTL on chromosome 5. Fine
146 mapping on chromosome 1 and 3 QTLs refined the QTL boundaries (**Figure S2 and S3**, see
147 Experimental Procedures). Based on sequence identity between the QTL in Col-0 and in an
148 Oy-0 RenSeq dataset (Van de Weyer *et al.*, 2019), we identified four NLRs associated with
149 the QTLs in Oy-0: three TIR-NLR paralogs on chromosome 1 (*WRR4A*, *WRR4B* and one
150 absent in Col-0 that we called *WRR4D*) and a CC-NLR absent in Col-0 on chromosome 3 (that
151 we called *Candidate to be WRR11*, *CWR11*) (**Figure S1B-C**).

152 We expressed these genes, with their own promoters and terminators, in the fully susceptible
153 accession *Ws-2*. Only *WRR4A^{Oy-0}* conferred full resistance (**Figure 1b**). *CWR11*, the only NLR
154 from the *WRR11* locus, does not confer AcEx1 resistance. The *WRR11* locus is orthologous
155 to the position 17.283-18.535 Mb on chromosome 3 of Col-0 (**Figure S3**). The *RPW8* alleles
156 of Col-0 are encoded between positions 18.722-18.734 Mb. Oy-0 carries 15 copies of *RPW8*
157 (Van de Weyer *et al.*, 2019), so it could be that some copies recombined on the other side of
158 the *WRR11* locus. We cloned one homolog of *RPW8.1* from Oy-0 and expressed it with its
159 own promoter and terminator in *Ws-2* (**Figure 1**). Most transgenic lines were susceptible to
160 AcEx1 but four lines were resistant. These lines were also smaller than the other lines,
161 suggesting an autoimmune phenotype. Since most transgenic lines are susceptible and
162 ectopic expression of *RPW8* is known to result in autoimmunity (Xiao *et al.*, 2003), we did not
163 further investigate the role of *RPW8^{Oy-0}* paralogs in AcEx1 resistance. The gene underlying
164 *WRR11* locus resistance remains unknown.

165 We conducted a bulk segregant analysis using an F2 population between HR-5 (resistant) and
166 *Ws-2* (susceptible). RenSeq on bulked F2 susceptible segregants revealed a single locus on
167 chromosome 1, that maps to the same position as the chromosome 1 QTL in Oy-0 (**Figure**
168 **S4A**). Since *WRR4A^{Oy-0}* confers resistance to AcEx1, we expressed its HR-5 ortholog, in

169 genomic context, in the fully susceptible accession Ws-2, and found that *WRR4A*^{HR-5} also
170 confers full resistance to AcEx1 (**Figure 1b**).

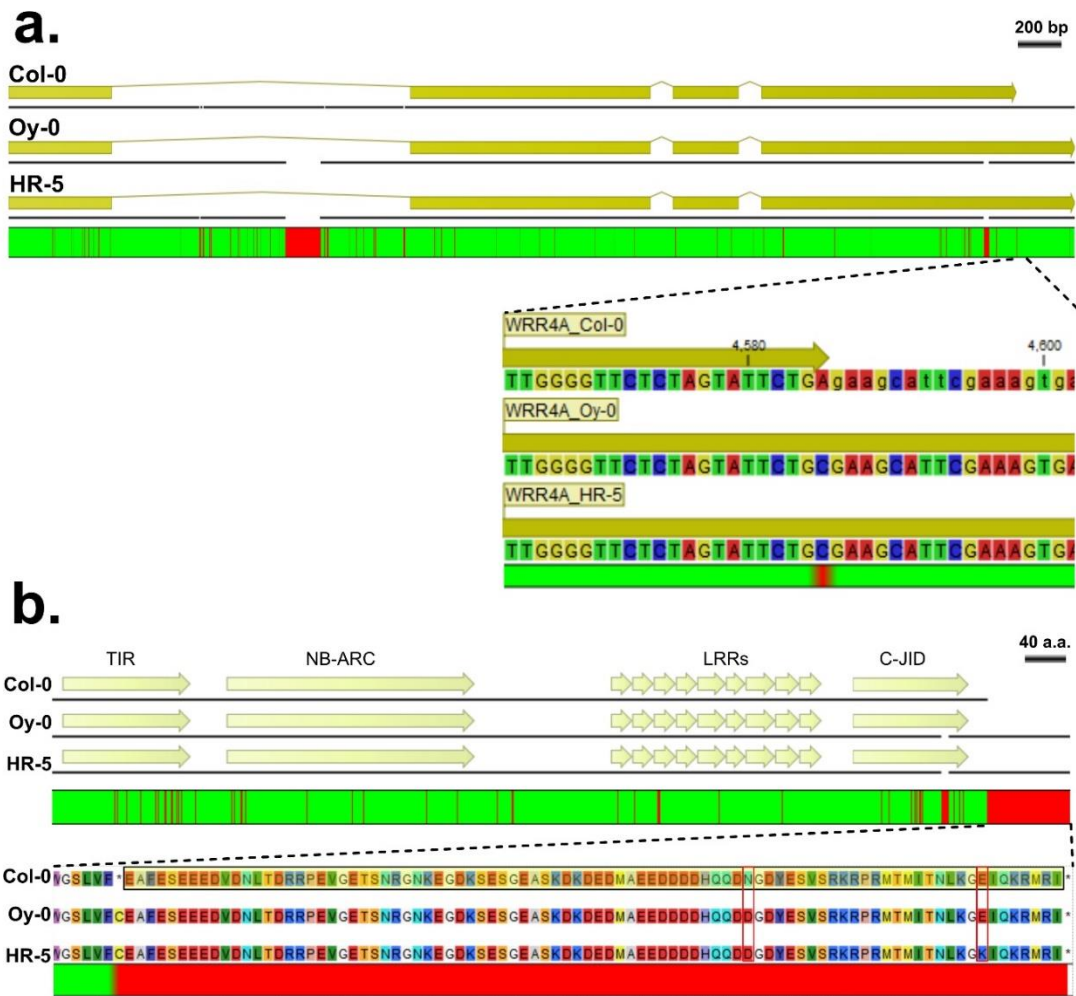
171 In conclusion, *WRR4A* from Col-0 can weakly recognise AcEx1 but does not provide full
172 resistance. We identified two *WRR4A* alleles, in Oy-0 and HR-5, that confer complete AcEx1
173 resistance.

174

175 ***WRR4A*^{Col-0} carries an early stop codon compared to *WRR4A*^{Oy-0}**

176 To understand why the Oy-0 and HR-5 alleles of *WRR4A* confer full resistance to AcEx1, while
177 the Col-0 allele does not, we compared the gene and protein sequences (**Figure 2**). First, we
178 defined the cDNA sequence of *WRR4A*^{Oy-0}. The splicing sites are identical between the two
179 alleles. There are 46 polymorphic amino acids between Col-0, HR-5 and Oy-0. Col-0 shares
180 96.03% identity with Oy-0 and 96.23% with HR-5, while Oy-0 and HR-5 share 97.15% identity.
181 *WRR4A*^{Col-0} carries a 156-nucleotide insertion in the first intron compared to Oy-0 and HR-5.
182 A striking polymorphism is a TGC->TGA mutation in *WRR4A*^{Col-0}, resulting in an early stop
183 codon compared to *WRR4A*^{Oy-0} and *WRR4A*^{HR-5} (**Figure 2**), located 178 amino acids after the
184 last LRR, resulting in an 89 amino acid extension in *WRR4A*^{Oy-0} and *WRR4A*^{HR-5}. The
185 nucleotide sequence for this extension is almost identical between HR-5, Oy-0 and Col-0 (two
186 polymorphic sites). Thus, by mutating TGA to TGC in Col-0, we could engineer an allele with
187 the extension, that we called *WRR4A*^{Col-0_LONG} (**Figure 3a**). By mutating TGC to TGA in Oy-0,
188 we could engineer an Oy-0 allele without the extension, that we called *WRR4A*^{Oy-0_SHORT}. We
189 expressed these alleles, as well as the WT Col-0 and Oy-0 alleles, with their genomic context,
190 in the AcEx1-compatible accession Ws-2. None of the *WRR4A*^{Col-0_LONG} and *WRR4A*^{Oy-0_SHORT}
191 transgenic seeds germinated, suggesting autoactivity in Arabidopsis. We tried to generate
192 Arabidopsis Col-0 lines with *WRR4A*^{Col-0_STOP} using CRISPR adenine base editor (see
193 Experimental Procedures). Out of 24 transformed plants, none displayed editing activity at all.
194 Thus, we did not generate stable *WRR4A* stop codon mutants in Arabidopsis. However, we
195 cloned these alleles under the control of the 35S promoter and the Ocs terminator for transient
196 overexpression in *N. tabacum*.

197

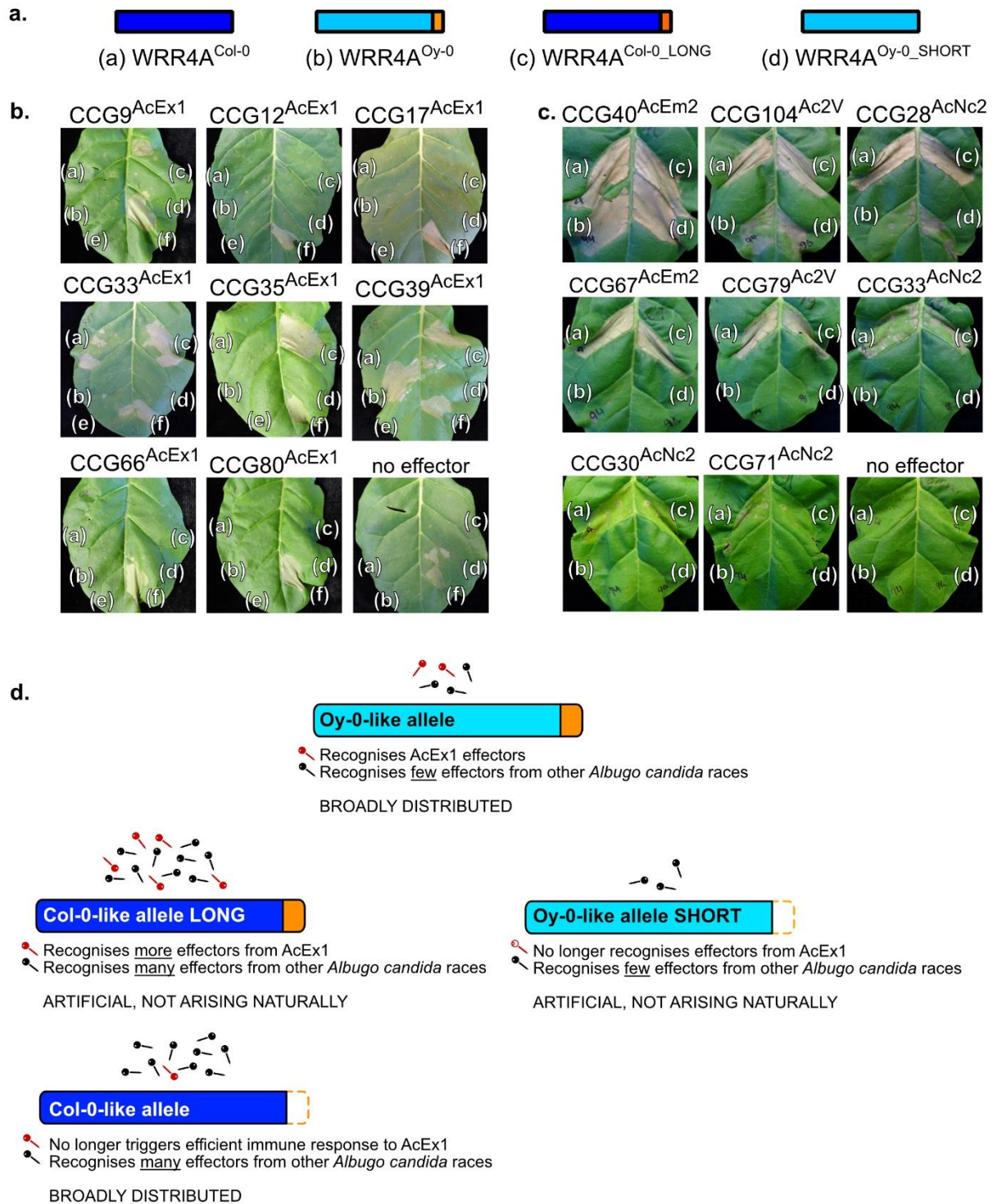


198

199 **Figure 2: Allelic variation between Col-0 and Oy-0 alleles of WRR4A**

200 **a.** Nucleotide sequence alignment from ATG to TGA. Plain yellow areas represent exons. Yellow lines represent
 201 introns. bp: base pair **b.** Amino acid alignment. a.a: amino acid. The C-terminal extension is framed in yellow for
 202 Col-0 to indicate that an early stop codon avoids translation of this sequence. **a.b.** Cartoons made with CLC
 203 Workbench Main. Green represents identity. Red represents polymorphism. Figures are on scale.

204



205

206 **Figure 3: Recognition of CCG effectors by WT and stop codon mutant alleles of *WRR4A***

207 CCG effector candidates were transiently expressed in 4-week old *N. tabacum* leaves, under the control of the 35S
 208 promoter and Ocs terminator, alone or with WT or mutant alleles of *WRR4A*. Leaves were infiltrated with
 209 *Agrobacterium tumefaciens* strain GV3101 in infiltration buffer at OD₆₀₀ = 0.4. Pictures were taken at 4 dpi. **a.**
 210 cartoon of the *WRR4A* alleles: (a) Col-0 WT, (b) Oy-0 WT, (c) Col-0 with TGA-TGC mutation, causing an Oy-0 like
 211 C-terminal extension, (d) Oy-0 with a TGC-TGA mutation causing a truncation of the C-terminal extension. **b.** AcEx1
 212 CCG effector candidates alone (e) or with one of the four *WRR4A* alleles as shown in Fig 3a (a), (b), (c), (d). MLA7

213 CC domain was used as an HR positive control (f). **c.** Seven CCGs from other races of *Albugo candida* known to
214 be recognized by Col-0 allele of WRR4A were tested with the three others WRR4A alleles. Co-expression of CCG
215 with their cognate R-protein WRR4A Col-0 was used as a positive control (a). Expression of the WRR4A alleles
216 without effector was used as negative controls (bottom right picture). **d.** Summary of the CCG recognition by
217 WRR4A alleles. Red nails: AcEx1 effectors, black nails: CCGs from other *A. candida* races.

218

219 Since many TIR-NLRs carry a PL C-JID, we conducted a Hidden Markov Model (HMM) search
220 for C-JID and found one in WRR4A (www.ebi.ac.uk/Tools/hmmer/search/hmmsearch on
221 *Arabidopsis thaliana* using C-JIC HMM previously reported (Ma *et al.*, 2020), e-value = 5.7e-
222 14). This C-JID is present in both Oy-0 and Col-0 alleles (**Figure 2b**). The C-terminal extension
223 in WRR4A^{Oy-0} relative to WRR4A^{Col-0} does not show homology with known protein domains.

224

225 **Extension in WRR4A confers specific recognition of AcEx1 candidate effectors**

226 We tried to identify AcEx1 effectors specifically recognised by WRR4A^{Oy-0}. We tested for
227 hypersensitive response (HR), as typical marker of NLR activation, upon transient WRR4A^{Oy-0}
228 expression along with AcEx1 effector candidates in *N. tabacum* leaves. Secreted
229 CxxCxxxxxG (or CCGs) proteins are expanded in the genomes of *Albugo* species and are
230 effector candidates (Kemen *et al.*, 2011, Furzer *et al.*, 2021). We identified 55 CCGs in the
231 AcEx1 genome (Jouet *et al.*, 2018, Redkar *et al.*, 2021), and PCR-amplified and cloned 21 of
232 them, prioritizing those that showed allelic variation with other races. Eight CCGs (*i.e.* CCG9,
233 CCG12, CCG17, CCG33, CCG35, CCG39, CCG66, CCG80) induced a WRR4A^{Oy-0}-
234 dependent HR in *N. tabacum* in a preliminary screen with high *Agrobacterium* concentration
235 (OD₆₀₀ = 0.5) and were further investigated (**Figure S5**). We co-delivered four alleles of
236 WRR4A with the eight candidates at lower *Agrobacterium* concentrations (OD₆₀₀ = 0.4) to test
237 the robustness of their recognition (**Figure 3**). At this concentration, only CCG39 is
238 consistently recognised by WRR4A^{Oy-0} and explains AcEx1 resistance in Oy-0. WRR4A^{Col-0}
239_{LONG} can also recognise CCG39, but WRR4A^{Oy-0}_{SHORT} cannot. Hence, the C-terminal
240 extension fully explains the acquisition of recognition of CCG39. We also observed a weak
241 recognition of CCG33 by WRR4A^{Col-0}, as shown in complementary work (Redkar *et al.*, 2021)
242 and which could contribute to the WRR4A-dependent chlorotic response to AcEx1 in Col-0
243 (**Figure 1**). In addition, WRR4A^{Col-0}_{LONG} recognises CCG9 and CCG35 (**Figure 3b**).
244 Recognition of CCG9 and CCG35 is not explained solely by the C-terminal extension (as
245 WRR4A^{Oy-0} does not recognise them) or by the core region of the Col-0 allele (as WRR4A^{Col-0}
246 does not recognise them).

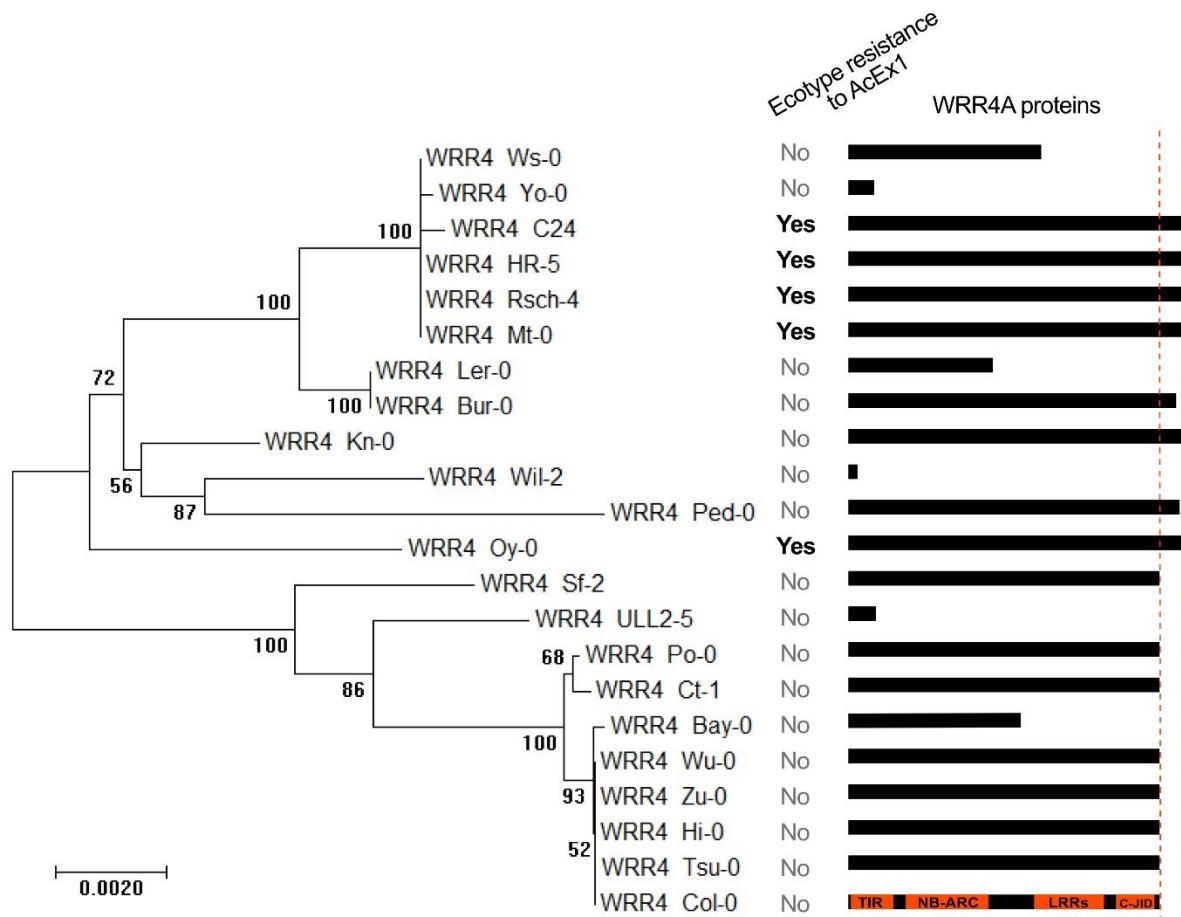
247 *WRR4A*^{Col-0} can recognise eight CCG effectors from other races of *A. candida* (Redkar *et al.*,
248 2021). We found that *WRR4A*^{Oy-0} is able to recognise CCG40, CCG104 and CCG28, but not
249 CCG67, CCG79, CCG33, CCG30 and CCG71 (**Figure 3c**). *WRR4A*^{Col-0_LONG} recognises all
250 the CCGs indistinguishably from *WRR4A*^{Col-0}, indicating no influence of the C-terminal
251 extension on their recognition.

252 In conclusion, we identified one AcEx1 effector specifically recognised by *WRR4A*^{Oy-0}. The C-
253 terminal extension is required and sufficient for its recognition. We also found that *WRR4A*^{Oy-0}
254 does not recognise several of the Col-0-recognised CCG from other races.

255

256 ***WRR4A* alleles carrying a C-terminal extension are associated with AcEx1 resistance**

257 The NLR repertoire of 64 Arabidopsis accessions has been determined using resistance gene
258 enrichment Sequencing (RenSeq) (Van de Weyer *et al.*, 2019). We found 20 susceptible and
259 5 resistant genotypes that belong to the 64 accessions (**Table S2**). We retrieved *WRR4A* from
260 these 25 accessions (<http://ann-nblrrrome.tuebingen.mpg.de/apollo/jbrowse/>). The read
261 coverage was insufficient to resolve *WRR4A* sequence in Bur-0 (susceptible) and Mt-0
262 (resistant). *WRR4A* is absent from the *WRR4* cluster in Ws-2, Edi-0 and No-0. Consistently,
263 these accessions are fully susceptible to AcEx1. From the DNA sequence of the 20 other
264 accessions, we predicted the protein sequence, assuming that the splicing sites correspond
265 to those in Col-0 and Oy-0 (**Figure 4**). There are two well-defined clades of *WRR4A* alleles,
266 with a bootstrap value of 100. One clade includes Col-0; the other clade includes Oy-0. The
267 Col-0-like and Oy-0-like clades are also discriminated in a phylogeny based on predicted
268 protein sequences (**Figure S6**). All alleles from the Col-0 clade carry TGA (apart from ULL2-
269 5, TGC, but *WRR4A* is pseudogenised in this accession), while all alleles from Oy-0 clade
270 carry TGC, at the Col-0 stop codon position. Several alleles from both clades, including Bay-
271 0, ULL2-5, Wil-2, Ler-0, Ws-0 and Yo-0, carry an early stop codon (*i.e.* upstream of the Col-0
272 stop codon position), so the resulting proteins are likely not functional. Consistently, all the
273 accessions from the Col-0 clade and all the accessions carrying an early stop codon are
274 susceptible to AcEx1. The only exception is Kn-0, that carries an Oy-0-like allele of *WRR4A*
275 but is susceptible to AcEx1. Otherwise, the presence of an Oy-0-like C-terminal extension
276 associates with resistance.



277

278 **Figure 4: An early stop codon in *WRR4A* is associated with AcEx1 susceptibility**

279 *WRR4A* genomic sequence of 20 *Arabidopsis* accessions were extracted from [http://ann-](http://ann-nblrrrome.tuebingen.mpg.de/apollo/jbrowse/)
 280 [nblrrrome.tuebingen.mpg.de/apollo/jbrowse/](http://ann-nblrrrome.tuebingen.mpg.de/apollo/jbrowse/) (Van de Weyer *et al.*, 2019). Nucleotide sequences corresponding
 281 from ATG to TGA of the Oy-0 allele (including introns) were aligned using MUSCLE (software: MEGA7). A
 282 phylogenetic tree was generated using the Maximum Likelihood method and a bootstrap (100 replicates) was
 283 calculated (software: MEGA7). The tree is drawn to scale, with branch lengths measured in the number of
 284 substitutions per site. The resistance / susceptibility phenotypes are indicated. Cartoons on the right represent
 285 *WRR4A* predicted protein, on scale. TIR, NB-ARC, LRR and C-JID are indicated in the Col-0 allele. Dashed orange
 286 line represents the Col-0 stop codon. Dashed blue line represents the Oy-0 stop codon.

287

288 ***AcEx1* resistance can be transferred from *Arabidopsis* to *Camelina***

289 *AcEx1* can grow on *Camelina sativa* (Figure 5). Like *Arabidopsis*, it can be transformed using
 290 the floral dip method (Liu *et al.*, 2012). We generated a *WRR4A^{Oy-0}*-transgenic camelina line.
 291 Out of twelve individuals, eight showed complete resistance, three displayed a chlorotic
 292 response (likely *WRR4A*-mediated HR) and one enabled limited pustule formation. All twelve
 293 WT camelina control plants showed mild to severe white rust symptoms. This indicates that
 294 *WRR4A^{Oy-0}* can confer resistance to *AcEx1* in *C. sativa*.

***Albugo candida* race Ex1**
5-week old plants, 12 dpi



***Camelina sativa* (cv Celine)**



Camelina sativa* (cv Celine) + *WRR4A-Oy-0

295

296 **Figure 5: WRR4A confers resistance to AcEx1 in camelina crop**

297 Five-week old camelina (cultivar Celine) plants were sprayed inoculated with AcEx1 race of the white rust
298 oomycete pathogen *A. candida*. Pictures were taken 12 dpi (day post inoculation). **Top row:** Twelve wild-type
299 plants all show mild to severe white rust symptoms. **Bottom row:** twelve lines transformed with *WRR4A^{Oy-0}* were
300 tested. One shows mild white rust symptoms, three show local chlorotic response and eight show complete green
301 resistance. White dash line indicates sporulation; red dash line indicates a chlorotic response with no pustule
302 formation.

303

304

305 **Discussion:**

306 **Col-0 and HR-5 *WRR4A* alleles recognise effectors from AcEx1**

307 A screen for novel sources of resistance to AcEx1 identified accessions HR-5 and Oy-0 as
308 worthy of further investigation. Positional cloning from Oy-0 and then allele mining in HR-5
309 showed that this immunity is mediated by alleles of *WRR4A* in HR-5 and Oy-0 with distinct
310 recognition capacities compared to the Col-0 allele. In Oy-0, two additional dominant loci,
311 *WRR11* on chromosome 3 and *WRR15* on chromosome 5 contribute resistance to AcEx1 but
312 the molecular basis of these resistances was not defined. Further investigation on *WRR11*
313 was conducted but did not reveal the causal gene (Castel, 2019, Chapter 3).

314 WRR4A^{Oy-0} recognizes at least one AcEx1 effector that is not recognized by WRR4A^{Col-0}
315 (**Figure 3**). Conceivably, WRR4A^{Oy-0} could be combined with WRR4A^{Col-0} and WRR4B^{Col-0} to
316 expand the effector recognition spectrum of a stack of WRR genes that could be deployed in
317 *B. juncea* or *C. sativa*.

318

319 **WRR4A alleles fall into two clades that can or cannot confer AcEx1 resistance**

320 Analysis of WRR4A allele diversity in Arabidopsis revealed WRR4A^{Oy-0}-like and WRR4A^{Col-0}-
321 like alleles. Since WRR4A^{Col-0}- like alleles show near-identity in nucleotide sequence after the
322 premature stop codon to WRR4A^{Oy-0}- like alleles, the latter are likely to be ancestral, and the
323 WRR4A^{Col-0}- like early stop codon occurred once, in the most recent common ancestor of Sf-
324 2 and Col-0. Other early stop codons, resulting in loss-of-function proteins, occurred randomly
325 in both Oy-0- and Col-0-containing clades. About a third of the investigated accessions contain
326 an early stop codon resulting in a likely non-functional allele (**Figure 4**). The full-length Oy-0-
327 like alleles are associated with resistance to AcEx1, while the Col-0-like alleles are associated
328 with susceptibility (**Figure 3**). The only exception is Kn-0, which displays a full length Oy-0-
329 like allele but is susceptible to AcEx1. Susceptibility in Kn-0 could be explained by SNPs, lack
330 of expression or mis-splicing of WRR4A.

331

332 **The Col-0 allele C-terminal truncation correlates with gain of recognition for some** 333 **CCGs and loss of recognition for others, suggesting an evolutionary trade-off**

334 We propose that, in the absence of AcEx1 selection pressure, the Col-0-like early stop codon
335 occurred to provide a new function, along with the loss of AcEx1 effector recognition. This new
336 function enables recognition of more CCGs from other *A. candida* races.

337 By combining the C-terminal extension on WRR4A^{Oy-0} with the core region of WRR4A in Col-
338 0 (**Figure 3d**), recognition of additional AcEx1 CCGs was enabled. Furthermore, Arabidopsis
339 natural accessions carrying the core region of the Col-0-like allele also lack the C-terminal
340 extension (**Figure 4 and S6**). This could be an example of intramolecular genetic suppression
341 (Kondrashov *et al.*, 2002; Schülein *et al.*, 2001; Brasseur *et al.*, 2001; Davis *et al.*, 1999). The
342 combination between the core region of the Col-0 allele with the C-terminal extension may
343 form a hyper-active WRR4A allele with excessive fitness cost for the plant. The early stop
344 codon may have occurred in Col-0 to compensate for hyper-activation of an ancestral WRR4A
345 allele. Hyper-activation of the immune system is deleterious, as shown for example by hybrid
346 incompatibility caused by immune receptors (Wan *et al.*, 2021).

347 Many plant TIR-NLRs show homology in their post-LRR domains (Saucet *et al.*, 2020). The
348 recently published structure of Roq1, a TIR-NLR from *N. benthamiana*, reveals a clear-cut C-
349 terminal jelly roll/Ig-like domain (C-JID) after the LRR (Martin *et al.*, 2020). It interacts with the
350 Roq1 cognate effector XopQ. Unlike the LRR, the C-JID does not play a role in auto-inhibition.
351 Similarly, in RPP1 (TIR-NLR from Arabidopsis), a C-JID is observed after the LRR (Ma *et al.*,
352 2020). It physically interacts with the cognate effector ATR1 in the active resistosome. We
353 found that WRR4A also contains a PL C-JID. Both WRR4A^{Col-0}- and WRR4A^{Oy-0}- carry the C-
354 JID, so it does not explain the unique CCG recognition of each allele.

355

356 **Arabidopsis WRR4A resistance to AcEx1 can be transferred to the crop camelina**

357 *Camelina sativa* was recently engineered to produce LC-PUFAs, an essential component in
358 the feed used in fish farming (Petrie *et al.*, 2014). Currently, fish farming uses wild fish-derived
359 fish oil. Fish oil-producing camelina offers a solution to reduce the need for wild fish harvesting,
360 potentially reducing pressure on world marine fish stocks (Betancor *et al.*, 2018). There are
361 challenges in delivering products derived from transgenic crops but fish oil-producing crops
362 could reduce the environmental impact of fish farming. White rust causes moderate symptoms
363 on camelina. However, *A. candida* is capable of immunosuppression (Cooper *et al.*, 2008). *A.*
364 *candida*-infected fields constitute a risk for secondary infection of otherwise incompatible
365 pathogens. To safeguard camelina fields against white rust, both chemical and genetic
366 solutions are possible. Genetic resistance offers the advantage of a lower cost for farmers and
367 reduces the need for fungicide release in the environment. Since the first report of white rust
368 on camelina in France in 1945, no genetic resistance has been characterised. All the strains
369 collected on camelina can grow equally irrespective of camelina cultivar (Séguin-Swartz *et al.*,
370 2009). This absence of phenotypic diversity precludes discovery of resistance loci using
371 classic genetic tools. We found that WRR4A^{Oy-0} confers resistance to AcEx1 in camelina
372 (**Figure 5**). Arabidopsis WRR4A resistance is functional in *B. juncea* and *B. oleracea*,
373 suggesting that the mechanism of activation and the downstream signalling of WRR4A is
374 conserved, at least in Brassicaceae.

375 In conclusion, we found a novel example of post LRR polymorphism within an NLR family,
376 associated with diversified effector recognition spectra. By investigating the diversity of
377 WRR4A, we identified an allele that confers white rust resistance in the camelina crop.

378

379 **Experimental procedures:**

380 **Plant material and growth conditions**

381 *Arabidopsis thaliana* (*Arabidopsis*) accessions used in this study are Øystese-0 (Oy-0, NASC:
382 N1436), HR-5 (NASC: N76514), Wassilewskija-2 (Ws-2, NASC: N1601) and Columbia (Col-
383 0, NASC: N1092). Col-0_ *wrr4a-6* mutant is published (Borhan *et al.*, 2008). Seeds were sown
384 directly on compost and plants were grown at 21°C, with 10 hours of light and 14 hours of
385 dark, 75% humidity. For seed collection, 5-weeks old plants were transferred under long-day
386 condition: 21°C, with 16 hours of light and 8 hours of dark, 75% humidity. For *Nicotiana*
387 *tabacum* (cultivar Petit Gerard) and *Camelina sativa* (cultivar Celine), seeds were sown
388 directly on compost and plants were grown at 21°C, with cycles of 16 hours of light and 8
389 hours of dark, at 55% humidity.

390

391 ***Albugo candida* Infection assay**

392 For propagation of *Albugo candida*, zoospores from the infected leaf inoculum were
393 suspended in water (~10⁵ spores/ml) and incubated on ice for 30 min. The spore suspension
394 was then sprayed on plants using a Humbrol® spray gun (~700 µl/plant) and plants were
395 incubated at 4°C in the dark overnight to promote spore germination. Infected plants were kept
396 under 10-hour light (20 °C) and 14-hour dark (16°C) cycles. Phenotypes were monitored 14 to
397 21 days after inoculation.

398

399 **QTL analysis**

400 QTL mapping of the bipartite F8 Oy-0 x Col-0 population (470 Recombinant Inbred Lines,
401 RILs, publiclines.versailles.inra.fr/page/27) (Simon *et al.*, 2008) was performed on a genetic
402 map of 85 markers across the five linkage groups that accompanied the population using
403 RQTL (Broman *et al.*, 2003). Standard interval mapping using a maximum likelihood
404 estimation under a mixture model (Lander and Botstein, 1989) was applied for interval
405 mapping. Analysis revealed two major QTLs: on chromosome 1 and on chromosome 3.

406 Chromosome 1 QTL is located between 20,384 Mb and 22,181 Mb (**Figure S2**). It includes
407 the TIR-NLR cluster *WRR4* and the CC-NLR cluster *RPP7*. Six RILs (three resistant and three
408 susceptible) recombine within the QTL and were used for fine mapping. We designed a Single
409 Nucleotide Polymorphism (SNP, 21,195 Mb, Fw: TCAGATTGTAAGTCTCGAAGG, Rv:
410 CCATCAAGCACACTGTATTCC, amplicon contains two SNPs, Oy-0: A and G, Col-0: G and
411 C) and an Amplified Fragment Length Polymorphism (AFLP, 21,691 Mb, Fw:
412 AAGGCAATCAGATTAAGCAGAA, Rv: GCGGGTTTCCTCAGTTGAAG, Oy-0: 389 bp, Col-0:

413 399 bp) markers between *WRR4* and *RPP7*. Four lines eliminate *RPP7* from the QTL. The
414 only NLR cluster in chromosome 1 QTL is *WRR4*.

415 Chromosome 3 QTL is located between 17,283 Mb and 19,628 Mb (**Figure S3**). It includes
416 the atypical *resistance*-gene cluster *RPW8*, the CC-NLR *ZAR1* and the paired TIR-NLRs
417 *At3g51560-At3g51570*. Six RILs (three resistant and three susceptible) recombine within the
418 QTL and were used for fine mapping. We designed an AFLP (18,016 Mb, Fw:
419 gctacgccactgcatttagc, Rv: CCAATTCCGCAACAGCTTTA, Oy-0: 950 bp, Col-0: 1677 bp) and
420 a Cleaved Amplified Polymorphic Sequence (CAPS, 18,535 Mb, Fw:
421 TCAAGCCTGTTAAGAAGAAGAAGG, Rv: GCCCTCCACAAAGATTCTGAAGTA, enzyme:
422 Ddel, Oy-0: uncleaved, Col-0: cleaved) markers between the QTL border and *RPW8*. We
423 designed a CAPS marker (18,850 Mb, Fw: TCTCGGGGAAAATATGATTAGA, Rv:
424 GGTTGATTTTTATTGTGGTAGTCGT, enzyme: SmaI, Oy-0: cleaved, Col-0: uncleaved)
425 between *RPW8* and *ZAR1*. We designed a SNP (18,937 Mb, Fw:
426 CCACAAGGTCGGAATCTGTAGC, Rv: TGCACAGAAGTAACCCACCAAC, Oy-0: C, Col-0:
427 T) and a CAPS (19,122 Mb, Fw: ACCACCACCTCGATGCATTTTC, Rv:
428 CCTTCCCTGCGAAAGACACTC, enzyme: BsrI, Oy-0: uncleaved, Col-0: cleaved) markers
429 between *ZAR1* and the TIR-NLR pair. Three recombinants eliminate the TIR-NLR pair, two
430 eliminate *ZAR1* and one eliminates *RPW8*. None of the Col-0 NLR clusters orthologs are
431 present in the QTL. The gene underlying chromosome three resistance is located between
432 the border of the QTL and *RPW8*.

433

434 ***Bulk segregant analysis and RenSeq***

435 We generated an F2 population from a cross between HR-5 (resistant) and Ws-2
436 (susceptible). We phenol/chloroform extracted DNA from 200 bulked F2 lines fully susceptible
437 to AcEx1. The bulked DNA sample was prepared as an Illumina library and enriched using the
438 c. The sample was sequenced in a pooled MiSeq run (data available on request). Firstly, reads
439 were aligned with BWA mem (Li and Durbin, 2009) to the and SNPs called with Samtools (Li
440 *et al.*, 2009). The genome was scanned for regions of high linkage with the next generation
441 mapping tools at <http://bar.utoronto.ca/ngm/> (Austin *et al.*, 2011). Secondly, the reads were
442 mapped using BWA to the RenSeq PacBio assembly generated for HR-5 (Van de Weyer *et*
443 *al.*, 2019). Highly linked regions were confirmed visually with the integrated genome viewer
444 (Robinson *et al.*, 2017).

445 ***Gene cloning***

446 Genes were cloned with the USER method (NEB) following the manufacturer
447 recommendations, with their natural 5' and 3' regulatory sequences into LBJJ233-OD
448 (containing a FAST-Red selectable marker, pre-linearized with PacI and Nt. BbvCI restriction
449 enzymes). For overexpression, genes were cloned into LBJJ234-OD (containing a FAST-Red
450 selectable marker and a 35S / Ocs expression cassette, pre-linearized with PacI and Nt. BbvCI
451 restriction enzymes). Primers, template and vectors are indicated in (Table S3). *WRR4A*^{Col-0}
452 has been previously published (Cevik *et al.*, 2019).

453 All the plasmids were prepared using a QIAPREP SPIN MINIPREP KIT on *Escherichia coli*
454 DH10B thermo-competent cells selected with appropriate antibiotics. Positive clones
455 (confirmed by size selection on electrophoresis gel and capillary sequencing) were
456 transformed in *Arabidopsis thaliana* via *Agrobacterium tumefaciens* strain GV3101.
457 Transgenic seeds were selected under fluorescent microscope for expression of the FAST-
458 Red selectable marker (Shimada *et al.*, 2010).

459

460

461 ***CRISPR adenine base editor***

462 An sgRNAs targeting *WRR4A* stop codon in Col-0 (TTCTGAGAagcattcgaaag[nGA]) was
463 assembled by PCR to a sgRNA backbone and 67 bp of the *U6-26* terminator. It was then
464 assembled with the *AtU6-26* promoter in the Golden Gate compatible level 1 *pICH47751*. We
465 designed a mutant allele of a plant codon optimized Cas9 with a potato intron (Addgene:
466 117515) with D10A (nickase mutant) and R1335V/L1111R/D1135V/G1218R/
467 E1219F/A1322R/T1337R, to change the PAM recognition from NGG to NG (Nishimasu *et al.*,
468 2018). We assembled this Cas9 (golden gate compatible Bpil: GACA-GCTT) along with a
469 barley codon optimized TadA module (golden gate compatible Bpil: AATG-GCTT) in a level 0
470 vector *pICH41308*. It was then assembled with the YAO promoter (Addgene: 117513) and the
471 E9 terminator (Addgene: 117519) in a level 1 vector *pICH47811* (with expression in reverse
472 orientation compared to the other level 1 modules). It was then assembled with a FAST-Red
473 selectable marker (Addgene: 117499) and the sgRNA level 1 cassette into a level 2 vector
474 *pICSL4723*, using the end-linker *pICH41766*. Level 0 vector was cloned using Bpil enzyme
475 and spectinomycin resistance. Level 1 vectors were cloned using Bsal enzyme and
476 carbenicillin resistance. Level 2 vectors were cloned using Bpil enzyme and kanamycin
477 resistance. It was expressed via *Agrobacterium tumefaciens* strain GV3101 in *Arabidopsis*
478 Oy-0. In the first generation after transformation, we did not detect any mutant from 24
479 independent transformants. It indicates an absence of activity of the construct. It can be

480 explained by the Cas9 mutations that were not tested before on this specific allele nor in
481 combination with TadA.

482

483 ***Transient expression in N. tabacum leaves***

484 *A. tumefaciens* strains were streaked on selective media and incubated at 28 °C for 24 hours.
485 A single colony was transferred to liquid LB medium with appropriate antibiotic and incubated
486 at 28 °C for 24 hours in a shaking incubator (200 rotations per minute). The resulting culture
487 was centrifuged at 3000 rotations per minute for 5 minutes and resuspended in infiltration
488 buffer (10 mM MgCl₂, 10 mM MES, pH 5.6) at OD₆₀₀ = 0.4 (2 x10⁸ cfu/ml). For co-expression,
489 each bacterial suspension was adjusted to OD₆₀₀ = 0.4 for infiltration. The abaxial surface of
490 4-weeks old *N. tabacum* were infiltrated with 1 ml needle-less syringe. Cell death was
491 monitored three days after infiltration.

492

493 ***Resolution of WRR4A^{Oy-0} cDNA sequence***

494 RNA was extracted from three Col-0_*rpw8* and three Col-0 WT individual plants using the
495 RNeasy Plant Mini Kit (QIAGEN) and treated with RNase-Free DNase Set (QIAGEN). Reverse
496 transcription was carried out using the SuperScript IV Reverse Transcriptase (ThermoFisher).
497 PCR was conducted using Fw: TCTGATGTCCGCAACCAAAC (in the first exon) and Rv:
498 GTCCTCTTCGGCCATATCTTC (in the last exon) with the Taq Polymerase enzyme (NEB)
499 following the manufacturer protocol. The 2848 nt amplicon sequence, corresponding to the
500 cDNA sequence (*i.e.* with already spliced introns) was resolved by capillary sequencing. It
501 indicates that the splicing sites are identical between *WRR4A^{Oy-0}* and the splicing sites
502 reported in the database TAIR10 for *WRR4A^{Col-0}*.

503

504 **Acknowledgements:**

505 We thank the Gatsby Foundation (UK) for funding to the Jones lab. We thank Mark Youles in
506 TSL Synbio for his excellent support with Golden Gate cloning and for providing modules. This
507 research was supported in part by the NBI Computing infrastructure for Science (CiS) group
508 and Dan MacLean's group by providing computational infrastructure. B.C., S.F., O.F. and V.C.
509 were supported by Biotechnology and Biological Sciences Research Council (BBSRC) grant
510 BB/L011646/1. A.R. was supported by EMBO LTF (ALTF-842- 2015). B.C, S.W and J.D.G.J.
511 were supported in part by ERC Advanced Investigator grant to JDGJ 'ImmunityByPairDesign'
512 Project ID 669926.

513

514 **Data availability statement:**

515 All relevant data can be found within the manuscript and its supporting materials. The
516 sequences of the genomic clones of WRR4A^{Oy-0} and WRR4A^{HR-5} are deposited at NCBI
517 GenBank as MW533532 and MW533533 respectively.

518

519 **Author contributions:**

520 BC, SF, OF, AR, VC, EH and JJ designed research; BC, SF, OF, AR, SW, VC performed
521 research; BC, SF, OF, AR, VC, EH and JJ analysed data; and BC and JJ wrote the paper.
522 All authors read and approved the final manuscript.

523

524 **Conflicts of interest statement:**

525 The authors declare that they have no conflict of interests

526

527 **Supporting materials:**

528 Figure S1: Detailed map of candidate loci in Oy-0

529 Figure S2: Fine mapping of chromosome 1 QTL

530 Figure S3: Fine mapping of chromosome 3 QTL

531 Figure S4: Detailed map of candidate loci in HR-5

532 Figure S5: Some AcEx1 CCG candidate effectors are recognised by WRR4A^{Oy-0}

533 Figure S6: Phylogeny of WRR4A based on protein sequences

534 Table S1: Arabidopsis v1 RenSeq bait library (Arbor Bioscience, MI, USA) as described by
535 (Jupe *et al.*, 2013)

536 Table S2: Phenotype of 283 Arabidopsis accessions in response to AcEx1 infection

537 Table S3: Primers used in this study

538

539 **References:**

- 540 **Andolfo, G., Jupe, F., Witek, K., Etherington, G.J., Ercolano, M.R. and Jones, J.D.G.**
541 (2014) Defining the full tomato NB-LRR resistance gene repertoire using genomic and
542 cDNA RenSeq. *BMC Plant Biol.*, **14**, 120. Available at:
543 <http://www.biomedcentral.com/1471-2229/14/120>.
- 544 **Arora, H., Padmaja, K.L., Paritosh, K., Mukhi, N., Tewari, A.K., Mukhopadhyay, A.,**
545 **Gupta, V., Pradhan, A.K. and Pental, D.** (2019) BjuWRR1, a CC-NB-LRR gene
546 identified in Brassica juncea, confers resistance to white rust caused by *Albugo*
547 *candida*. *Theor. Appl. Genet.*, **132**, 2223–2236. Available at:
548 <https://doi.org/10.1007/s00122-019-03350-z>
- 549 **Austin, R.S., Vidaurre, D., Stamatiou, G., et al.** (2011) Next-generation mapping of
550 *Arabidopsis* genes. *Plant J.*, **67**, 715–725.
- 551 **Baggs, E., Dagdas, G. and Krasileva, K. V.** (2017) NLR diversity, helpers and integrated
552 domains: making sense of the NLR IDentity. *Curr. Opin. Plant Biol.*, **38**, 59–67.
553 Available at: <http://dx.doi.org/10.1016/j.pbi.2017.04.012>.
- 554 **Belhaj, K., Cano, L.M., Prince, D.C., et al.** (2017) *Arabidopsis* late blight: infection of a
555 nonhost plant by *Albugo laibachii* enables full colonization by *Phytophthora infestans*.
556 *Cell. Microbiol.*, **19**, e12628.
- 557 **Betancor, M.B., Li, K., Bucerzan, V.S., et al.** (2018) Oil from transgenic *Camelina sativa*
558 containing over 25 % n-3 long-chain PUFA as the major lipid source in feed for Atlantic
559 salmon (*Salmo salar*). *Br. J. Nutr.*, **119**, 1378–1392.
- 560 **Borhan, M.H., Gunn, N., Cooper, A., Gulden, S., Tör, M., Rimmer, S.R. and Holub, E.B.**
561 (2008) WRR4 encodes a TIR-NB-LRR protein that confers broad-spectrum white rust
562 resistance in *Arabidopsis thaliana* to four physiological races of *Albugo candida*. *Mol.*
563 *Plant-Microbe Interact.*, **21**, 757–768.
- 564 **Borhan, M.H., Holub, E.B., Kindrachuk, C., Omid, M., Bozorgmanesh-Frad, G. and**
565 **Rimmer, S.R.** (2010) WRR4, a broad-spectrum TIR-NB-LRR gene from *Arabidopsis*
566 *thaliana* that confers white rust resistance in transgenic oilseed brassica crops. *Mol.*
567 *Plant Pathol.*, **11**, 283–291.
- 568 **Brasseur, G., Rago, J.P. Di, Slonimski, P.P. and Lemesle-Meunier, D.** (2001) Analysis of
569 suppressor mutation reveals long distance interactions in the bc1 complex of
570 *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta - Bioenerg.*, **1506**, 89–
571 102. **Broman, K.W., Wu, H., Sen, S. and Churchill, G.A.** (2003) R/qtl: QTL mapping in

- 572 experimental crosses. *Bioinformatics*, **19**, 889–890.
- 573 **Castel, B.** (2019) Natural and CRISPR-induced genetic variation for plant immunity.
574 University of East Anglia, PhD thesis. Available at:
575 <https://ueaeprints.uea.ac.uk/id/eprint/71447/>.
- 576 **Cesari, S.** (2017) Multiple strategies for pathogen perception by plant immune receptors.
577 *New Phytol.*, **219**, 17–24. Available at: <http://doi.wiley.com/10.1111/nph.14877>.
- 578 **Cevik, V., Boutrot, F., Apel, W., et al.** (2019) Transgressive segregation reveals
579 mechanisms of Arabidopsis immunity to Brassica-infecting races of white rust (*Albugo*
580 *candida*). *Proc. Natl. Acad. Sci.*, **116**, 2767–2773.
- 581 **Cooper, A.J., Latunde-Dada, A.O., Woods-Tör, A., Lynn, J., Lucas, J.A., Crute, I.R. and**
582 **Holub, E.B.** (2008) Basic compatibility of *Albugo candida* in *Arabidopsis thaliana* and
583 *Brassica juncea* causes broad-spectrum suppression of innate immunity. *Mol. Plant-*
584 *Microbe Interact.*, **21**, 745–756.
- 585 **Davis, J.E., Voisine, C. and Craig, E.A.** (1999) Intragenic suppressors of Hsp70 mutants:
586 Interplay between the ATPase- and peptide-binding domains. *Proc. Natl. Acad. Sci. U.*
587 *S. A.*, **96**, 9269–9276.
- 588 **Engler, C., Gruetzner, R., Kandzia, R. and Marillonnet, S.** (2009) Golden gate shuffling: A
589 one-pot DNA shuffling method based on type II restriction enzymes. *PLoS One*, **4**,
590 e5553.
- 591 **Engler, C., Youles, M., Gruetzner, R., Ehnert, T.M., Werner, S., Jones, J.D.G., Patron,**
592 **N.J. and Marillonnet, S.** (2014) A Golden Gate modular cloning toolbox for plants.
593 *ACS Synth. Biol.*, **3**, 839–843.
- 594 **Fairhead, S.** (2016) Translating genetics of oomycete resistance from *Arabidopsis thaliana*
595 into Brassica production. University of Warwick, PhD thesis. Available at:
596 <http://wrap.warwick.ac.uk/90258>
- 597 **Furzer, O.J., Cevik, V., Fairhead, S., Bailey, K., Redkar, A., Schudoma, C., MacLean, D.,**
598 **Holub, E.B., Jones, J.D.G.** (2021) PacBio Sequencing of the *Albugo candida* Ac2V
599 genome reveals the expansion of the “CCG” class of effectors. bioRxiv.
- 600 **Gupta, A.K., Raj, R., Kumari, K., Singh, S.P., Solanki, I.S. and Choudhary, R.** (2018)
601 Management of Major Diseases of Indian Mustard Through Balanced Fertilization,
602 Cultural Practices and Fungicides in Calcareous Soils. *Proc. Natl. Acad. Sci. India,*
603 *Sect. B - Biol. Sci.*, **88**, 229–239.

- 604 **Jones, J.D.G. and Dangl, J.L.** (2006) The plant immune system. *Nature*, **444**, 323–329.
- 605 **Jouet, A., Saunders, D., McMullan, M., et al.** (2018) Albugo candida race diversity, ploidy
606 and host-associated microbes revealed using DNA sequence capture on diseased
607 plants in the field. *New Phytol.*, **221**, 1529–1543. Available at:
608 <http://doi.wiley.com/10.1111/nph.15417>.
- 609 **Jupe, F., Witek, K., Verweij, W., et al.** (2013) Resistance gene enrichment sequencing
610 (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant
611 genomes and rapid mapping of resistance loci in segregating populations. *Plant J.*, **76**,
612 530–544.
- 613 **Kemen, E., Gardiner, A., Schultz-Larsen, T., et al.** (2011) Gene Gain and Loss during
614 Evolution of Obligate Parasitism in the White Rust Pathogen of Arabidopsis thaliana.
615 *PLoS Biol.*, **9**, e1001094. Available at: <http://dx.plos.org/10.1371/journal.pbio.1001094>.
- 616 **Kroj, T., Chanclud, E., Michel-Romiti, C., Grand, X. and Morel, J.B.** (2016) Integration of
617 decoy domains derived from protein targets of pathogen effectors into plant immune
618 receptors is widespread. *New Phytol.*, **210**, 618–626.
- 619 **Kuang, H., Woo, S.S., Meyers, B.C., Nevo, E. and Michelmore, R.W.** (2004) Multiple
620 genetic processes result in heterogeneous rates of evolution within the major cluster
621 disease resistance genes in lettuce. *Plant Cell*, **16**, 2870–2894.
- 622 **Lander, E.S. and Botstein, D.** (1989) Mapping mendelian factors underlying quantitative
623 traits using RFLP linkage maps. *Genetics*, **121**, 185–199. Available at:
624 <http://www.ncbi.nlm.nih.gov/pubmed/2563713>.
- 625 **Lee, R.R.Q. and Chae, E.** (2020) Plant Communications Patterns of NLR Cluster Variation
626 in Arabidopsis thaliana Genomes. *Plant Commun.*, **1**, 100089. Available at:
627 <https://doi.org/10.1016/j.xplc.2020.100089>.
- 628 **Li, H. and Durbin, R.** (2009) Fast and accurate short read alignment with Burrows-Wheeler
629 transform. *Bioinformatics*, **25**, 1754–1760.
- 630 **Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G.,
631 Abecasis, G. and Durbin, R.** (2009) The Sequence Alignment/Map format and
632 SAMtools. *Bioinformatics*, **25**, 2078–2079.
- 633 **Liu, X., Brost, J., Hutcheon, C., et al.** (2012) Transformation of the oilseed crop Camelina
634 sativa by Agrobacterium-mediated floral dip and simple large-scale screening of
635 transformants. *Vitr. Cell. Dev. Biol. - Plant*, **48**, 462–468.

- 636 **Ma, S., Lapin, D., Liu, L., et al.** (2020) Direct pathogen-induced assembly of an NLR
637 immune receptor complex to form a holoenzyme. *Science*, **370**, eabe3069. Available at:
638 <https://dx.doi.org/10.1126/science.abd9993>.
- 639 **Martin, R., Qi, T., Zhang, H., Liu, F., King, M., Toth, C. and Staskawicz, B.J.** (2020)
640 Structure of the activated Roq1 resistosome directly recognizing the pathogen effector
641 XopQ. *Science*, **370**, eabd9993. Available at: <https://doi.org/10.1126/science.abd9993>.
- 642 **McMullan, M., Gardiner, A., Bailey, K., et al.** (2015) Evidence for suppression of immunity
643 as a driver for genomic introgressions and host range expansion in races of *Albugo*
644 *candida*, a generalist parasite. *Elife*, **4**, e04550. Available at:
645 <http://elifesciences.org/lookup/doi/10.7554/eLife.04550>.
- 646 **Meyers, B.C., Shen, K.A., Rohani, P., Gaut, B.S. and Michelmore, R.W.** (1998) Receptor-
647 like genes in the major resistance locus of lettuce are subject to divergent selection.
648 *Plant Cell*, **10**, 1833–1846.
- 649 **Monteiro, F. and Nishimura, M.T.** (2018) Structural , Functional , and Genomic Diversity of
650 Plant NLR proteins : An Evolved Resource for Rational Engineering of Plant Immunity.
651 *Annu. Rev. Phytopathol.*, **56**, 12.1-12.25.
- 652 **Napier, J.A., Haslam, R.P., Tsalavouta, M. and Sayanova, O.** (2019) The challenges of
653 delivering genetically modified crops with nutritional enhancement traits. *Nat. Plants*, **5**,
654 563–567. Available at: <http://dx.doi.org/10.1038/s41477-019-0430-z>.
- 655 **Napier, J.A., Usher, S., Haslam, R.P., Ruiz-Lopez, N. and Sayanova, O.** (2015)
656 Transgenic plants as a sustainable, terrestrial source of fish oils. *Eur. J. Lipid Sci.*
657 *Technol.*, **117**, 1317–1324.
- 658 **Nishimasu, H., Shi, X., Ishiguro, S., et al.** (2018) Engineered CRISPR-Cas9 nuclease with
659 expanded targeting space. *Science.*, **361**, 1259–1262.
- 660 **Pedersen, W.L.** (1988) Resistance To Maintain Residual Effects. *Annu. Rev. Phytopathol.*,
661 **26**, 369–378.
- 662 **Petrie, J.R., Shrestha, P., Belide, S., et al.** (2014) Metabolic engineering *Camelina sativa*
663 with fish oil-like levels of DHA. *PLoS One*, **9**, e85061.
- 664 **Pound G.S. and Williams P.H.** (1963) Biological races of *Albugo candida*. *Phytopathol.*, **53**,
665 1146-1149.
- 666 **Prince, D.C., Rallapalli, G., Xu, D., et al.** (2017) *Albugo*-imposed changes to tryptophan-
667 derived antimicrobial metabolite biosynthesis may contribute to suppression of non-host

- 668 resistance to *Phytophthora infestans* in *Arabidopsis thaliana*. *BMC Biol.*, **15**, 20.
669 Available at: <http://bmcbiol.biomedcentral.com/articles/10.1186/s12915-017-0360-z>.
- 670 **Redkar, A., Cevik, V., Bailey, K., Furzer, O.J., Fairhead, S., Borhan, M.H., Holub, E.B.,**
671 **Jones, J.D.G.** (2021) The *Arabidopsis* WRR4A and WRR4B paralogous NLR proteins
672 both confer recognition of multiple *Albugo candida* effectors. bioRxiv.
- 673 **Robinson, J.T., Thorvaldsdóttir, H., Wenger, A.M., Zehir, A. and Mesirov, J.P.** (2017)
674 Variant review with the integrative genomics viewer. *Cancer Res.*, **77**, e31–e34.
- 675 **Roux, C. Le, Huet, G., Jauneau, A., et al.** (2015) A Receptor Pair with an Integrated Decoy
676 Converts Pathogen Disabling of Transcription Factors to Immunity. *Cell*, **161**, 1074–
677 1088. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0092867415004420>.
- 678 **Ruiz-Lopez, N., Haslam, R.P., Napier, J.A. and Sayanova, O.** (2014) Successful high-level
679 accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic
680 oilseed crop. *Plant J.*, **77**, 198–208.
- 681 **Sarris, P.F., Duxbury, Z., Huh, S.U., et al.** (2015) A Plant Immune Receptor Detects
682 Pathogen Effectors that Target WRKY Transcription Factors. *Cell*, **161**, 1089–1100.
683 Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0092867415004419>.
- 684 **Saucet, S.B., Esmenjaud, D. and Ghelder, C. Van** (2021) Integrity of the post-LRR domain
685 is required for TNLs' function. *Mol. Plant-Microbe Interact.*, **34**, 286-296.
- 686 **Schülein, R., Zühlke, K., Krause, G. and Rosenthal, W.** (2001) Functional Rescue of the
687 Nephrogenic Diabetes Insipidus-causing Vasopressin V2 Receptor Mutants G185C and
688 R202C by a Second Site Suppressor Mutation. *J. Biol. Chem.*, **276**, 8384–8392.
- 689 **Séguin-Swartz, G., Eynck, C., Gugel, R.K., et al.** (2009) Diseases of *Camelina sativa*
690 (false flax). *Can. J. Plant Pathol.*, **31**, 375–386.
- 691 **Shimada, T.L., Shimada, T. and Hara-Nishimura, I.** (2010) A rapid and non-destructive
692 screenable marker, FAST, for identifying transformed seeds of *Arabidopsis thaliana*.
693 *Plant J.*, **61**, 519–528. Available at: [http://doi.wiley.com/10.1111/j.1365-](http://doi.wiley.com/10.1111/j.1365-313X.2009.04060.x)
694 [313X.2009.04060.x](http://doi.wiley.com/10.1111/j.1365-313X.2009.04060.x).
- 695 **Simon, M., Loudet, O., Durand, S., Bérard, A., Brunel, D., Sennesal, F.X., Durand-**
696 **Tardif, M., Pelletier, G. and Camilleri, C.** (2008) Quantitative trait loci mapping in five
697 new large recombinant inbred line populations of *Arabidopsis thaliana* genotyped with
698 consensus single-nucleotide polymorphism markers. *Genetics*, **178**, 2253–2264.
- 699 **Steuernagel, B., Periyannan, S.K., Hernández-Pinzón, I., et al.** (2016) Rapid cloning of

- 700 disease-resistance genes in plants using mutagenesis and sequence capture. *Nat.*
701 *Biotechnol.*, **34**, 652–655.
- 702 **Wan, W., Kim, S., Castel, B., Charoennit, N. and Chae, E.** (2021) Genetics of
703 autoimmunity in plants: an evolutionary genetics perspective. *New Phytol.*, **229**, 1215–
704 1233.
- 705 **Weber, E., Engler, C., Gruetzner, R., Werner, S. and Marillonnet, S.** (2011) A modular
706 cloning system for standardized assembly of multigene constructs. *PLoS One*, **6**,
707 e16765.
- 708 **West, A.L., Miles, E.A., Lillycrop, K.A., Han, L., Sayanova, O., Napier, J.A., Calder, P.C.**
709 **and Burdge, G.C.** (2019) Postprandial incorporation of EPA and DHA from transgenic
710 *Camelina sativa* oil into blood lipids is equivalent to that from fish oil in healthy humans.
711 *Br. J. Nutr.*, **121**, 1235–1246.
- 712 **Weyer, A.-L. Van de, Monteiro, F., Furzer, O.J., et al.** (2019) A Species-Wide Inventory of
713 NLR Genes and Alleles in *Arabidopsis thaliana*. *Cell*, **178**, 1260–1272. Available at:
714 <https://linkinghub.elsevier.com/retrieve/pii/S0092867419308372>.
- 715 **Witek, K., Jupe, F., Witek, A.I., Baker, D., Clark, M.D. and Jones, J.D.G.** (2016)
716 Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT
717 sequencing. *Nat. Biotechnol.*, **34**, 656–660. Available at:
718 <http://dx.doi.org/10.1038/nbt.3540>.
- 719 **Xiao, S., Brown, S., Patrick, E., Brearley, C. and Turner, J.G.** (2003) Enhanced
720 Transcription of the *Arabidopsis* Disease Resistance Genes RPW8.1 and RPW8.2 via a
721 Salicylic Acid – Dependent Amplification Circuit Is Required for Hypersensitive Cell
722 Death. *Plant Cell*, **15**, 33–45.