A butterfly egg-killing hypersensitive response in Brassica nigra is controlled by a single locus, PEK, containing a cluster of TIR-NBS-LRR receptors

Nina Fatouros[1], Niccolò Bassetti[1], Lotte Caarls[1], Klaas Bouwmeester[1], Patrick Verbaarschot[1], Ewan van Eijden[1], Bas Zwaan[2], Guusje Bonnema[3], and Eric Schranz[1]

[1]Wageningen University & Research Leerstoelgroep Biosystematiek
[2]Wageningen University & Research
[3]Wageningen University & Research Laboratorium voor Plantenveredeling

July 15, 2023

Abstract

The hypersensitive response (HR) characterizes monogenic qualitative resistance traits in several pathosystems. Knowledge on its role in plant resistance to insects is so far limited to a few resistance (*R*) gene-based resistances against piercing-sucking insects. Egg deposition by cabbage white butterflies (*Pieris* spp.), pests of cabbage crops (*Brassica* spp.), can trigger an HR-like cell death, which reduces egg survival and represents an effective plant resistance trait before feeding damage occurs. Here, we identified natural variation of HR-like to *Pieris* egg deposition in the black mustard (*B. nigra* L.) and performed genetic mapping. HR-like segregated as a Mendelian trait and a single dominant locus on chromosome B3, named *PEK* (*P ieris e gg- k illing*) was identified. In the ˜50 kb region, eleven candidate genes, are located, including a cluster of genes encoding intracellular receptor proteins, TIR-NBS-LRR (TNLs). The PEK locus was found to be highly polymorphic between the parental accessions of our mapping populations and among *B. nigra* reference genomes. Our study is the first that identifies a single locus potentially involved in HR-like cell death induced by insect eggs. Further fine-mapping, comparative genomics and validation of the *PEK* locus will shed light on the role of TNL receptors in egg-induced HR-like cell death.

Introduction

Insect pests represent a major threat for global food security (Oerke, 2006). Pests control largely relies on the use of pesticides which, on the other side, are increasingly reported to have harmful ecological effects (Forister et al., 2019). Future crop yield stability will thus depend on plant breeding efforts to improve crop resistance traits against pests (Crall et al., 2018). Hypersensitive response (HR) is one of the most studied traits associated with plant defence against pathogens and pests and it is often underlined by single resistance (R) genes (Wagner et al., 2021). So far only a handful of R genes have been identified against insect herbivores, mostly consisting of cell surface receptors (pattern recognition receptors, PRRs) or intracellular receptors (nucleotide-binding leucin rich-repeat, NLRs) (Kouretlis & van der Hoorn, 2018). Resistance traits against insects based on R genes are mostly limited to piercing-sucking insects such as gall midges (Bentur et al., 2016; Harris et al., 2012), or phloem-feeding insects such as aphids (Botha, Li, & Lapitan, 2005; Dogimont, Chovelon, Pauquet, Boualem, & Bendahmane, 2014; Klingler, Nair, Edwards, & Singh, 2009; Nicolis & Venter, 2018; Rossi et al., 1998; Sun et al., 2020), whiteflies (Nombela, Williamson, & Muñiz, 2003) and planthoppers (Tamura et al., 2014; Liu et al., 2015; Zhao et al., 2016). Remarkably, reports on resistance genes to chewing insects are even more scarce, with only a few studies showing that PRR surface receptors also mediate defenses against chewing caterpillars (Gilardoni, Hettenhausen, Baldwin, & Bonaventure, 2011;

L. Hu et al., 2018; Steinbrenner et al., 2020). Indeed, immunity against feeding insect herbivores appears to be mainly controlled by polygenic quantitative traits (Kliebenstein 2017).

Given the lack of effective *R* genes against chewing insects, resistance mechanisms targeting insect eggs have been proposed as a complementary defence strategy (Fatouros, Cusumano, Danchin, & Colazza, 2016; Tamiru, Khan, & Bruce, 2015). Clearly, the recognition and killing of insect eggs is advantageous to plants as it prevents the destructive feeding by the hatching larvae (Hilker & Fatouros, 2015, 2016). The investigation of egg-killing traits thus represents an alternative and unexplored source of novel *R* genes to increase crop resistance to pests (Fatouros et al., 2016).

Cabbage white butterflies, such as the gregarious *Pierisbrassicae* and the solitary *P. rapae* (Lepidoptera: Pieridae), are pests of crucifer crops (*Brassica* spp.) and a serious agricultural challenge (Kumar, 2017; Ryan et al., 2019).*Pieris* eggs induce a HR-like cell death in their host plants of the Brassicaceae family resembling a HR induced by pathogens (Caarls et al., 2023; Griese et al., 2021; Shapiro & De Vay, 1987). Under field conditions, a severe cell death reduces egg survival up to more than 40% on black mustard *B. nigra* (Griese et al., 2021). Therefore, the egg-induced cell death represents a trait with a high potential as a novel plant defense against eggs, hence reducing the impact of successive larval stages. While it is known that plants respond to*Pieris* eggs with a salicylic acid (SA)-dependent immune response (Bonnet et al., 2017; Bruessow, Gouhier-Darimont, Buchala, Metraux, & Reymond, 2010; Caarls et al., 2023; Little, Gouhier-Darimont, Bruessow, & Reymond, 2007), the genes involved in detection and activation of egg-induced HR remain unknown.

A few recent studies began to investigate the genetic basis of*Pieris spp.* egg-induced HR-like cell death using the model species *A. thaliana* (Groux et al., 2021) and the crop *B. rapa* (Bassetti et al., 2022), which both benefit from extensive resources for classical forward genetics. A genome-wide association study (GWAS) in *A. thaliana* identified two loci, an L-type*lectin receptor-like kinase-I.1* (*LecRK-I.1*) and a putative Ca^{2+} channel *glutamate receptor 2.7*(*GLR2.7*) (Groux, 2019; Groux et al., 2021). Further, a QTL mapping in *B. rapa* identified three QTLs *Pbc1-3* associated with cell death size (Bassetti et al., 2022). The QTLs included many genes involved in plant immunity, but they underlined large genomic regions yet to be fine-mapped. A partial overlap among the loci identified in both plant species was suggested, given that*Bra* LecRK-I.1 is included within *Pbc3* (Bassetti et al., 2022). Overall, the study of plant-insect egg molecular interaction is a relatively recent field, and more research is clearly needed to understand to which extent the genetic regulation of *Pieris*egg-plant interaction is conserved between plant species.

Egg-induced HR-like cell death observed in *A. thaliana* and*B. rapa* manifests as a light necrosis that has no or little effect on egg survival (Groux, 2019; Groux et al., 2021). In contrast, egg deposition on *B. nigra* triggers a severe HR, spreading from the leaf abaxial up to the adaxial side, which correlates with a substantial killing of different *Pieris* eggs (Caarls et al., 2023; Fatouros et al., 2014; Griese et al., 2021; Griese et al., 2020). Previously, we found that the severity and occurrence of egg-induced cell death in *B. nigra* differs between plants of the same accession (Caarls et al., 2023) and between accessions (Caarls et al., 2023; Griese, Dicke, Hilker, & Fatouros, 2017; Pashalidou, Fatouros, Loon, Dicke, & Gols, 2015), suggesting the existence of natural variation for egg-induced HR. A treatment with a solution of compounds derived from the eggs, an egg wash, was shown to mimic egg-induced responses in plants (Caarls et al., 2023) and it provides suitable treatment to screen large genetic populations. In summary, *B. nigra* represents an ideal plant species to study the genetics of egg-induced HR because the phenotype is strong, stable, easy to score, varies between accessions, and has a proven egg-killing effect.

In this study, we investigated the inheritance and genetic basis of*P. brassicae* egg-induced HR-like cell death in *B. nigra* . Given the lack of advanced genetic populations, we crossed plant material collected from the field. We found variation in the response between and within field-collected *B. nigra* accessions, and then used crosses between strong- and low-responding individual plants to study the inheritance of the trait. We found that *Pieris*butterfly egg-induced HR *B. nigra* segregates as a Mendelian trait, and then performed genetic mapping through bulk-segregant analysis paired with whole genome sequencing (BSA-seq). Recombinant analysis was used to further fine-map the genetic region and identify a single dominant locus of ˜50 kb

which we named*Pieris e* gg-*k* illing (PEK). Within PEK, a gene cluster of TIR-NBS-LRRs, a type of NLR receptors was present, showed copy number variants (CNVs) between different *B. nigra*genomes.

Material and methods

Plant materials

The inheritance of the HR-like cell death was studied using *B. nigra* accessions collected from a local population in the floodplain of the Rhine River near Wageningen, The Netherlands (N51.96, E05.68). The accessions SF3-O1, SF19-O1, SF25-O1, SF29-O1, SF47-O1 and SF48-O1 originated from one multiplication by open pollination ("O1") of accessions used in previous studies (Griese, Dicke, Hilker, & Fatouros, 2017). The accessions DG1, DG12 and DG29 originated from open pollinated wild plants collected in 2018. A single DG1 plant showing no HR in response to eggs was selfed to obtain accession DG1-S1. A single DG1-S1 plant was then crossed with a single SF48-O1 plant to obtain an F1 population (Fig. 2a). Single plants from the F_1 that showed HR-like cell death were backcrossed to other DG1-S1 plants to obtain segregating backcross families (BC_1). Selfing of individual plants was done to generate BC_1-S_1 and BC_1-S_2 populations.

Plants were grown in a greenhouse under standardized conditions (21° day / 18° night, RH 50 - 70%, LD 16:8 h). Seeds were vernalized at 4° C for two days to induce even germination and then were sown in small trays with sowing soil (Lentse potgrond, Lent, The Netherlands). Seedlings were transplanted one week after germination into 17 cm diameter pots with potting soil (Lentse potgrond, Lent, The Netherlands). Plants were grown for five weeks before treatment with *P. brassicae* egg wash.

Insect material

Pieris brassicae L. (Lepidoptera: Pieridae) butterflies were obtained from a rearing of the Laboratory of Entomology, Wageningen University. Insects were kept in a greenhouse under standardized conditions (21 ± 4° C, RH: 60 – 80 %, L16: D8). Larvae were reared on Brussel sprout (*Brassica oleracea* var. *gemmifera*) cv. Cyrus, while the adults were fed with a 10 % honey solution and allowed to oviposit on clean plants of the same genotype.

Egg wash treatments

For the treatment of different *B. nigra* accessions (Fig. 1), egg wash was prepared following a recently published protocol (Caarls et al., 2023). In short, *Pieris brassicae* eggs were collected on filter paper pinned underneath a *B. oleracea* leaf in a cage containing twenty mated females. Egg clutches laid on the paper were cut out and submersed in 1 mL 2-(N-morpholino) methanesulfonic acid (MES) buffer per 400 eggs, overnight (16h) without disturbance. The solution (egg wash) was pipetted into a new tube the next morning. For this experiment, two 10 µl drops of egg wash were applied to each of the youngest fully developed leaves. Drops of an equivalent amount of MES buffer were applied as negative control.

For the treatment of the genetic mapping populations and to compare egg-induced HR to pathogen-induced HR, a protocol resulting in a more concentrated egg wash was used (Bassetti et al., 2022). In brief,*P. brassicae* egg clutches were collected from Brussel sprout leaves within 24 h after oviposition. Eggs were carefully removed with a stainless-steel lab spatula without breaking them and placed in an Eppendorf tube together with demineralised water in a ratio of ˜1000 eggs per 1 ml of water. After an overnight incubation at room temperature, the liquid phase was retained and stored at -20 °C. Then, two 5 µl drops of egg wash were applied to each of the youngest fully developed leaves. Drops of an equivalent amount of demineralised water were applied as negative control.

Pathogen extracts treatments

The fungal pathogens *Rhizoctonia solani* (Rs) and *Alternaria brassicicola* isolate MUCL2097 (Ab), were cultured on Potato Dextrose agar (PDA) plates at 25 °C. To prepare fungal extracts, the mycelium was

first grown until it covered a whole Petri dish (Ø 100 mm), then it was cut into mycelial plugs (1 cm^2), inoculated into 100 ml Potato Dextrose medium and incubated at 25 °C and 200 rpm. Mycelium was harvested from liquid cultures after 1.5 weeks, dried from excess medium and placed into a 50 ml tube containing 5 ml demineralised water. All tubes were kept on ice and ultrasonicated 6 times for 1 minute at maximum amplitude, using an ultrasonication probe. After a centrifugation step of 5 min at 4000 rpm, the supernatant was aliquoted in Eppendorf tubes and stored at -20 °C until use.

The bacterial pathogen *Xanthomonas campestris* pv.*campestris* race 4 (Xcc) was maintained on yeast extract-dextrose-carbonate agar (YDCA) plates at 28 °C. To prepare bacterial extract, Xcc was grown overnight in 15 ml liquid LB medium at 28 °C and 200 rpm. Cultures of Xcc at OD600 of 0.6 - 0.7 were centrifuged for 10 min at 3000 rpm, resuspended in a minimal amount of demineralised water and ultrasonicated as described above. Tubes were then centrifuged again for 10 min at 3000 rpm and the supernatant was aliquoted in Eppendorf tubes and stored at -20 °C until use. All pathogen extracts were applied using a 1 ml syringe.

Assessment of egg wash-induced cell death

Egg wash-induced HR-like cell death was scored as "HR severity" on a scale from 0 to 4 as previously described (Caarls et al., 2023): 0, no visible response; 1, brown spots underneath egg wash-treated spot, only visible at abaxial side leaf; 2, cell death also visible at adaxial side of leaf, spot smaller than 2 mm diameter; 3; cell death covering the size of the egg wash-treated spot; 4, cell death spreading lesion beyond the treated spot.

DNA extraction, pooling, sequencing

For all the population of our crossing scheme, Genomic DNA was extracted from young leaves previously sampled, snap frozen and stored at -80C. DNA was extracted following a modified CTAB method from Maloof lab (https://openwetware.org/wiki/Maloof_Lab:96well_CTAB). DNA concentration and purity was estimated with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, MA, USA). The DNA integrity was confirmed was assessed using a 1% agarose gel with ethidium bromide. Prior to sequencing, DNA concentration was measured with a Qubit Fluorometer (Invitrogen, MA, USA).

For the bulk segregant analysis (BSA) resistant (R, plants showing HR) and susceptible (S, plants without HR) bulks ($n = 10$) were prepared by pooling equal amounts of DNA from each individual plant. Further, the three accessions that originated the initial crossing were also used for DNA isolation (the S parent DG1-S1, the R parent F1_#130 and the donor of HR SF48-O1, "R donor" (Fig. 2). For the WGS experiment, 1 ug of genomic DNA of each sample (three accessions and two bulks) was used for library preparation. Library preparation and whole genome sequencing were carried out by Novogene (Cambridge, UK). Sequencing libraries were generated using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, UK) following the manufactures' protocol. The genomic DNA was randomly fragmented to a size of 350bp by Bioruptor, then DNA fragments were size-selected with sample purification beads. The selected fragments were then end-polished, A-tailed, and ligated with the full-length adapter. After these treatments, these fragments were filtered with beads. At last, the library was analysed for size distribution by Agilent 2100 Bioanalyzer (Agilent technologies, CA, USA) and quantified using real-time PCR. Libraries were sequenced on an Illumina NovaSeq 6000 platform using 150 bp paired end (PE) reads.

K-mer based genetic mapping

K -mer based genetic mapping was performed following the recommendations of Comparative subsequence sets analysis (CoSSA) workflows (Prodhomme et al., 2019). First, sequencing read quality was assessed with FastQC (Andrews, 2010). Then, k- mer tables were built with a k -mer size of 31 nucleotides using GlistMaker of the GenomeTester4 v4.0 suite (Kaplinski, Lepamets, & Remm, 2015).k- mer that occurred only once were removed as likely resulting from sequencing errors. To identify resistant (R) haplotype-specific k -mers, GlistCompare of GenomeTester4 was used to perform basic set operations such as unions, intersections of differences between k -mer tables of different samples (Supporting information: Fig. S1). An additional

filtering step was carried out to retain R haplotype-specific k -mers. The sequencing yielded 14.4 Gb for the R bulk and an approximate 24x depth considering a haploid *B. nigra* genome of ˜550 Mb. Given that *B. nigra* genome is diploid (2n = 2x = 16) and assuming uniform sequencing coverage, k -mers originated from the Rhaplotype should have sequencing depth of ˜12x. Thus, we decided to retain k -mers with a depth between 10x and 20x which represented k -mers derived from R-specific haplotype. Retained$k$ -mers were aligned to *B. nigra* reference genomes NI100 v2.0, C2 v1.0 and Sangam v1.0 using BWA *aln* (v0.7.17) allowing 3 mismatches (Li & Durbin, 2009). The number of mapped k -mers mapped per 1 Mb bins were counted using bedtools v2.25 (Quinlan & Hall, 2010).

KASP markers genotyping

Kompetitive Allele Specific Polymorphisms (KASP) PCR markers were used to validate the results of k -mer based genetic mapping. For each sample, DNA concentration was adjusted to 5-50 ng/µl. Primers were designed on the sequences flanking the SNPs identified by k -mers of the R-specific haplotype. KASP genotyping assays were performed according to the manufacturer's instructions (LGC Genomics, UK). In brief, 2 µL DNA at a concentration of 5–50 ng/µL was added to 96-well plate with KASP PCR mix (5 µL 2× KASP Master mix, 0.6 µL 10mM primer mix, 2.4 Milli-Q water). The PCR was performed in a CFX96 Touch Real-Time PCR Detection System combined with CFX Maestro Software for data reading (Bio-Rad, Hercules, CA, USA).

Variant calling within *PEK* locus

Single nucleotide polymorphisms (SNPs) and Insertion/Deletion (InDels) variants were called using a workflow based on GATK Best Practices (et al., . Reads were aligned to the *B. nigra* reference genome C2 v1.0, to which the mitochondrial sequence (Genbank accession no. NC_029182) and chloroplast sequence (Genbank accession no. NC_030450) were added, using BWA mem v0.7.17 with default parameters (Li, 2013). The resulting alignment files were sorted and indexed using SAMtools v.1.11 (Li et al., 2009). Alignment files were filtered to restric variant calling to the *PEK* locus. Duplicate read pairs were marked using the MarkDuplicates tool of the GATK suite v.4.1.9.0. Variants (SNPs and InDels) were called in each sample on a window of x Mb around the *PEK* locus using GATK HaplotypeCaller. Samples were then jointly genotyped using GATK GenomicsDBImport and GATK GenotypeGVCFs, with default parameters. SNPs filtration was performed with the following parameters: QD < 2, QUAL < 30, SOR > 3, FS > 60, MQ < 40, MQRankSum < -12.5, ReadPosRankSum < -8. InDels filtration was performed with the following parameters: QD < 2, QUAL < 30, SOR > 3, FS > 200, ReadPosRankSum < -20. Finally, only variants that in agreement with our genetic model were retained: i) heterozygous in resistant (HR+) material; ii) homozygous DG1-S1 allele in susceptible (HR-) material. The functional effect of the retained variants was predicted using SnpEff with default parameters (Cingolani et al., 2012).

Copy number variations (CNVs) at *PEK* locus between three *B. nigra* reference genomes was performed with the tool GEvo on the CoGe web platform (Lyons et al., 2008). All genomes were accessed in September 2021 from the following databases: *B. nigra* genomes NI100 v2.0 and C2 v1.0 were downloaded from http://cruciferseq.ca/, genome Sangam v1.0 from http://brassicadb.cn/. Pairwise alignment of genomes was performed with default settings: Alignment algorithm "(B)LastZ: Large regions"; Word size: 8; Gap start penalty: 400; Gap extend penalty: 30; Chaining: NO; Score threshold: 3000; Max threshold: 0; Minimum HSP length: 50. CNVs were identified by visualizing the region between markers flanking*PEK* locus (M27 and M28) and identifying conserved regions/genes that were highlighted as High Scoring Pairs (HSP).

Comparative genomics of *PEK* locus between *B. nigra*, A. *thaliana* and *B. rapa*

Synteny analysis between the *B. nigra PEK* locus, *A. thaliana* and *B. rapa* genomes was performed using the tool SynMap on the CoGe web platform (Haug-Baltzell, Stephens, Davey, Scheidegger, & Lyons, 2017; Lyons et al., 2008). SynMap legacy version was used with similar settings as previously described (Bassetti, 2022). All genomes were accessed in September 2021 from the following databases: *B. nigra* genomes NI100 v2.0 and C2 v1.0 were downloaded from http://cruciferseq.ca/, genome Sangam v1.0 from http://brassicadb.cn/, *B. rapa* genome Chiifu v3 from http://brassicadb.cn/, *A. thaliana* Col-0 vTAIR10

from https://www.arabidopsis.org/.

Statistical analysis of phenotypic data and marker-trait association

All statistical analyses were conducted on R v4.0.1 (R Core Team 2021). Scoring of HR-like cell death in severity categories were analysed with a non-parametric method (Kruskal-Wallis test) on HR scores (0, 1, 2, 3, and 4) and different accessions were included as categorical fixed factors. Differences in mean HR severity were tested using Kruskal-Wallis test, followed by Wilcoxon Rank Sum test with Benjamini-Hochberg correction. Chi-square tests were used to test the goodness of fit of the segregation of phenotypic data and KASP markers data. Marker-trait association was analyzed with the R/qtl package (Broman et al., 2003) using the *scanone* function for binary data (presence/absence of HR).

Results

Natural variation in egg-induced HR between *B. nigra* accessions

To study natural variation in egg-induced HR-like under controlled conditions, we collected seeds from a local *B. nigra* population that previously showed variation in HR-like cell death in field conditions (Fatouros et al., 2014; Griese et al., 2017). Induction of HR-like cell death was tested using treatment with an egg wash, which we previously showed to mimic *Pieris* eggs (Caarls et al., 2023). Overall, we observed variation between plants of each accession in occurrence and severity of the HR-like cell death (Fig. 1). In addition, we found significant differences between accessions for the severity of the HR-like cell death response (Kruskall-Wallis test, $p < 0.05$). In some accessions, for example SF48-O1, almost all plants showed an HR (score 2-4) and for some, e.g., DG1, only 1 out of 7 plants responded with an HR to the wash. The variation in egg-induced HR within and between accessions suggests the existence of genetic variation within the population that could be used for genetic mapping.

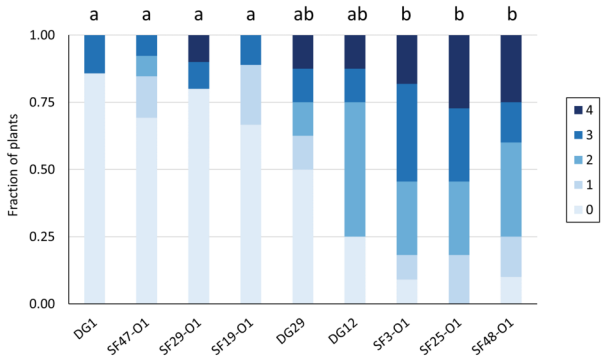

Figure 1. Intraspecific variation of HR-like cell death in*B. nigra* accessions. HR-like cell death induced by egg wash in nine accessions. Treatments were applied on the abaxial side of the leaf as drops. Egg-wash induced symptoms were scored in severity: 0, no visible response; 1, brown spots underneath egg

wash-treated spot, only visible at abaxial side leaf; 2, cell death also visible at adaxial side of leaf, spot smaller than 2 mm diameter; 3; cell death covering the size of the egg wash-treated spot; 4, cell death spreading lesion beyond the treated spot. Controls (MES buffer) did not induce visible symptoms (not shown). For each treatment/accession combination N=10. Different letters indicate significant difference between accessions (Kruskall-Wallis test, p -value < 0.05).

Variation in egg-induced HR is independent of cell death induced by other biotic stresses

To understand whether a *B. nigra* plant showing absence of egg-induced HR-like cell death was also impaired in the ability to develop cell death against other biotic stresses, we tested two *B. nigra* accessions that either consistently develop a strong HR-like response (SF48-O1) or no cell death at all (DG1-S1). We treated them with egg wash and extracts of the Gram-negative hemibiotrophic bacterium*Xanthomonas campestris* pv. *Campestris* (Xcc) and the necrotrophic fungi *Alternaria brassicicola* and *Rhizoctonia solani* . We found that DG1-S1, the accession unable to develop cell death upon egg wash, could still develop a cell death against pathogen extracts (Supporting information: Fig. S1). In other words, egg wash induced different HR-like severity between the two accessions unlike the three pathogen extracts (Kruskal–Wallis test,$p < 0.01$) (Fig. 1, Supporting information: Fig. S2).

Egg-killing HR is inherited as a single dominant Mendelian locus

We studied the inheritance of *P. brassicae* egg-induced HR in a crossing scheme derived from a cross between accessions DG1-S1 and SF48-O1 (Fig. 2a) that were selected from our germplasm screening (Fig. 1). The parental accessions consistently showed contrasting phenotypes (Fig. 2a), particularly DG1-S1 showing no/weak HR (score 0-1) and SF48-O1 showing a strong HR (score 2-4) upon treatment with egg wash. Based on our previous studies (Griese 2017, Griese 2021), no/weak HR (score 0-1) did not affect egg survival resulting in "susceptible" (S) plants, while strong HR (score 2-4) caused egg-killing resulting in "resistant" (R) plants. When egg-killing HR was scored as presence/absence in the F_1 population (F1-1, $n = 150$), it segregated with a clear bimodal distribution with a 1:1 ratio between plants without and with HR (χ^2 test, $p> 0.05$) (Fig. 1b, Table 1). Segregation in the F_1 was not surprising considering that wild *B. nigra* are self-incompatible and thus highly heterozygous. This suggests that HR may be heterozygous at least in the R donor accession. A backcross population (BC1-3, $n = 66$) between a resistant F_1 plant and the susceptible parent (DG1-S1) showed again a 1:1 segregation (χ^2 test, $p> 0.05$) (Fig. 2b, Table 1). Recurring of a 1:1 segregation pattern resembled the outcome of a test-cross for a single heterozygous locus. This was confirmed after selfing a resistant BC_1plant with HR which resulted in a BC_1S_1population (BC1S1-1, $n = 695$) with a 3:1 ratio between R and S plants (χ^2 test, $p > 0.05$) (Fig. 2b, Table 1). Overall, the segregation of *P. brassicae*egg-killing HR in our crossing scheme was consistent with a trait controlled by a single dominant Mendelian locus, which should be heterozygous in the HR donor SF48-O1 plant.

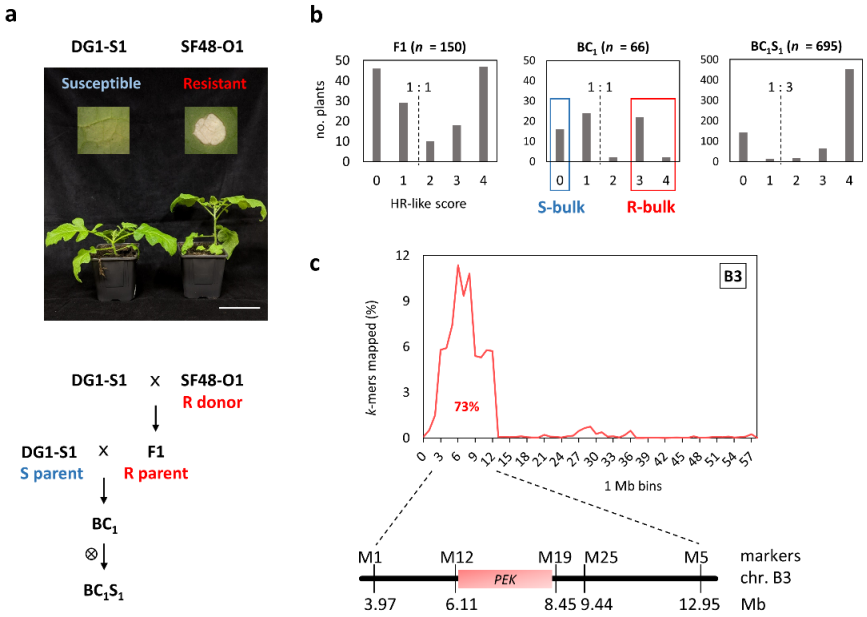

Figure 2. Butterfly egg-killing HR segregates as a Mendelian trait and it is mapped to a single locus on chromosome B3 . A) *B. nigra* parental accessions, five-weeks old plants and relative HR phenotype (above). Crossing scheme used to study inheritance of HR-like induced by *P. brassicae e* gg wash (below). Magnification bar = 10 cm. B) HR phenotypic distribution within the populations obtained from the crossing scheme. C) Distribution of *k* -mers unique to resistant (R) samples mapped on chromosome B3 (genome C2). *k* -mers are plotted on each 1 Mb bin as percentage of the total *k* -mer set. A single peak consisting of ˜73% R-specific*k* -mers located the *PEK* locus at interval 3-13 Mb (top panel). Validation of the locus was carried out with KASP markers on the BC_1 population (n = 66) and four informative recombinants restricted the *PEK* locus between 6.11 and 8.45 Mb (bottom panel).

The single locus model was further supported by the phenotypic segregation in other crosses involving selfings of parental plants, F_2 and BC_1 populations (Supporting information: Table S1). Selfing of the susceptible parent DG1-S1 resulted in progeny with no HR (DG1-S2, n = 15). Similarly, a backcross between F_1 plants with no HR and DG1-S1 (BC1-5, n = 70) resulted also in progenies with no HR. These results further suggested that absence of HR resulted from fixing a homozygous recessive allele at a single locus. Conversely, backcrosses between F_1 with HR-like and DG1-S1 showed again a 1:1 segregation (BC1-4, n = 69; BC1-6, n = 70), as previously observed. Finally, F_2s derived from resistant F_1s showed a 3:1 segregation as expected (F2-2, n= 8; F2-3, n = 12).

Genetic mapping of egg-killing HR to a single *PEK* locus through BSA-seq

To identify genomic regions associated with *Pieris* egg-induced HR, we performed a *B* ulk *s* egregant *a* nalysis coupled with whole genome *s* equencing (BSA-seq) on the backcross population BC1-3 (Fig. 2b). We generated two bulks (n = 10) with either susceptible plants (S-Bulk) or with resistant plants (R-Bulk). Genomic DNA of S-bulk, R-bulk, the S parent (DG1-S1), the R parent (F1_#130) and the donor of HR (SF48-O1, "R donor") was sequenced using Illumina 150 bp paired-end reads yielding between 14 and 22 Gb data for each sample (Supporting information: Table S2). As we estimated a haploid genome size of our *B. nigra* material of ˜550 Mb, our sequencing resulted in a read depth between 26x and 35x for each diploid genome of parents, thus a coverage ranging between 13x and 17.5x of a haploid genome. Given the heterozygosity in our plant material, we performed a *k*- mer based BSA-seq approach (CoSSA) which was recently developed for a highly heterozygous tetraploid potato (Prodhomme *et al.* 2019). Our genetic model

pointed at a monogenic dominant locus which was heterozygous in the R parent F1_#130, homozygous in the S parent DG1-S1 and segregating 1:1 in the backcross population BC1-3. Thus we expected a single resistant (R) allele in the R parent conferring HR. Accordingly, we first generated k -mer tables ($k = 31$) for each sample independently. Then we selected k -mers from the R allele ("R haplotype") by using basic set algebra to retain k -mers that were unique to the R-bulk and originated from R parent and R donor (Supporting information: Fig. S3). The resulting R haplotype-specific$k$ -mer set was filtered to retain unique k -mers with a frequency similar to half of sequencing depth for a haploid genome (15x+ 5) to discard k -mers derived from repeated regions (Supporting information: Table S3).

The unique R haplotype-specific k -mers were aligned to 1 Mb bins of the *B. nigra* reference genome C2 (Supporting information: Fig. S4). Approximately ˜85% of the R-specific k -mers were successfully aligned, while the rest likely represented sequences from plant material that were too divergent or absent from the *B. nigra* reference genome. A unique single peak consisting of ˜73% of the R haplotype-specific k -mers was found in a 10 Mb interval on the proximal end of chromosome B3, spanning from 3 Mb to 13 Mb (Fig. 2c). All other k -mers (˜27%) mapped at a very low frequency (below 0.4% for each 1 Mb bin) throughout the rest of the genome (Supporting information: Fig. S4). Similarly, alignment of R haplotype-specific$k$ -mers to other *B. nigra* genomes resulted also on a single peak, namely on chromosome B3 of accession NI100 and chromosome B7 of accession Sangam (not shown). Location of *PEK* on a different chromosome in Sangam is likely due to a different chromosome naming by the authors of the genome, as we found that chromosome B7 of Sangam is perfectly syntenic to chromosome B3 of C2 and NI100 accessions (Supporting information: Fig. S5). In conclusion, a BSA-seq approach allowed us to map the HR cell death trait in a BC_1population and identified a single genetic locus on chromosome B3. This confirms the genetic model that we hypothesized based on the phenotypic segregation.

The *PEK* locus is responsible for egg-killing

To validate the locus identified by BSA-seq on the top of chromosome B3, we designed KASP markers that targeted the whole 10 Mb region (Supporting information: Table S4). We genotyped the entire BC1 population (BC1-3, $n = 66$) with five KASP markers. Each KASP marker co-segregated with the HR cell death phenotype, without showing segregation distortion (χ^2 test, $p > 0.05$) (Supporting information: Table S5). The S parent DG1-S1 and susceptible BC_1 plants were homozygous (*pek*- DG1/*pek*- DG1) while the R donor SF48-O1, the R parent F1_#130 and resistant BC_1 plants were heterozygous (*PEK* -SF48/*pek* -DG1). Four informative recombinants between M1 and M25 were then genotyped with additional KASP markers, which restricted the locus to the interval 6.11-8.45 Mb between M12 and M19 (Fig. 2c). Validation with KASP markers confirmed that HR was associated with heterozygosity at a single locus on chromosome B3, which we named*Pieris -e* gg *k* illing (*PEK*) locus.

We then proceeded with fine-mapping the *PEK* locus through recombinant analysis using a BC_1S_1population (BC1S1-1, $n = 695$) that was generated by selfing a resistant BC1-3 plant with heterozygous genotype at the *PEK*locus, thus carrying both SF48-O1 allele (*PEK* -SF48) and DG1-S1 allele (*pek* -DG1). The whole BC_1S_1population was genotyped with five KASP markers (M1, M13, M19, M25, M5) spanning the interval. Each marker showed a 1:2:1 allelic segregation ratio (χ^2 test, $p > 0.05$) which was expected given the 3:1 phenotypic segregation ratio between R and S plants (Table 2). The markers were placed on a genetic map of 20.6 cM with a total recombination rate of 2.54 cM/Mb (Fig. 3a). As previously observed in BC1-3, all markers covering the region were associated with HR and marker M13 (6.06 Mb) showed the highest LOD score (Table 2). In total, we could identify 114 recombinants between markers M1 and M5, of which 64 informative recombinants between markers M1 and M19 (Fig. 3b). These 64 plants showed recombination between heterozygous (*PEK-SF48/pek*- DG1) and homozygous (*pek*- DG1/*pek*- DG1) genotypes. Interestingly, all the susceptible BC_1S_1 plants had homozygous *pek*- DG1 allele at marker M13. Additional KASP markers were designed between M1 and M19 and four key recombinants (Haplotypes 6 and 7) restricted the*PEK* locus between M27 and M28. Although we did not find a maker co-segregating with the HR phenotype, fine-mapping restricted the*PEK* locus to a ˜50 kb interval on chromosome B3.

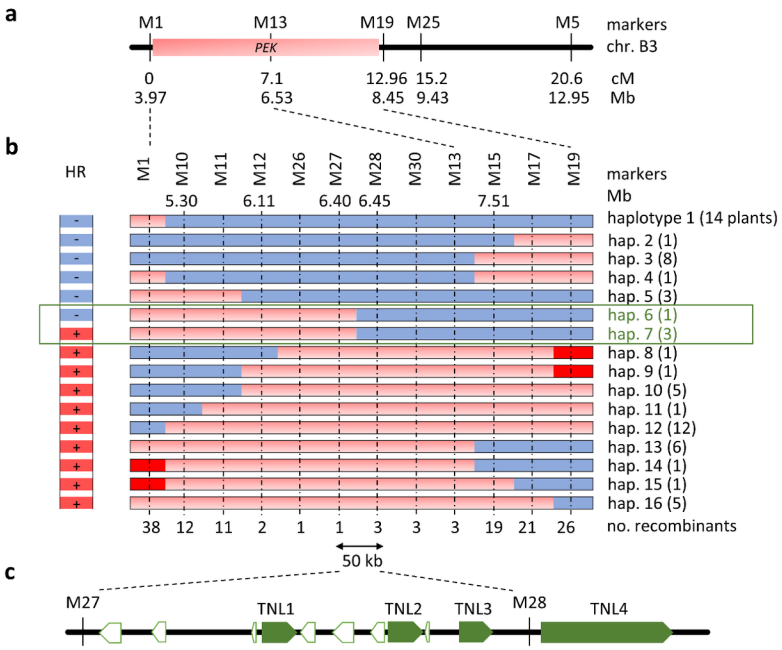

Figure 3. *PEK* locus is fine-mapped to a ˜50 kb interval including a cluster of TIR-NBS-LRR receptors. a) Fine mapping with five makers on a BC1S1 population ($n = 695$) located the *PEK* locus between 3.97 and 8.45 Mb. Marker names, genetic distance (cM) and physical distance 9 are given. b) Genotype and phenotype of 64 recombinants used for recombinant analysis. Recombinants with same genotype are represented by a single haplotype ("hap."). Blue bars represent homozygous DG-S1 allele (S/S), light red heterozygous (R/S), dark red homozygous SF48-O1 genotype (R/R). Phenotype of each haplotype (HR presence/absence) is shown on the left side: "-" means susceptible (S, no HR), "+" means resistant (R, strong HR). Numbers under each dashed line indicate the recombinants between each marker and the phenotype. c) *PEK* locus is fine-mapped to a ˜50 kb interval (genome C2 v1.0) containing eleven genes and a cluster of four TIR-NBS-LRR (green). A full list of genes within the *PEK* locus is reported in Table 3.

We performed an additional recombinant screening on four BC_1S_2 populations that were generated from four R plants with heterozygous genotype (*PEK*- SF48/*pek* -DG1) at the locus (Supporting information: Fig. S6). All four BC_1S_2s showed the expected 3:1 phenotypic segregation ratio between R and S plants (Supporting information: Table S1). Informative recombinants between M12 and M15 were identified in all populations (7 in BC1S2-2, 12 in BC1S2-3, 2 in BC1S2-4, 8 in BC1S2-5) and genotyped with additional markers (M26, M27, M28, M30). In all populations, S plants had homozygous*pek*- DG1 allele at markers M26, M27 and M28. One R recombinant in population BC1S2-3 was homozygous *pek*- DG1/*pek*- DG1 at M28, thus confirming the right border of the locus.

Main candidate genes within *PEK* are a cluster of TNLs receptors

To identify candidate genes for the HR phenotype, we checked the annotations within the *PEK* locus in the *B. nigra* reference genome C2. The region contains 11 genes with many duplicated loci such as three *B. nigra* homologs of a methionine aminopeptidase 1D (MAP1D, AT4G3700), three homologs of an unknown membrane protein (AT4G37030) and three TIR-NBS-LRR. A fourth TIR-NBS-LRR (TNL) is present just outside marker M28. Based on gene annotations, the four TNLs are the only genes that we could associate to known plant immunity functions (Cui, Tsuda, & Parker, 2015). Thus, they represent good candidate genes for the HR phenotype. Further, we used the sequencing data from the BSA-seq to study the variation within the *PEK* locus. In total we identified 785 variants (SNPs and InDels) within each of the eleven genes, but we could not pinpoint yet putative casual variants associated to the trait (Supporting information: Table

S6).

Finally, we investigated whether the *PEK* locus overlaps with loci that were previously associated with *P. brassicae* egg-induced cell death in *A. thaliana* (Groux et al., 2021) and *B. rapa*(Bassetti et al., 2022). Overall, *PEK* appeared syntenic to the bottom of *A. thaliana* chromosome 4 (Supporting information: Fig. S7a) and to the center of *B. rapa* chromosome A08 (Supporting information: Fig. S7b). None of these regions were previously associated with butterfly egg-induced HR, as genes *AtLecRK-I.1* (AT3G45330) and *AtGLR2.7* (AT2G29120) located on *A. thaliana*chromosomes 2 and 3 (Groux, 2019; Groux et al., 2021), while *B. rapa* QTLs *Pbc1-3* located on chromosomes A02, A03 and A06 (Bassetti et al., 2022). In conclusion, the *PEK* locus here identified in *B. nigra* represents a novel genomic region associated with a butterfly egg-induced HR which results in egg-killing.

PEK locus shows copy number variations (CNVs) among*B. nigra* genomes

The *PEK* locus contained multiple duplicated genes, including a cluster of TIR-NBS-LRR, a class of NLR intracellular receptors. NLRs are often organized in genomic clusters as the result of tandem duplications, unequal crossing over and gene conversion (Kuang *et al.* 2004). Thus, we suspected that the locus may be highly dynamic and polymorphic among *B. nigra* genomes. Indeed, we found extensive copy number variations (CNVs) for some of the genes when comparing the available *B. nigra* genomes NI100, C2, and Sangam (not shown). Specifically, the TNLs were present in two copies in NI100, four copies in C2, and seven copies in Sangam. Similarly, we found CNVs also for*Bn* MAP1D which is present in two copies in NI100, three copies in C2, and four copies in Sangam. Collectively, our data showed that the*PEK* locus is highly polymorphic among available *B. nigra*genomes.

Discussion

Hypersensitive response induced by *Pieris* spp. egg deposition is an appealing model system to study the interaction between plants and insect eggs as well as an effective plant defence trait to chewing herbivores. Here, we report for the first time that *P. brassicae*egg-killing HR-like cell death in *B. nigra* segregates as a Mendelian dominant trait underlined by a single locus. Through BSA-seq and fine-mapping, we identified the *PEK* locus on a ˜50kb interval on the proximal arm of *B. nigra*chromosome B3. We found a cluster of TIR-NBS-LRR (TNLs) intracellular receptors which are the only genes associated with plant immunity and are considered likely candidate genes. So far, no TNL receptor genes have been shown to be involved in immunity responses against insects. It is intriguing to further understand whether they directly recognize an egg-derived component and/or co-act downstream of egg recognition.

Segregation of the HR phenotype throughout our crossing scheme supported the evidence for a Mendelian trait underlined by a single dominant locus. As we crossed two plants from heterogenous wild *B. nigra*accessions, we observed phenotypic segregation of different morphological traits in the F_1. Segregation of HR was consistent with a single dominant locus originating from a heterozygous donor resistant (R) plant. In fact, we showed a 1:1 segregation ratio of F_1 and BC_1 populations, followed by a 3:1 segregation ratio of F_2, BC_1S_1 and BC_1S_2 derived from selfings of heterozygous resistant plants. Accordingly, selfing of plants without HR (S plants) resulted in progenies that were also unable to develop HR. We successfully identified the *PEK* locus using a BSA-seq approach which was already proven to be advantageous for quickly identifying single Mendelian loci in genetic populations with little recombination (Liu, Yeh, Tang, Nettleton, & Schnable, 2012), as well as in highly heterozygous species (Dakouri et al., 2018; Prodhomme et al., 2019). So far, HR has been frequently associated with monogenic qualitative resistance to bacteria, fungi, nematodes, and viruses (Kourelis & van der Hoorn, 2018). In plant-insect interactions, however, HR seemed less prominent as defense response and mostly occurring against cell content feeders such as aphids, gall midges or planthoppers (Botha et al., 2005; Himabindu, Suneetha, Sama, & Bentur, 2010; Klingler et al., 2009; Stuart, Chen, Shukle, & Harris, 2012). It is thus remarkable that an HR cell death evolved to target insect eggs and, also, that it is underlined by a single major effect locus as previously shown mainly for HR-based resistance traits against pathogens.

Through recombinant analysis, we fine mapped the *PEK* locus to a ˜50 kb region. Further fine-mapping in BC_1S_2 populations did not increase the resolution into the locus. *PEK* contains eleven genes, among which a cluster of intracellular receptors of the TNL type. The other genes within the *PEK* locus were annotated either as "unknown function", as an unspecified "membrane proteins" (BniB03g15430.C2) or were orthologs of a methionine aminopeptidase 1D (MAP1D, AT4G37030). MAP1D is an enzyme responsible for the cleavage of the initiator Methionine residue at the N-terminal of proteins (Ross, Giglione, Pierre, Espagne, & Meinnel, 2005). MAPs have been indicated as first step required for the stabilization and/or degradation of chloroplasts proteins (Apel, Schulze, & Bock, 2010), but a putative involvement in plant defense is yet to be proven. The involvement of MAP proteins in plant immunity has not been proven. However, given the involvement of TNLs in perception and signalling of plant immunity against pathogens (Cui, Tsuda, & Parker, 2015; Monteiro & Nishimura, 2018), and that cloned *R* genes providing resistance based on HR are often NLRs (Kourelis & van der Hoorn, 2018), we consider these TNLs as the main candidate genes for egg-induced HR.

How can we explain a putative role for a TNL intracellular receptor within the plant-egg interaction? So far, TNLs and other NLRs have been associated with qualitative disease resistance traits, often based on HR, and indeed most of the cloned resistance (R) genes are NLRs (Kourelis & van der Hoorn, 2018). Their main function is to activate defense upon perception of "effector proteins", a diversified array of proteins generally injected inside plant cells by pathogens and piercing-sucking insects to modulate and/or suppress the initial plant pattern-triggered immunity (PTI) response (Basu, Varsani, & Louis, 2018; Toruño, Stergiopoulos, & Coaker, 2016). NLRs either directly or indirectly detect these effectors at different cellular localizations to trigger so-called effector-triggered immunity (ETI) which often leads to HR (Monteiro & Nishimura, 2018). If the TNLs within the *PEK*locus are responsible for egg-induced HR, hypothetical "egg effectors" should be able to diffuse from the egg glue through the cell wall (Fig. 4). Interestingly, ongoing efforts suggest that the egg-associated elicitor of HR may not be a protein (Caarls et al. in prep). Alternatively, the TNLs within *PEK* may not be involved in egg recognition but rather in sensing perturbations of cellular homeostasis (Cui et al., 2015). For example, the TNL SNC1 of *A. thaliana*activates upon misregulation of MPK3/6 signalling and unregulated SA accumulation (Wang et al., 2013). Furthermore, certain autoimmune phenotypes in which cell death is regulated by sphingolipids appears to be monitored also by a TNL (Berkey, Bendigeri, & Xiao, 2012; Palma et al., 2010).

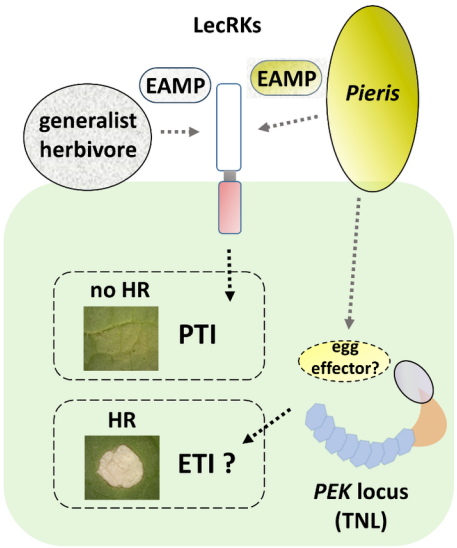

Figure 4. Proposed model of plant perception and immune response to Lepidoptera eggs (*Mamestra brassicae* and *Pieris*spp.) in *Brassica* spp. and *A. thaliana*. General elicitors or egg-associated molecular patterns (EAMPs), that are conserved among Lepidoptera eggs, e.g. PCs, induce a weak plant immune response resembling PTI (Caarls et al. 2023; Stahl et al. 2020). The recognition is mediated by different LecRKs in *A. thaliana*(Gouhier-Darimont, Stahl, Glauser, & Reymond, 2019; Groux et al., 2021). A putative effector present in *Pieris* eggs instead induces an ETI and following HR-like cell death in some *Brassica* spp, putatively mediated by TNLs within the PEK locus.

While waiting for functional validation of the *PEK* locus, a hypothetical role for TNLs in the perception and/or modulation of the plant response to *Pieris* spp. eggs could provide an extra step in the PTI-ETI model for eggs (Fig. 4). Insect eggs induce signalling pathways of PTI response similarly to the recognition of PAMPs from bacteria, fungi, and other biotic stresses (Jones & Dangl, 2006; Reymond, 2021). According to this model, egg elicitors that are conserved across insect species, also known as egg-associated molecular patterns (EAMPs), are detected by plant cell surface receptors (PRRs). An example of such conserved elicitors/EAMPs are the phosphatidylcholines (PCs), phospholipids which are present in all insect eggs (Stahl et al., 2020). PCs are, however, not sufficient to elicit HR in *B. nigra* and the elicitor(s) of HR seem to rather be present in a water-soluble phase from the glue-like secretion surrounding the eggs (Caarls et al., 2023). So far, at least two PRRs, LecRK-I.1 and LecRK-I.8, were found to mediate *Pieris* egg-induced PTI and HR-like phenotypes in *A. thaliana* (Gouhier-Darimont et al., 2019; Groux et al., 2021). Thus, LecRKs could potentially also modulate the PTI phenotypes that were induced in *A. thaliana* by eggs of *P. brassicae, Spodoptera littoralis* and *Drosophila melanogaster* (Bruessow et al., 2010) and in *Brassica nigra* by eggs of *Pieris* spp. and *Mamestra brassicae* (Caarls et al., 2023). *Pieris* spp. eggs induce a visible HR, while we observed no HR under eggs of generalists, e.g. *M. brassicae* or non-adapted Pierid butterflies (Caarls et al., 2023; Griese et al., 2021). In this scenario, one or more *Pieris* egg "effector" compounds, could be detected by unknown plant receptors, such as the *PEK* locus, and trigger a second immune response leading to a macroscopic HR-like cell death which acts as a defence trait.

Previously, we showed that *B. nigra* expresses a strong HR in response to *Pieris* spp. eggs resulting in egg-killing and that this response is consistently stronger compared to the responses observed in other Brassicaceae species (Griese et al., 2021). From a plant breeding perspective, the molecular markers flanking the*PEK* locus may be sufficient to attempt the introgression of egg-killing HR-like cell death into elite *Brassica* crops lines, as interspecific crosses between *Brassica* crop cultivars and crop wild relatives (CWR) from secondary/tertiary gene pools are sometimes done for other resistance traits (Hu et al., 2021; Katche, Quezada-Martinez, Katche, Vasquez-Teuber, & Mason, 2019; Lv, Fang, Yang, Zhang, & Wang, 2020).

From a scientific perspective, the characterization of genetic diversity of loci containing PRR or NLR receptors across a broad phylogenetic context helped to resolve the macroevolutionary history of pathogen and insect resistance traits and generate hypothesis on putative functional domains (Snoeck, Guayazan-Palacios, & Steinbrenner, 2022; Wang et al., 2021). In this study, we found within *PEK* locus many SNPs and/or InDels between our parental accessions. Additionally, we found copy number variants (CNVs) at the locus between *B. nigra* reference genomes, but the identification of the casual variants will likely require *de novo* assembly of the locus within our plant material. The high diversification in causal polymorphisms at NLR loci is well described (Dolatabadian & Fernando, 2022) and it resulted from evolution through a massive expansion and lineage diversification that allows adaptation to a multitude of biotic stresses (Shao, Xue, Wang, Wang, & Chen, 2019). Consequently, NLR loci are hardly ever well represented by a single plant genome and, further, are likely to be misassembled (Barragan & Weigel, 2021). It is thus fundamental to characterize the genomic context of *PEK* locus across the Brassicaceae to study whether the locus' genetic diversity across the plant family correlates with interspecific variation in HR phenotype. Such study may potentially reveal different types of polymorphisms at the *PEK* locus, as the interspecific variation in HR may have arisen from differences in life-history traits of plant species and/or exposure to insect eggs. For example, the weak type of HR developed by*A. thaliana* could be the result of lower selective pressure by the herbivore since as a short-day flowering species it is not a host of*Pieris* spp. (Harvey, Gols, Wagenaar, & Bezemer, 2007) and only occasionally of *Anthocaris cardamines* (Wiklund, 1984). Conversely, *B. rapa* crop

morphotypes show a weak HR (Bassetti, 2022) as the result of a different mechanism, for example negative selection during domestication as can occur with inducible defence traits in other crops (Turcotte, Turley, & Johnson, 2014; Whitehead, Turcotte, & Poveda, 2017).

In conclusion, here we report that intraspecific variation for HR induced by *P. brassicae* eggs is associated with a single locus in*B. nigra* . Through classical forward genetics we identified the*PEK* locus, which contains a cluster of TNL receptors. The locus seems highly polymorphic between the known *B. nigra* genomes and we expect this to be the case also for our accessions. This implies the need for improved genome assembly before future fine-mapping. This future work will enable cloning and functional testing of the first*B. nigra* gene involved in defense against insect eggs.

Acknowledgements

We are grateful to Dr. Charlotte Prodhomme and Corentin Clot for insightful discussion on the CoSSA pipeline settings for *k*- mer based genetic mapping. Thanks to Dr. Chengcheng Cai and Dr. Robin van Velzen for discussing whole genome sequencing data analysis. Further, we are grateful to Dr. Isobel Parkin and Dr. Kumar Paritosh for access to early versions of *B. nigra* genomes. We are grateful to the employees of Unifarm (WUR) for rearing and caring of the plants used in the experiment. We thank Pieter Rouweler, André Gidding, and the late Frans van Aggelen for rearing of *Pieris brassicae.* This research was made possible by support of the Dutch Technology Foundation TTW, which is part of the Dutch Research Council (NWO) (NWO/TTW VIDI grant 14854 to N.E.F.).

Availability of data

The datasets supporting the conclusions of this article are temporary available at this Zenodo repository (https://doi.org/10.5281/zenodo.8131352). All sequencing raw data have been deposited in the European Nucleotide Achieve (ENA) under accession number PRJEB64240.

References

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. In.

Apel, W., Schulze, W. X., & Bock, R. (2010). Identification of protein stability determinants in chloroplasts. *Plant J, 63* (4), 636-650. doi:10.1111/j.1365-313X.2010.04268.x

Barragan, A. C., & Weigel, D. (2021). Plant NLR diversity: the known unknowns of pan-NLRomes. *Plant Cell, 33* (4), 814-831. doi:10.1093/plcell/koaa002

Bassetti, N. (2022). *E(gg)xit strategy of plant defence: Evolution and genetics of a butterfly egg-triggered cell death.* (Ph.D.), Wageningen University, Wageningen.

Bassetti, N., Caarls, L., Bukovinszkine'Kiss, G., El-Soda, M., van Veen, J., Bouwmeester, K., . . . Fatouros, N. E. (2022). Genetic analysis reveals three novel QTLs underpinning a butterfly egg-induced hypersensitive response-like cell death in Brassica rapa. *BMC Plant Biology, 22* (1), 140. doi:10.1186/s12870-022-03522-y

Basu, S., Varsani, S., & Louis, J. (2018). Altering Plant Defenses: Herbivore-Associated Molecular Patterns and Effector Arsenal of Chewing Herbivores. *Mol Plant Microbe Interact, 31* (1), 13-21. doi:10.1094/mpmi-07-17-0183-fi

Bentur, J. S., Rawat, N., Divya, D., Sinha, D. K., Agarrwal, R., Atray, I., & Nair, S. (2016). Rice-gall midge interactions: Battle for survival. *J Insect Physiol, 84* , 40-49. doi:10.1016/j.jinsphys.2015.09.008

Berkey, R., Bendigeri, D., & Xiao, S. (2012). Sphingolipids and Plant Defense/Disease: The "Death" Connection and Beyond. *Frontiers in Plant Science, 3* . doi:10.3389/fpls.2012.00068

Bonnet, C., Lassueur, S., Ponzio, C., Gols, R., Dicke, M., & Reymond, P. (2017). Combined biotic stresses trigger similar transcriptomic responses but contrasting resistance against a chewing herbivore in*Brassica nigra* . *BMC Plant Biol., 17* (1), 127. doi:10.1186/s12870-017-1074-7

Botha, A.-M., Li, Y., & Lapitan, N. L. V. (2005). Cereal host interactions with Russian wheat aphid: A review. *Journal of Plant Interactions, 1* (4), 211-222. doi:10.1080/17429140601073035

Broman KW, Wu H, Sen S, & Churchill G. (2003) QTL mapping in experimental crosses. *Bioinformatics* , 19: 889–890.

Bruessow, F., Gouhier-Darimont, C., Buchala, A., Metraux, J. P., & Reymond, P. (2010). Insect eggs suppress plant defence against chewing herbivores. *Plant J, 62* (5), 876-885. doi:10.1111/j.1365-313X.2010.04200.x

Caarls, L., Bassetti, N., Verbaarschot, P., Mumm, R., van Loon, J. J. A., Schranz, M. E., & Fatouros, N. E. (2023). Hypersensitive-like response induced by cabbage white butterflies is specifically induced by molecules from egg-associated secretions in *Brassica* plants*Frontiers in Ecology and Evolution, 10* , 1070859. doi: doi: 10.3389/fevo.2022.1070859

Cingolani, P., Platts, A., Wang le, L., Coon, M., Nguyen, T., Wang, L., . . . Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin), 6* (2), 80-92. doi:10.4161/fly.19695

Crall, J. D., Switzer, C. M., Oppenheimer, R. L., Ford Versypt, A. N., Dey, B., Brown, A., . . . & de Bivort, B. L. (2018). Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science* , 362(6415), 683-686._DOI: 10.1126/science.aat1598 _

Cui, H., Tsuda, K., & Parker, J. E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. *Annu Rev Plant Biol, 66* , 487-511. doi:10.1146/annurev-arplant-050213-040012

Dakouri, A., Zhang, X., Peng, G., Falk, K. C., Gossen, B. D., Strelkov, S. E., & Yu, F. (2018). Analysis of genome-wide variants through bulked segregant RNA sequencing reveals a major gene for resistance to Plasmodiophora brassicae in Brassica oleracea. *Scientific Reports, 8* (1), 17657. doi:10.1038/s41598-018-36187-5

Dogimont, C., Chovelon, V., Pauquet, J., Boualem, A., & Bendahmane, A. (2014). The Vat locus encodes for a CC-NBS-LRR protein that confers resistance to Aphis gossypii infestation and A. gossypii-mediated virus resistance. *Plant J, 80* (6), 993-1004. doi:10.1111/tpj.12690

Dolatabadian, A., & Fernando, W. G. D. (2022). Genomic Variations and Mutational Events Associated with Plant-Pathogen Interactions.*Biology (Basel), 11* (3). doi:10.3390/biology11030421

Fatouros, N. E., Cusumano, A., Danchin, E. G. J., & Colazza, S. (2016). Prospects of herbivore egg-killing plant defenses for sustainable crop protection. *Ecology and Evolution, 6* (19), 6906-6918.

Fatouros, N. E., Pineda, A., Huigens, M. E., Broekgaarden, C., Shimwela, M. M., Figueroa Candia, I. A., . . . Bukovinszky, T. (2014). Synergistic effects of direct and indirect defences on herbivore egg survival in a wild crucifer. *Proceedings of the Royal Society. B: Biological Sciences, 281* (1789).

Forister ML, Pelton EM, Black SH (2019) Declines in Insect Abundance and Diversity: We Know Enough to Act Now. *Conserv Sci Pract* 1 (8): 1–8_https://doi.org/10.1111/csp2.80 _

Gilardoni, P. A., Hettenhausen, C., Baldwin, I. T., & Bonaventure, G. (2011). Nicotiana attenuata LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during Manduca sexta herbivory. *Plant Cell, 23* (9), 3512-3532. doi:10.1105/tpc.111.088229

Gouhier-Darimont, C., Stahl, E., Glauser, G., & Reymond, P. (2019). The Arabidopsis Lectin Receptor Kinase LecRK-I.8 Is Involved in Insect Egg Perception. *Front Plant Sci, 10* , 623. doi:10.3389/fpls.2019.00623

Griese, E., Caarls, L., Bassetti, N., Mohammadin, S., Verbaarschot, P., Bukovinszkine'Kiss, G., . . . Fatouros, N. E. (2021). Insect egg-killing: a new front on the evolutionary arms-race between brassicaceous plants and pierid butterflies. *New Phytol., 230* , 341-353. doi:10.1111/nph.17145

Griese, E., Dicke, M., Hilker, M., & Fatouros, N. E. (2017). Plant response to butterfly eggs : Inducibility, severity and success of egg-killing leaf necrosis depends on plant genotype and egg clustering /631/158/2456 /631/158/856 article. *Scientific Reports, 7* (1).

Griese, E., Pineda, A., Pashalidou, F. G., Iradi, E. P., Hilker, M., Dicke, M., & Fatouros, N. E. (2020). Plant responses to butterfly oviposition partly explain preference-performance relationships on different brassicaceous species. *Oecologia, 192* (2), 463-475. doi:10.1007/s00442-019-04590-y

Groux, R. (2019). *Molecular mechanisms of insect egg-triggered cell death* (PhD), Université de Lausanne Lausanne.

Groux, R., Stahl, E., Gouhier-Darimont, C., Kerdaffrec, E., Jimenez-Sandoval, P., Santiago, J., & Reymond, P. (2021). Arabidopsis natural variation in insect egg-induced cell death reveals a role for LECTIN RECEPTOR KINASE-I.1. *Plant Physiol, 185* (1), 240-255. doi:10.1093/plphys/kiaa022

Harris, M. O., Freeman, T. P., Anderson, K. M., Harmon, J. P., Moore, J. A., Payne, S. A., . . . Stuart, J. J. (2012). Hessian fly Avirulence gene loss-of-function defeats plant resistance without compromising the larva's ability to induce a gall tissue. *Entomologia Experimentalis et Applicata, 145* (3), 238-249. doi:https://doi.org/10.1111/eea.12010

Harvey, J. A., Gols, R., Wagenaar, R., & Bezemer, T. M. (2007). Development of an insect herbivore and its pupal parasitoid reflect differences in direct plant defense. *Journal of Chemical Ecology, 33* (8), 1556-1569.

Haug-Baltzell, A., Stephens, S. A., Davey, S., Scheidegger, C. E., & Lyons, E. (2017). SynMap2 and SynMap3D: web-based whole-genome synteny browsers. *Bioinformatics, 33* (14), 2197-2198. doi:10.1093/bioinformatics/btx144

Hilker, M., & Fatouros, N. E. (2015). Plant responses to insect egg deposition. *Annual Review of Entomology, 60* , 493-515.

Hilker, M., & Fatouros, N. E. (2016). Resisting the onset of herbivore attack: plants perceive and respond to insect eggs. *Curr. Opin. Plant Biol., 32* , 9-16. doi:10.1016/j.pbi.2016.05.003

Himabindu, K., Suneetha, K., Sama, V. S. A. K., & Bentur, J. S. (2010). A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. *Euphytica, 174* , 179-187.

Hu, D., Jing, J., Snowdon, R. J., Mason, A. S., Shen, J., Meng, J., & Zou, J. (2021). Exploring the gene pool of Brassica napus by genomics-based approaches. *Plant Biotechnol J, 19* (9), 1693-1712. doi:10.1111/pbi.13636

Hu, L., Ye, M., Kuai, P., Ye, M., Erb, M., & Lou, Y. (2018). OsLRR-RLK1, an early responsive leucine-rich repeat receptor-like kinase, initiates rice defense responses against a chewing herbivore.*New Phytol, 219* (3), 1097-1111. doi:10.1111/nph.15247

Jones, J. D., & Dangl, J. L. (2006). The plant immune system.*Nature, 444* (7117), 323-329. doi:10.1038/nature05286

Kaplinski, L., Lepamets, M., & Remm, M. (2015). GenomeTester4: a toolkit for performing basic set operations - union, intersection and complement on k-mer lists. *GigaScience, 4* (1), 58. doi:10.1186/s13742-015-0097-y

Katche, E., Quezada-Martinez, D., Katche, E. I., Vasquez-Teuber, P., & Mason, A. S. (2019). Interspecific Hybridization for Brassica Crop Improvement. *Crop Breeding, Genetics and Genomics, 1* (1), e190007. doi:10.20900/cbgg20190007

Kliebenstein, D. J. (2017). Quantitative Genetics and Genomics of Plant Resistance to Insects. In *Annual Plant Reviews online* (pp. 235-262).

Klingler, J. P., Nair, R. M., Edwards, O. R., & Singh, K. B. (2009). A single gene, AIN, in Medicago truncatula mediates a hypersensitive response to both bluegreen aphid and pea aphid, but confers resistance only to bluegreen aphid. *J Exp Bot, 60* (14), 4115-4127. doi:10.1093/jxb/erp244

Kourelis, J., & van der Hoorn, R. A. L. (2018). Defended to the Nines: 25 Years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function. *Plant Cell, 30* (2), 285-299. doi:10.1105/tpc.17.00579

Kumar, S. (2017). Assessment of avoidable yield losses in crop brassicas by insect-pests. *Journal of entomology and zoology studies, 5* , 1814-1818.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics, 25* (14), 1754-1760. doi:10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. et al. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* , **25** , 2078– 2079.

Li, H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Prepr. *arXiv* : 1303.3997.

Little, D., Gouhier-Darimont, C., Bruessow, F., & Reymond, P. (2007). Oviposition by pierid butterflies triggers defense responses in*Arabidopsis* . *Plant Physiol., 143* , 784-800.

Liu, S., Yeh, C. T., Tang, H. M., Nettleton, D., & Schnable, P. S. (2012). Gene mapping via bulked segregant RNA-Seq (BSR-Seq). *PLoS One, 7* (5), e36406. doi:10.1371/journal.pone.0036406

Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., . . . Wan, J. (2015). A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature Biotechnology, 33* (3), 301-305. doi:10.1038/nbt.3069

Lv, H., Fang, Z., Yang, L., Zhang, Y., & Wang, Y. (2020). An update on the arsenal: mining resistance genes for disease management of Brassica crops in the genomic era. *Horticulture Research, 7* (1), 34. doi:10.1038/s41438-020-0257-9

Lyons, E., Pedersen, B., Kane, J., Alam, M., Ming, R., Tang, H., . . . Freeling, M. (2008). Finding and comparing syntenic regions among Arabidopsis and the outgroups papaya, poplar, and grape: CoGe with rosids. *Plant Physiol, 148* (4), 1772-1781. doi:10.1104/pp.108.124867

Monteiro, F., & Nishimura, M. T. (2018). Structural, Functional, and Genomic Diversity of Plant NLR Proteins: An Evolved Resource for Rational Engineering of Plant Immunity. *Annual Review of Phytopathology, 56* (1), 243-267. doi:10.1146/annurev-phyto-080417-045817

Nicolis, V., & Venter, E. (2018). Silencing of a Unique Integrated Domain Nucleotide-Binding Leucine-Rich Repeat Gene in Wheat Abolishes Diuraphis noxia Resistance. *Mol Plant Microbe Interact, 31* (9), 940-950. doi:10.1094/mpmi-11-17-0262-r

Nombela, G., Williamson, V. M., & Muñiz, M. (2003). The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly Bemisia tabaci. *Mol Plant Microbe Interact, 16* (7), 645-649. doi:10.1094/mpmi.2003.16.7.645

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144* (1), 31-43. doi:10.1017/S0021859605005708

Palma, K., Thorgrimsen, S., Malinovsky, F. G., Fiil, B. K., Nielsen, H. B., Brodersen, P., . . . Mundy, J. (2010). Autoimmunity in Arabidopsis acd11 is mediated by epigenetic regulation of an immune receptor.*PLoS Pathog, 6* (10), e1001137. doi:10.1371/journal.ppat.1001137

Pashalidou, F. G., Fatouros, N. E., Loon, J. J. A. v., Dicke, M., & Gols, R. (2015). Plant-mediated effects of butterfly egg deposition on subsequent caterpillar and pupal development, across different species of wild Brassicaceae. *Ecological Entomology, 40* (4), 444-450.

Prodhomme, C., Esselink, D., Borm, T., Visser, R. G. F., van Eck, H. J., & Vossen, J. H. (2019). Comparative Subsequence Sets Analysis (CoSSA) is a robust approach to identify haplotype specific SNPs; mapping and pedigree analysis of a potato wart disease resistance gene Sen3.*Plant Methods, 15* , 60. doi:10.1186/s13007-019-0445-5

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics, 26* (6), 841-842. doi:10.1093/bioinformatics/btq033

R Core Team (2021). R: a language and environment for statistical computing. https://www.r-project.org/.

Reymond, P. (2021). Receptor kinases in plant responses to herbivory.*Curr. Opin. Biotechnol., 70* , 143-150. doi:10.1016/j.copbio.2021.04.004

Ross, S., Giglione, C., Pierre, M., Espagne, C., & Meinnel, T. (2005). Functional and developmental impact of cytosolic protein N-terminal methionine excision in Arabidopsis. *Plant Physiol, 137* (2), 623-637. doi:10.1104/pp.104.056861

Rossi, M., Goggin, F. L., Milligan, S. B., Kaloshian, I., Ullman, D. E., & Williamson, V. M. (1998). The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proc Natl Acad Sci U S A, 95* (17), 9750-9754. doi:10.1073/pnas.95.17.9750

Ryan, S. F., Lombaert, E., Espeset, A., Vila, R., Talavera, G., Dinca, V., . . . Shoemaker, D. (2019). Global invasion history of the agricultural pest butterfly Pieris rapae revealed with genomics and citizen science. *Proc Natl Acad Sci U S A, 116* (40), 20015-20024. doi:10.1073/pnas.1907492116

Shao, Z. Q., Xue, J. Y., Wang, Q., Wang, B., & Chen, J. Q. (2019). Revisiting the Origin of Plant NBS-LRR Genes. *Trends Plant Sci, 24* (1), 9-12. doi:10.1016/j.tplants.2018.10.015

Shapiro, A. M., & De Vay, J. E. (1987). Hypersensitivity reaction of*Brassica nigra* L. (Cruciferae) kills eggs of *Pieris*butterflies (Lepidoptera, Pieridae). *Oecologia, 71* (4), 631-632.

Snoeck, S., Guayazan-Palacios, N., & Steinbrenner, A. D. (2022). Molecular tug-of-war: Plant immune recognition of herbivory. *Plant Cell, 34* (5), 1497-1513. doi:10.1093/plcell/koac009

Stahl, E., Brillatz, T., Ferreira Queiroz, E., Marcourt, L., Schmiesing, A., Hilfiker, O., . . . Reymond, P. (2020). Phosphatidylcholines from*Pieris brassicae* eggs activate an immune response in*Arabidopsis* . *eLife, 9* , e60293. doi:10.7554/eLife.60293

Steinbrenner, A. D., Muñoz-Amatriaín, M., Chaparro, A. F., Aguilar-Venegas, J. M., Lo, S., Okuda, S., . . . Schmelz, E. A. (2020). A receptor-like protein mediates plant immune responses to herbivore-associated molecular patterns. *Proceedings of the National Academy of Sciences, 117* (49), 31510-31518. doi:10.1073/pnas.2018415117

Stuart, J. J., Chen, M. S., Shukle, R., & Harris, M. O. (2012). Gall midges (Hessian flies) as plant pathogens. *Annu Rev Phytopathol, 50* , 339-357. doi:10.1146/annurev-phyto-072910-095255

Sun, M., Voorrips, R. E., Van't Westende, W., van Kaauwen, M., Visser, R. G. F., & Vosman, B. (2020). Aphid resistance in Capsicum maps to a locus containing LRR-RLK gene analogues. *Theor Appl Genet, 133* (1), 227-237. doi:10.1007/s00122-019-03453-7

Tamiru, A., Khan, Z. R., & Bruce, T. J. A. (2015). New directions for improving crop resistance to insects by breeding for egg induced defence. *Curr. Opin. Insect Sci.* doi:10.1016/j.cois.2015.02.011

Tamura, Y., Hattori, M., Yoshioka, H., Yoshioka, M., Takahashi, A., Wu, J., . . . Yasui, H. (2014). Map-based Cloning and Characterization of a Brown Planthopper Resistance Gene BPH26 from Oryza sativa L. ssp.

indica Cultivar ADR52. *Scientific Reports, 4* (1), 5872. doi:10.1038/srep05872

Toruño, T. Y., Stergiopoulos, I., & Coaker, G. (2016). Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners. *Annu Rev Phytopathol, 54* , 419-441. doi:10.1146/annurev-phyto-080615-100204

Turcotte, M. M., Turley, N. E., & Johnson, M. T. J. (2014). The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytol, 204* (3), 671-681. doi:10.1111/nph.12935

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the National Academy of Sciences* , 118(2), e2023989118._https://doi.org/10.1073/pnas.2023989118

Wang, H., Lu, Y., Liu, P., Wen, W., Zhang, J., Ge, X., & Xia, Y. (2013). The ammonium/nitrate ratio is an input signal in the temperature-modulated, SNC1-mediated and EDS1-dependent autoimmunity of nudt6-2 nudt7. *Plant J, 73* (2), 262-275. doi:10.1111/tpj.12032

Wang, W., Chen, L., Fengler, K., Bolar, J., Llaca, V., Wang, X., . . . Ma, J. (2021). A giant NLR gene confers broad-spectrum resistance to Phytophthora sojae in soybean. *Nat Commun, 12* (1), 6263. doi:10.1038/s41467-021-26554-8

Whitehead, S. R., Turcotte, M. M., & Poveda, K. (2017). Domestication impacts on plant–herbivore interactions: a meta-analysis.*Philosophical Transactions of the Royal Society B: Biological Sciences, 372* (1712), 20160034. doi:doi:10.1098/rstb.2016.0034

Wiklund, C. (1984). Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia, 63* , 23-29.

Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A. et al. (2013) From fastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics* , **43** , 11.10.1– 11.10.33.

Zhao, Y., Huang, J., Wang, Z., Jing, S., Wang, Y., Ouyang, Y., . . . He, G. (2016). Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. *Proc Natl Acad Sci U S A, 113* (45), 12850-12855. doi:10.1073/pnas.1614862113

Tables

Table 1. Segregation ratios of phenotypes in the populations used for genetic mapping of PEK locus. For each population, plants were treated with egg wash and scored as resistant (R) with a HR scores 2-4 and susceptible (S) with a HR score 0-1. Phenotypic distribution was analyzed with a Chi-square (χ^2) test.

Population	Generation	Observed	Observed	Expected	Expected	Expected	χ^2 (p-
		R	**S**	**R**	**S**	**Ratio (R : S)**	
F1-1	F1	75	75	75	75	1 : 1	1
BC1-3	BC_1	26	40	33	33	1 : 1	0.0
BCS1-1	BC_1S_1	533	155	516	172	3 : 1	0.1

Table 2. Segregation ratios and association with HR phenotype of five markers used to genotype the whole BC_1S_1 population (n = 695). Alleles at each marker are given a symbol based on the

phenotype of the accession from which they derive: "R" is for the SF48-O1 allele, the resistant parent. "S" is for the DG1-S1 allele, the susceptible parent.

Marker	position on B3 (Mb)	Observed	Observed	Observed	Expected (1:2:1)	Expected (1:2:1)	Expected (1:2:1)	χ^2 test (p-value)	A w P (s
		R/R	R/S	S/S	R/R	R/S	S/S		
M1	3.97	170	346	159	168.75	337.5	168.75	0.675	7
M13	6.53	173	341	159	168.25	336.5	168.25	0.702	1
M19	8.45	166	354	158	169.50	339	169.5	0.469	8
M25	9.43	161	362	156	169.75	339.5	169.75	0.217	6
M5	12.95	156	363	151	167.50	335	167.5	0.093	5

[a] LOD threshold of MQM for a significant marker-trait association was estimated 1.88 after 1000 permutations and 5% error rate

Table 3. List of genes included within the *Pek* locus in the *B. nigra* genome C2. *B. nigra* genes ID within*PEK* locus as annotated in genome C2. Start and end location of coding sequence (CDS) is given. *A. thaliana* homologs were assigned with BLASTp (E-value = 1e- 5).

B. nigra gene ID	start CDS (bp)	end CDS (bp)	strand	*A. thaliana* homolog	*A. thaliana* homolog	*A. th*
				gene ID	**gene symbol**	**gene**
BniB03g015420.1C2	6,411,323	6,409,090	-	AT4G37080	-	Prote
BniB03g015430.1C2	6,414,903	6,416,090	-	AT4G37030 [a] AT4G37040	- MAP1D	Mem
BniB03g015440.1C2	6,425,471	6,425,693	-	AT4G37030	-	Mem
BniB03g015450.1C2	6,426,485	6,430,131	+	TIR-NBS-LRR [b]	-	multi
BniB03g015460.1C2	6,430,513	6,432,096	-	AT4G37040	MAP1D	Met a
BniB03g015470.1C2	6,436,216	6,433,822	-	AT4G37030	-	Mem
BniB03g015480.1C2	6,439,477	6,437,932	-	AT4G37020	-	eukar
BniB03g015490.1C2	6,439,814	6,443,405	+	TIR-NBS-LRR [b]	-	multi
BniB03g015500.1C2	6,443,871	6,444,160	-	AT4G37040	MAP1D	Met a
BniB03g015510.1C2	6,447,293	6,450,764	+	TIR-NBS-LRR [b]	-	multi
BniB03g015520.1C2	6,456,006	6,469,883	+	TIR-NBS-LRR [b]	-	multi

[a] This *B. nigra* gene appears to be a fusion between two *A. thaliana* genes.

[b] These *B. nigra* genes are canonical TIR-NBS-LRRs, hence it's difficult to assign an *A. thaliana*ortholog without in-depth phylogenetic analysis.