

# Two adjacent NLR genes conferring quantitative resistance to clubroot disease in *Arabidopsis* are regulated by a stably inherited epiallelic variation

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## ABSTRACT

Clubroot caused by the protist *Plasmodiophora brassicae* is a major disease affecting cultivated Brassicaceae. Using a combination of quantitative trait locus (QTL) fine mapping, CRISPR-Cas9 validation, and extensive analyses of DNA sequence and methylation patterns, we revealed that the two adjacent neighboring NLR (nucleotide-binding and leucine-rich repeat) genes *AT5G47260* and *AT5G47280* cooperate in controlling broad-spectrum quantitative partial resistance to the root pathogen *P. brassicae* in *Arabidopsis* and that they are epigenetically regulated. The variation in DNA methylation is not associated with any nucleotide variation or any transposable element presence/absence variants and is stably inherited. Variations in DNA methylation at the *Pb-At5.2* QTL are widespread across *Arabidopsis* accessions and correlate negatively with variations in expression of the two genes. Our study demonstrates that natural, stable, and transgenerationally inherited epigenetic variations can play an important role in shaping resistance to plant pathogens by modulating the expression of immune receptors.

**Key words:** methylation, clubroot, *Plasmodiophora brassicae*, *AT5G47260*, *AT5G47280*, ADR1-L3, *ddm1*

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## INTRODUCTION

Intraspecific diversity in plant immune interactions is associated with a high level of sequence variation at hundreds of NLRs (nucleotide-binding and leucine-rich repeats), one of the largest and most rapidly evolving plant gene families (Meyers et al., 2003; Yue et al., 2012; Shao et al., 2016). On the basis of their N-terminal domains, NLRs have been classified into four subclasses: Toll/interleukin-1 receptor type (TIR-NLR or TNL), coil-coiled type (CC-NLR or CNL), RPW8-type CC-NLR (CCRPW8 NLR or RNL), and G10-type CC-NLR (CCG10 NLR) (Contreras et al., 2023). Many NLR proteins are involved in recognition of a small range of effector proteins secreted by specific strains of plant pathogens, potentially triggering the

induction of strong plant defense responses that can rapidly stop pathogen invasion (Maekawa et al., 2011; Jones et al., 2016). The catalog of NLR genes expressed in a given plant genotype thus globally shapes the range of isolate-specific total resistances (incompatible interactions). However, this general rule has a few exceptions, including the existence of non-NLR-driven resistances (Thomas, 1998; Xiao et al., 2001; Larkan et al., 2013) and broad-spectrum NLR-driven resistances (Ernst et al., 2002; Qu et al., 2006).

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Effectors may be recognized in different ways: (1) NLRs can monitor the effect of pathogen effectors on their cellular targets; (2) pathogen effectors can be recognized by their direct interaction with one canonical NLR domain; or, alternatively, (3) effectors can be recognized by one non-canonical NLR domain, called an integrated decoy (ID), which mimics a protein domain of the effector target (Kourelis and van der Hoorn, 2018). Effector-activated CNLs then assemble into pentameric oligomers called resistosomes, driving a rapid intracellular inward  $\text{Ca}^{2+}$  flux that triggers downstream cellular defense responses (Förderer et al., 2022). Activated TNLs drive similar  $\text{Ca}^{2+}$ -mediated defense responses by an indirect pathway: assembled into tetrameric oligomers, their TIR domain mediates the biosynthesis of small signaling molecules, leading to downstream assembly of pentameric CCRPW8 NLR-based resistosomes that mediate  $\text{Ca}^{2+}$ -mediated defenses (Essuman et al., 2022). CCRPW8 NLRs thus play a central role in the integration of hub-connected TNL-based non-self-recognition processes and have therefore been called “helper NLRs” (Wu et al., 2017).

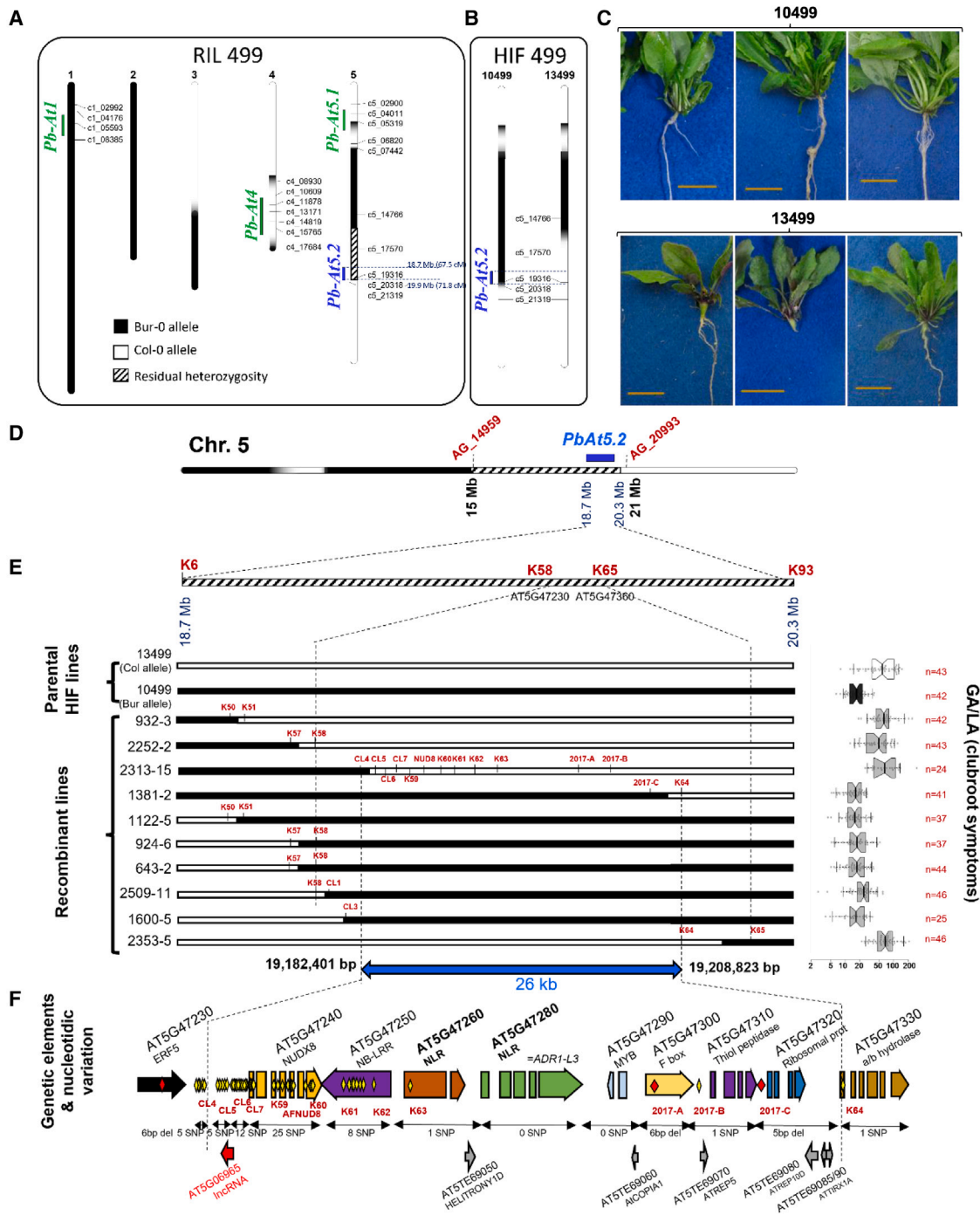
In contrast to *R*-gene-driven resistance, quantitative resistance is polygenic, i.e., it involves allelic variation at several quantitative trait loci (QTLs), which collectively contribute to post-invasive partial resistance in compatible plant–pathogen interactions. The nature of the few resistance QTLs cloned to date supports the premise that quantitative resistance genes (QRGs) are functionally more diverse than *R* genes (Nelson et al., 2017; Pilet-Nayel et al., 2017; Delplace et al., 2022). Among these QRGs, however, there are still genes encoding NLRs (Hayashi et al., 2010; Fukuoka et al., 2014; Xu et al., 2014; Debieu et al., 2016) and other receptors (Diener and Ausubel, 2005; Humi et al., 2015) or co-receptors (Huard-Chauveau et al., 2013). Thus, variation in NLR genes (or other non-self-recognition loci) also appears to contribute to variations in basal resistance levels during compatible interactions.

To trigger effective resistance, cellular levels of NLR proteins must reach minimum thresholds. However, high levels of NLRs can also lead to autoimmunity drawbacks, including spontaneous hypersensitive response and retarded plant growth (Li et al., 2015; Lai and Eulgem, 2018). NLR abundance is thus tightly controlled by multiple mechanisms at the transcriptional, post-transcriptional (i.e., alternative splicing), and post-translational levels (i.e., ubiquitin-dependent proteolytic regulation) (Zhang and Gassmann, 2007; Li et al., 2015; Lai and Eulgem, 2018). NLR regulation also involves a multitude of epigenetic-related cellular processes, including redundant networks of small RNAs (sRNAs) (Shivaprasad et al., 2012; Fei et al., 2013; Deng et al., 2018; Huang et al., 2019), histone modifications (Palma et al., 2010; Xia et al., 2013; Zou et al., 2014; Ramirez-Prado et al., 2018), histone-mark-dependent alternative splicing (Tsuchiya and Eulgem, 2013), and regulation of chromatin structure and DNA methylation (Li et al., 2010; Deleris et al., 2016). There is increasing evidence that epigenetic processes can play a role in the transitory imprinting of some plant biotic stress responses, at least for a few generations (Molinier et al., 2006; Slaughter et al., 2011; Luna et al., 2012; López Sánchez et al., 2016, 2021; Morán-Diez et al., 2021). It is, however, not yet clear to what extent stable transgenerational inheritance of epigenetically regulated gene expression contributes to the natural intraspecific diversity of plant–pathogen interactions.

The few available examples of transgenerational epigenetically controlled traits are found mainly in plant species, where the association between natural or induced differentially methylated regions (DMRs) and phenotypic traits was shown to be stably or (more often) metastably inherited across generations (Quadrana and Colot, 2016; Furci et al., 2019; Liégard et al., 2019). Such regions, designated epialleles, can have an effect on relevant agronomic traits: compatibility, accumulation of vitamin E, and fruit ripening in tomato; starch metabolism, disease resistance, and sex determination in melon; and fruit productivity in oil palm (Manning et al., 2006; Martin et al., 2009; Durand et al., 2012; Silveira et al., 2013; Quadrana et al., 2014; Ong-Abdullah et al., 2015; He et al., 2018; Bhat et al., 2020).

Plant DNA methylation can occur at cytosines in the three sequence contexts CG, CHG, and CHH (Henderson and Jacobsen, 2007) (where H can be A, C, or T), and its effect varies depending on the targeted genomic features (i.e., transposable elements [TEs], gene promoters, or gene bodies). DNA methylation patterns result from the dynamic combination of *de novo* methylation, maintenance methylation, and demethylation. *De novo* DNA methylation is catalyzed by the canonical and non-canonical RNA-directed DNA methylation (RdDM) pathways, which are both guided by small interfering RNAs (Cuerda-Gil and Slotkin, 2016; Zhang et al., 2018). Maintenance of DNA methylation relies mainly on RNA-independent pathways and requires the activity of DDM1, MET1, and VIM proteins at CG sites and of DDM1, KYP, CMT2/3, and the histone mark HK9me2 at CHG and CHH sites (Law and Jacobsen, 2010; Matzke and Mosher, 2014). Previous studies have noted that natural DMRs are over-represented on genes of the NLR disease-resistance gene family (Kawakatsu et al., 2016). However, it remains unclear whether natural epigenetic variation in NLR genes can influence the outcome of interactions between plants and pathogens.

Here, we report the identification of two adjacent NLR genes controlled by a naturally occurring stable epigenetic variation underlying a QTL involved in partial resistance to clubroot in *Arabidopsis*. Clubroot is a root gall disease caused by the telluric biotrophic pathogen *Plasmodiophora brassicae* (Rhizaria) and affects all Brassicaceae crops such as oilseed rape, kale, and turnip. The infection process involves a primary infection in root hairs that lasts only a few days. Secondary plasmodia then develop in root cortical cells, causing hyperplasia and hypertrophy that ultimately impair plant water and nutrient uptake. The reference accessions Columbia-0 (Col-0) and Burren-0 (Bur-0) are fully susceptible and partially resistant to *P. brassicae* isolate eH, respectively (Alix et al., 2007; Jubault et al., 2008b) (Supplemental Figure 1). Four main QTLs, which act additively, determine this difference. Here, we combined fine mapping of the QTL *Pb-At5.2*, which had the strongest effect on resistance, with CRISPR-Cas9 validation to identify two adjacent NLR genes, *AT5G47260* and *AT5G47280*, both involved in the control of clubroot partial resistance. Expression levels of the two genes vary between the susceptible and resistant parents and are linked to the DNA methylation status of a small region that includes these two genes and a neighboring TE sequence. The methylation status of the two resistance genes is stable over generations and is not associated with any structural variation in the intervening transposon. Epiallelic variation at this locus is frequent among natural *Arabidopsis* accessions,



**Figure 1. Fine mapping of *Pb-At5.2*.**

**(A)** Genetic map and residual heterozygosity in the recombinant inbred line (RIL) 499 derived from Bur-0 and Col-0 (Alix et al., 2007), and genetic and physical positions of clubroot resistance QTLs (Jubault et al., 2008b). Black, Bur-0 allele; white, Col-0 allele; hatched, heterozygous (Col-0/Bur-0).

**(B)** Allele configuration at *Pb-At5.2* in the two derived HIF lines 10499 and 13499.

**(C)** Photos showing that *Pb-At5.2* conferred partial resistance to the eH isolate in the HIF 499 genetic background. HIF 10499 and 13499 harbored Bur-0 and Col-0 alleles, respectively, at *Pb-At5.2*. Observations were made 21 days post inoculation.

**(D)** First round of fine mapping: allelic structure in the F1 lines derived from reciprocal crosses between 10499 and 13499. A total of 554 F3 lines with recombination in the confidence interval were screened using 10 SNP markers between AG\_14959 and AG\_20993. High-density genotyping (94 SNPs

(legend continued on next page)

and the low methylated state was correlated with the expression levels of the two NLR genes and with increased quantitative resistance to *P. brassicae* among 126 accessions. We showed that the RNA-independent pathway involving DDM1, MET1, VIM, and CMT2/3 maintains the hypermethylated epiallele in the clubroot-susceptible Col-0 accession. Overall, our findings demonstrate that quantitative resistance to a major root disease of Brassicaceae is associated, in *Arabidopsis*, with stable inheritance of a natural epigenetic variation involved in controlling the constitutive expression of an NLR gene pair.

## RESULTS

### Fine mapping of the *Pb-At5.2* locus responsible for clubroot resistance

In previous work, we used a population of F7 recombinant inbred lines (RILs) between the partially resistant accession Bur-0 and the susceptible Col-0, to map a QTL (*Pb-At5.2*) on chromosome 5 between 67.5 and 71.8 cM that explained a significant proportion ( $R^2 = 20\%$ ) of the resistance (Figure 1A) (Alix et al., 2007; Jubault et al., 2008b). In TAIR10, this interval (between *AT5G46260* and *AT5G47690*) contains 158 annotated sequences, including protein-coding genes, TE genes, pre-tRNAs, and small nuclear RNAs. The effect and confidence interval of this QTL were also confirmed previously in the heterogeneous inbred family (HIF) lines 10499 and 13499 (Lemarié et al., 2015). Both lines were derived from the RIL 499, which harbors the homozygous Bur-0 (resistance) allele on the QTLs *Pb-At1* and *Pb-At5.1*, the homozygous Col-0 (susceptibility) allele on the QTL *Pb-At4*, and residual heterozygosity in the *Pb-At5.2* region. The lines 10499 and 13499 inherited the homozygous Bur-0 (resistance) allele and the Col-0 (susceptibility) allele, respectively, at the QTL *Pb-At5.2* (Figure 1B and 1C; Supplemental Text 1).

The initial aim of the present work was to fine map *Pb-At5.2*, starting with reciprocal crosses between HIF lines 10499 and 13499. Clubroot symptoms in individuals of the F1 progeny were as severe as those in the susceptible parental line HIF 13499, suggesting that the Bur-0 resistance allele was recessive (Supplemental Figure 2). The boundaries of the *Pb-At5.2* resistance locus were further refined through several rounds of genotyping and clubroot phenotyping (generations F3–F5 downstream of crosses 10499/13499) (details are given in Figure 1D–1F, Supplemental Figure 3, Supplemental Text 1, Supplemental Data 1, and Supplemental Text 3). This enabled us to narrow down the confidence interval to 26 kb between the markers CLG4 (19 182 401 bp, in the promoter region of *AT5G47240*) and K64 (19 208 823 bp, in *AT5G47330*), with the genetic markers being defined using the available *de novo*

genome assembly of Bur-0 (Schneeberger et al., 2011). Comparison of the genetic sequences of Bur-0 and Col-0 in this 26-kb region revealed the absence of any structural variation and a low frequency of single-nucleotide polymorphisms (SNPs) (Figure 1F; details in Supplemental Text 3). This region contained eight annotated open reading frames (ORFs), including three NLR-encoding genes (*AT5G47250*, *AT5G47260*, and *AT5G47280*), six annotated TE sequences, and one long non-coding RNA gene (Figure 1F). The two F5 homozygous progeny lines 1381-2 and 2313-15, harboring the closest recombination events from both sides of the 26-kb interval (see Figure 1E), also showed partial resistance to a series of additional *P. brassicae* isolates (pathotypes 1, 4, and 7 following the classification of Some et al., 1996) and P1(+), which is representative of the new virulent strains that are emerging in Europe following breaking of clubroot resistance in oilseed rape varieties derived from the cultivar “Mendel” (Zamani-Noor et al., 2022). This result highlighted the broad spectrum of resistance conferred by the Bur-0 allele of *Pb-At5.2* (Supplemental Figure 4).

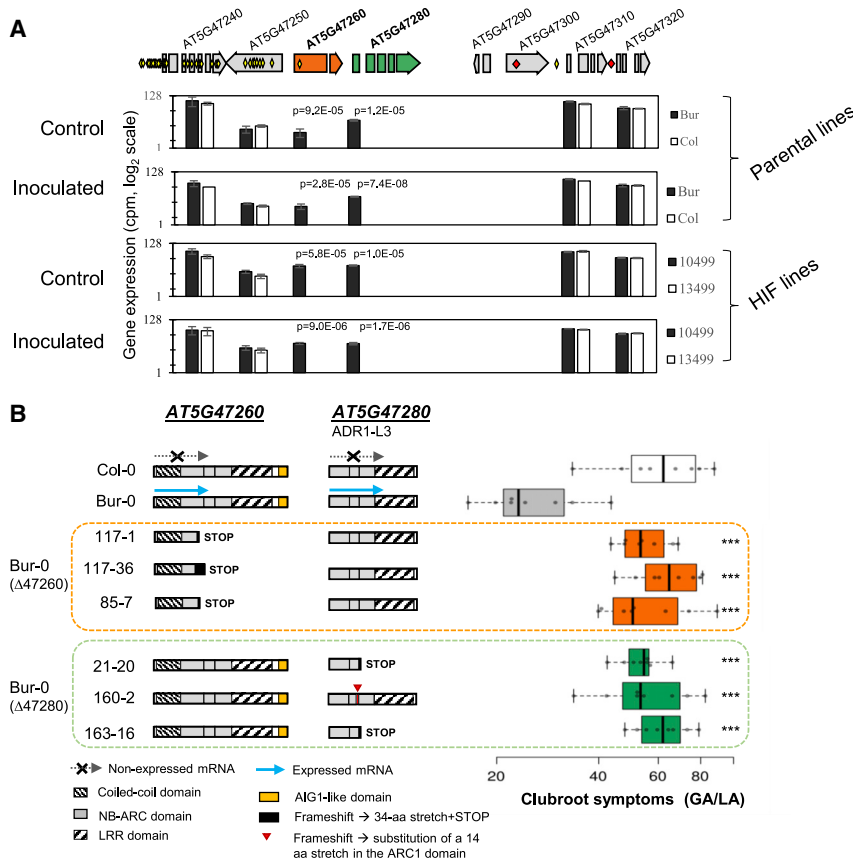
### RNA-seq analysis revealed a constitutive expression polymorphism of two NLR genes in the 26-kb QTL confidence interval

RNA-sequencing (RNA-seq) analysis was performed on Bur-0 and Col-0 accessions and on recombinant HIF lines 10499 and 13499. Pathogen-induced gene expression patterns differed markedly between genotypes harboring alleles *Pb-At5.2<sub>BUR</sub>* and *Pb-At5.2<sub>COL</sub>* (Supplemental Figure 5 and Supplemental Data 2). This regulation was consistent with our previously published studies, i.e., suggesting a role of camalexin biosynthesis and salicylic acid-mediated responses in *Pb-At5.2<sub>BUR</sub>*-mediated resistance and a role of jasmonic acid-driven induction of *ARGAH2* in *Pb-At5.2<sub>COL</sub>*-mediated basal resistance (for details see Supplemental Text 2). We then focused on the eight ORFs in *Pb-At5.2*. Genes *AT5G47290* and *AT5G47300* showed no expression, and genes *AT5G47240*, *AT5G47250*, *AT5G47310*, and *AT5G47320* showed similar expression levels in all four accessions (Figure 2A). In the 26-kb interval, only two genes, *AT5G47260* and *AT5G47280*, both encoding proteins from the non-TIR-NLR gene family, were differentially expressed between resistant and susceptible accessions: these two genes were constitutively expressed in Bur-0 and 10499 roots (i.e., with the Bur-0 allele), but their expression was undetectable in Col-0 and 13499 (i.e., with the Col-0 allele) (Figure 2A). *AT5G47280* encodes ADR1-L3, an NBS-LRR protein related to the small family of ADR1-type RNLs (although the encoded protein lacks the N-ter RPW8 domain). *AT5G47260*, encodes a CC-NBS-LRR-X protein with an ID-like C-ter extension domain (Figure 2B) homologous to members of the IAN family

from K1 to K94) in 88 recombinant F3 lines and clubroot phenotyping of their bulked segregating F4 progenies led to the identification of a new interval between markers K58 (*AT5G47230*) and K65 (*AT5G47360*).

(E) Second round of fine mapping: recombination positions in homozygous individuals obtained from selected recombinant lines. For each line, the GA/LA index (disease symptoms, log scale) is indicated on the right panel. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range (IQR) from the 25th and 75th percentiles; outliers are represented by dots; data points are plotted as open circles. The number of individual plants analyzed for each genotype is indicated (*n*). The notches are defined as  $\pm 1.58 \times \text{IQR}/\sqrt{n}$  and represent the 95% confidence interval for each median. GA/LA values statistically different from those of 13499 are indicated by asterisks (*t*-test,  $***p < 0.001$ ). Genetic markers are indicated for each recombination position. Markers between CL5 and 2017-C were used in every line but are shown only for 2313-15.

(F) New 26-kb interval of *Pb-At5.2* between markers CL4 (excluded) and K64 (excluded), containing eight annotated ORFs, six transposons, and one long non-coding RNA. Yellow and red diamonds indicate SNPs and nucleotide deletions, respectively.



**Figure 2. Identification and CRISPR-Cas9 validation of two NLR-encoding genes controlling clubroot resistance at QTL *Pb-At5.2*.**

**(A)** Sequence variations and expression levels of genes in the *Pb-At5.2* region. Gene expression values are from RNA-seq analyses conducted under inoculated and control conditions at 14 days post inoculation ( $\log_2$  normalized CPM) with parental lines Col-0/Bur-0 and HIF lines 13499/10499 (the last two were derived from RIL 499 and are homozygous *Pb-At5.2*<sub>Col/Col</sub> and *Pb-At5.2*<sub>Bur/Bur</sub> respectively) (Supplemental Data 2). False discovery rate-adjusted *p* values are shown if less than 0.05. Yellow and red diamonds indicate SNP and INDEL variations, respectively.

**(B)** Effect of *AT5G47260* or *AT5G47280* knockout on GA/LA index (disease symptoms) in the Bur-0 background. Cas9-mediated mutations were obtained in the Bur-0 genetic background. For each targeted gene, three independent homozygous mutations were used. Lines 117-1 and 21-20 no longer have the CRISPR-Cas9 cassette. For each line, the mean clubroot symptom score (GA/LA, log scale) was obtained by modeling raw data of eight biological replicates (with 10–12 individual plants per replicate). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the IQR from the 25th and 75th percentiles; data points are plotted as open circles. GA/LA values of edited lines that are statistically different from the Bur-0 GA/LA value are indicated by asterisks (Dunnnett’s test) as follows: \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001.

of AIG1 (= *AvrRpt2-Induced Gene1*, *AT1G33960*)-related proteins (Martin et al., 2023). These two adjacent genes are separated by a helitron, *AT5TE69050* (Figures 1F and 3A). *AT5G47280* contained no SNPs, and *AT5G47260* contained only one non-synonymous SNP (Supplemental Figures 7 and 8, Supplemental Text 3). There was also no sequence variation in the helitron *AT5TE69050* located between the two genes.

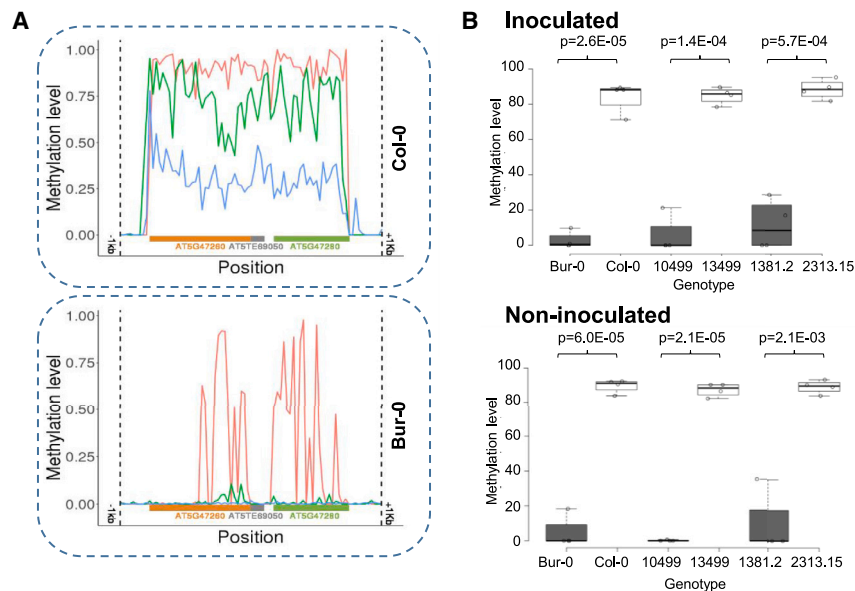
**CRISPR-Cas9 validation of the role of NLR genes *AT5G47260* and *AT5G47280* in clubroot resistance**

Given the contrasting expression levels of *AT5G47260* and *AT5G47280* in Bur-0 and Col-0, we next addressed their functional significance in clubroot resistance by generating knockout lines via CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) technology. We targeted the regions encoding the NB-ARC domain of both genes with two single guide RNAs in both resistant Bur-0 and HIF 10499 (which contains the Bur-0 allele) accessions. The CRISPR-Cas9-generated mutations in *AT5G47260* and *AT5G47280* gave rise to premature stop codons in most mutant lines and to substitution of a stretch of 14 amino acids in the ARC1 domain in line 160-2 (Figure 2B, Supplemental Figures 6–8, and Supplemental Text 4). For the following experiments, we used three mutants from separate transformation events for each gene and each background (Bur-0 or HIF 10499). For each gene, one mutant without a T-DNA insertion was obtained in the Bur-0 background. The *AT5G47260* and *AT5G47280* CRISPR-knockout mutants were then evaluated for clubroot resistance in

a complete randomized design. For both genes, clubroot symptoms were significantly higher in all lines edited in the Bur-0 genetic background than in the wild-type resistant Bur-0 accession and were as high as those of the susceptible accession Col-0 (Figure 2B), demonstrating the involvement of both *AT5G47260* and *AT5G47280* in clubroot resistance. Similar results were obtained for CRISPR-edited lines in the 10499 HIF genetic background (Supplemental Figure 9).

**Expression polymorphism of both NLR genes is associated with stably inherited methylation variation**

To understand why the two NLR genes *AT5G47260* and *AT5G47280* were differentially expressed in Bur-0 and Col-0, despite the absence of any sequence variation in their putative promoter regions, we analyzed the DNA methylation level of these regions in the two accessions using publicly available methylome data (Kawakatsu et al., 2016). The genomic interval between 19 188 411 and 19 196 559, which includes the two genes *AT5G47260* and *AT5G47280* and the intervening transposon *AT5TE69050*, was hypermethylated in Col-0 and hypomethylated in Bur-0 (Figure 3A). These contrasting methylation states were confirmed experimentally using DNA extracted from infected and non-infected roots of Col-0 and Bur-0 plants (Figure 3B) and CHOP-qPCR. The differences in DNA methylation were also found between the progeny HIF lines 10499 and 13499 and in the pair of HIF-derived homozygous near-isogenic lines 1381-2 and 2313-15 (Figure 3B), indicating that they are stably inherited independently of any DNA



**Figure 3. Methylation of the region surrounding the two NLR-encoding genes that control clubroot resistance at QTL *Pb-At5.2*.**

**(A)** Methylation profiles in the *AT5G47260* and *AT5G47280* region of Col-0 and Bur-0 accessions inferred from bisulfite data previously reported in Kawakatsu et al. (2016). Average methylation level was calculated within non-overlapping 100-bp windows starting 1 kb before the transcription start site (TSS) of *AT5G47260* and stopping 1 kb after the transcription site end (TSE) site of *AT5G47280*. Red: methylation in the CG context. Green: methylation in the CHH context. Blue: methylation in the CHG context.

**(B)** Methylation profiles on *AT5G47260* obtained by CHOP-qPCR in inoculated and non-inoculated roots of Bur-0/Col-0, 10499/13499, and the homozygous recombinant lines 2313-15 (*Pb-At5.2*<sub>Col/Col</sub>) and 1381-2 (*Pb-At5.2*<sub>Bur/Bur</sub>). The last two genotypes harbor the narrowest recombination events from either side of *Pb-At5.2* (between markers CLG4 and K64, details in Figure 1). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software;

whiskers extend 1.5 times the IQR from the 25th and 75th percentiles; outliers are represented by dots; data points are plotted as open circles.  $n = 4$  bulks of six plants, and  $p$  values are shown (two-sided  $t$ -test).

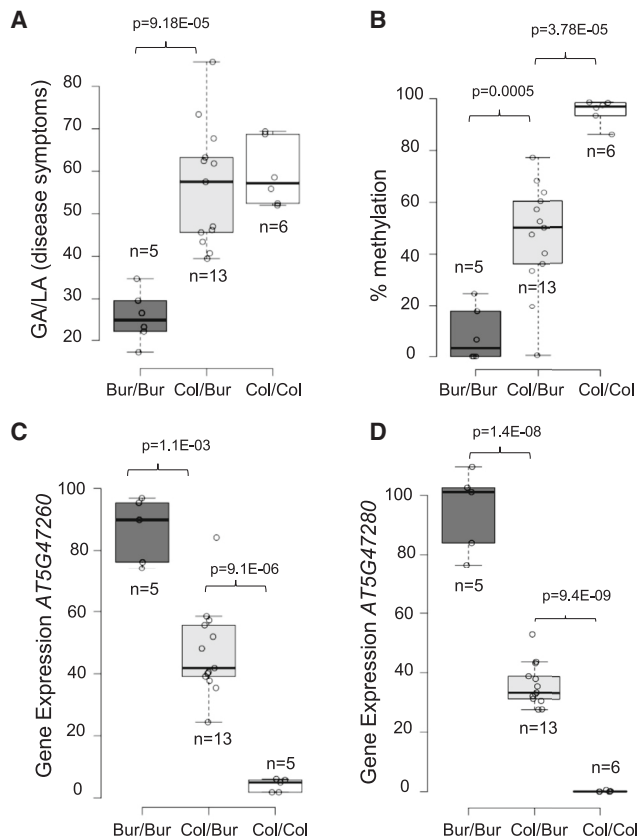
sequence polymorphism outside the locus. Moreover, the “Col-like” hypermethylation of *AT5G47260* and *AT5G47280* was systematically associated with low expression of the two NLR genes and a lower level of partial resistance to *P. brassicae* infection. To further investigate the inheritance of this epiallelic variation and its penetrance on gene expression and clubroot resistance, we investigated two groups of 100 individual plants, corresponding to the progenies derived from selfing the heterozygous 2509 and 1381 lines (harboring heterozygosity at the locus). Evaluation of plant disease for each individual plant in the two progenies revealed a 3:1 Mendelian segregation of the partial resistance phenotype. Clubroot symptoms in individuals with only one Bur-0 resistance allele were the same as in individuals with two susceptible Col-0 alleles (Figure 4A). In clubroot-inoculated roots of each individual plant from the 2509 progeny, the methylation state of the *Pb-At5.2* region was monitored by CHOP-qPCR on *AT5G47260*. The SNP allele status at *Pb-At5.2* was also investigated for each individual plant (for details of markers see Supplemental Data 1). Heterozygous Bur/Col individuals displayed intermediate parental methylation and expression values (Figure 4B–4D), providing a molecular explanation for the recessivity of the Bur-0 resistance allele. Together, these results suggested a link between partial resistance to *P. brassicae* and stably inherited epiallelic variation at *Pb-At5.2*, which controls the expression of two NLR genes.

### The Bur-like hypomethylated epiallele is well represented among *Arabidopsis* accessions and contributes to reduction in clubroot symptoms

To assess the relative contribution of changes in DNA sequence and DNA methylation at *Pb-At5.2* to clubroot resistance, we investigated natural allelic and epiallelic diversity across *Arabidopsis* accessions. We took advantage of recently published Illumina short genome sequence reads obtained from 1135 *Arabidopsis* accessions (1001 Genomes Consortium, 2016) to document the species-wide molecular diversity of the *Pb-At5.2* genomic region.

On the basis of quantitative horizontal and vertical coverage of short reads aligned to the Col-0 reference genome sequences, we identified two discrete groups of accessions. One group, containing 401 accessions, was characterized by high vertical and horizontal coverage (>0.75) and included the reference accession Col-0 as well as the partially clubroot-resistant Bur-0 (Figure 5A; for a detailed list of genotypes see Supplemental Data 3, sheet 1). The remaining 734 accessions contained diverse structural rearrangements, principally long deletions that translate into poor horizontal and vertical coverage compared with the reference Col-0 genome. Closer examination of coverage plots for the 401 Col-0/Bur-0-like accessions revealed a uniform haplotype structure that was present at high frequency at the species level (minor allele frequency [MAF] ~0.37). Nonetheless, the haplotype frequency varied among geographic groups, ranging from 52.7% in Spain to 17.7% in Asia (Supplemental Figure 10). We then analyzed DNA methylation levels in 287 accessions from among the 401 accessions that contained the Col-0/Bur-0-like *Pb-At5.2* and for which bisulfite data were publicly available (Kawakatsu et al., 2016). From these data, we could distinguish a group of 228 accessions, including Bur-0, that showed hypomethylation of *Pb-At5.2* and another group of 59 accessions, including Col-0, that displayed hypermethylation (Figure 5B). The prevalence of accessions with the Col-like (epi)haplotype varied considerably with geographic origin, ranging from 1.8% in Spain to 16.8% in central Europe (Supplemental Data 3 and Supplemental Figure 10). Consistent with a causal role for DNA methylation in the transcriptional regulation of *AT5G47260* and *AT5G47280*, reanalysis of publicly available RNA-seq data revealed a pronounced negative correlation between methylation level and *AT5G47260* and *AT5G47280* expression (Figure 5C). These results were further validated in infected roots from 20 natural accessions (Figure 5D).

Both Col-like and Bur-like epialleles were significantly represented among the natural accessions, offering interesting genetic



**Figure 4. Intermediate methylation and transcript levels of candidate genes in heterozygous plants are associated with full clubroot susceptibility.**

Eighty-three individual plants from the segregating progeny of recombinant line 2509 (heterozygous in Chr.5 region between genetic markers K58 and K93) were sampled at 21 days post inoculation. Leaves from each individual plant were used for genotyping (PCR marker CL\_N8), which defined  $n$  pools of >3 plants of each zygosity profile: Bur/Bur ( $n = 5$ ), Col/Bur ( $n = 13$ ), and Col/Col ( $n = 6$ ) (black, gray, and white boxes, respectively). Each plant pool was evaluated for (A) clubroot resistance (GA/LA), (B) percent methylation at the locus, and (C and D) candidate gene expression (*AT5G47260* and *AT5G47280*). Gene expression was quantified by RT-qPCR, and data were normalized over mean-Cp from the pools Bur/Bur following Pfaffl's method with two reference genes (Pfaffl, 2001). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the IQR from the 25th and 75th percentiles; outliers are represented by dots; data points are plotted as open circles.

material with which to determine the actual contributions of DNA sequence and DNA methylation to the control of clubroot partial resistance. One hundred and twenty-six accessions were selected for their methylation levels at the *AT5G47260*–*AT5G47280* region in data from Kawakatsu et al. (2016), including 42 accessions with the Col-like epiallele and 85 accessions with the Bur-like epiallele, and then assessed for their resistance to *P. brassicae* isolate eH. Whereas no DNA sequence polymorphisms in *Pb-At5.2* showed an association with clubroot resistance (Supplemental Data 4), the low DNA methylation state of the *AT5G47260/AT5G47280* locus was significantly associated with enhanced resistance levels (Figure 6). Together, these results corroborate and extend the conclusions

obtained by fine mapping of *Pb-At5.2* and provide strong evidence that natural epiallelic variations contribute to the quantitative differences in clubroot resistance observed among *Arabidopsis* accessions.

### ***Pb-At5.2* epivariation is independent of *cis*-genetic variations**

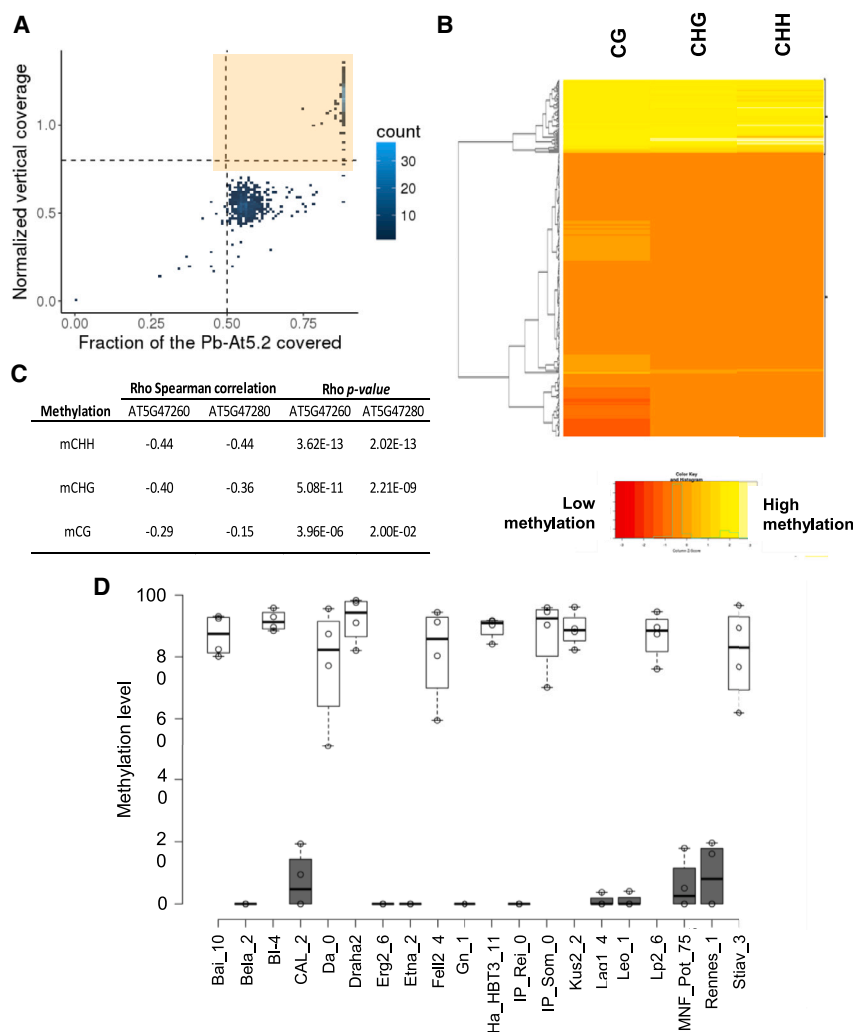
At the *Pb-At5.2* locus, the transposon *AT5TE69050* was present in both parental genotypes, with no sequence variation that might have been the primary cause of variation in DNA methylation on the two adjacent genes. Analysis of 34 out of the 287 accessions with the Col-0/Bur-0-like haplotype did not reveal the presence/absence of TE insertion variants within the 26-kb *Pb-At5.2* region (Quadrona et al., 2016; Stuart et al., 2016), with the exception of a helitron insertion in the accession NFA-10. Moreover, 18 and 16 of these accessions displayed hypermethylated and hypomethylated epialleles, respectively, indicating that variation in DNA methylation is not associated with TE presence/absence variants. In addition, the *cis*-nucleotide polymorphism located within the coding sequence of *AT5G47260* and detected in Bur-0 was absent in at least five other accessions sharing the hypomethylated epiallele (Supplemental Figure 11), indicating that the hypomethylated state of *Pb-At5.2* is not correlated with any specific DNA sequence polymorphism at the locus.

### **The hypermethylated epigenetic variant is maintained by the RNA-independent pathway**

Analysis of sRNAs identified in Col-0 (Stroud et al., 2013) revealed that the *AT5G47260/AT5G47280* region is targeted mostly by 24-nt sRNAs, which prompted us to generate sRNA profiles from non-inoculated roots of Col-0 and Bur-0 and from roots inoculated with *P. brassicae* isolate eH 14 and 21 days after inoculation. Consistent with the previously observed pattern of DNA methylation, we found high levels of sRNAs only in Col-0 (Figure 7A). To further explore the mechanisms involved in the maintenance of methylation at this locus, we made use of publicly available methylomes of Col-0 mutant plants defective in one or several DNA methylation pathways (Stroud et al., 2013). Despite the high levels of sRNAs detected over the *AT5G47260/AT5G47280* region, mutations affecting the RdDM pathway did not influence its DNA methylation level (Supplemental Figure 12). Conversely, DNA methylation was largely lost in mutants defective in sRNA-independent maintenance of DNA methylation, i.e., *ddm1*, *cmt2/3*, *met1*, and *suvh456* (Figure 7B). These results raise questions about the role of sRNAs targeted to the Col-like hypermethylated region whereas methylation maintenance depends solely on the RNA-independent pathway.

## **DISCUSSION**

To date, only a very small number of resistance QTLs have been characterized at the molecular level (Delplace et al., 2022). Detection and fine mapping of resistance QTLs is typically challenging not only because of the difficulties associated with measuring small variations in partial resistance in large numbers of individual progeny but also because resistance QTLs can be sensitive to environmental changes (Aoun et al., 2017; Laperche et al., 2017; Aigu et al., 2018). However, technical issues may have been only part of the problem.



**Figure 5. Natural epigenetic variation at *Pb-At5.2* affects expression of *AT5G47260/AT5G47280* in *Arabidopsis* accessions.**

**(A)** Screening for 1001 Genomes *Arabidopsis* accessions that display a Col/Bur-like genomic structure at *Pb-At5.2* (chr5: 19 185 600–19 200 600). x axis: horizontal coverage region covered by at least one read. y axis: vertical coverage in read percentage. The 401 accessions framed in the northeast intercardinal region delimited by dotted lines have a vertical read coverage >0.8 and a horizontal DNA-seq >0.5 (DNA-seq data from Alonso-Blanco et al. (2016)).

**(B)** Clustering of a series of accessions harboring Col/Bur-like genomic structure at *Pb-At5.2* by their level of methylation on *AT5G47260* and *AT5G47280*. Bisulfite data were obtained from the 1001 Genomes project (Supplemental Data 3, sheet 2). Average methylation level was calculated beginning 1 kb before the TSS of *AT5G47260* and stopping 1 kb after the TSE site of *AT5G47280* for each context.

**(C)** Spearman correlation between methylation and gene expression of *AT5G47260* and *AT5G47280* in a subset of 253 *Arabidopsis* accessions for which expression data were available (RNA-seq data from Kawakatsu et al., 2016). The correlation between gene expression and methylation level is given for all three DNA methylation contexts in the interval from 1 kb before the TSS of *AT5G47260* to 1 kb after the TSE site of *AT5G47280*.

**(D)** Confirmation of methylation profiles at *AT5G47260* in inoculated roots from 20 ecotypes. Methylation level was determined using CHOP-qPCR. Black and white bars indicate genotypes with Bur-like and Col-like methylation patterns, respectively. Center lines show the medians; box limits indicate the 25th and 75th percentiles as

determined by R software; whiskers extend 1.5 times the IQR from the 25th and 75th percentiles; outliers are represented by dots; data points are plotted as open circles.  $n = 4$  bulks of six plants.

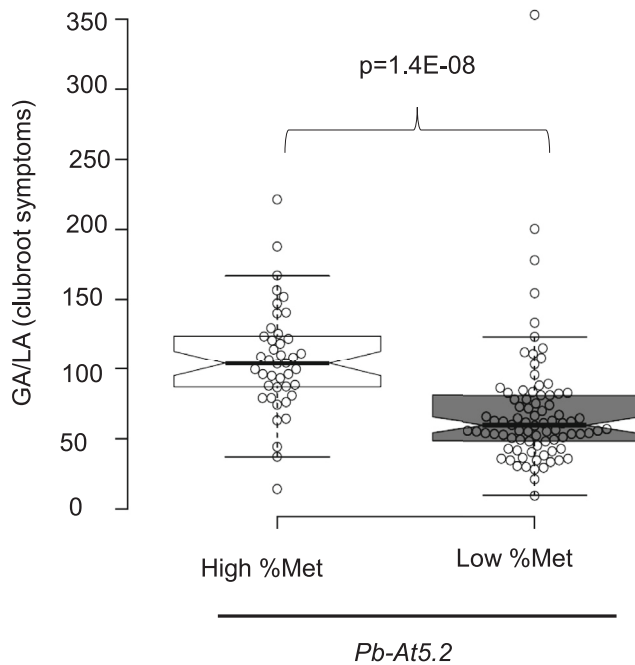
Recent developments in the field of epigenetics suggest that some inherited resistance factors may not be detected by classic genetic approaches that are based solely on DNA sequence variation. In the present work, a genome-wide association study (GWAS) failed to identify any nucleotide variation in the 26-kb interval of *Pb-At5.2* associated with clubroot response. By contrast, clubroot resistance was clearly related to epigenetic variation at two NLR genes in this interval. This work thus reveals for the first time an epigenetically driven expression polymorphism that makes a substantial contribution to the natural diversity of plant immune response.

Many examples of epialleles are metastable, i.e., they can be reversed by stochastic or unidentified factors (Weigel and Colot, 2012). Stability over multiple generations is a primary concern from both evolutionary and breeding perspectives. The epiallele described here seems to be extremely stable, as it was robustly detected in all our previous QTL investigations in *Arabidopsis*. This included two independent segregating progenies derived from Bur-0 and Col-0 (Jubault et al., 2008a) and additional studies with the HIF lines 10499/13499 (Lemarié et al., 2015; Gravot et al., 2016). The high level of methylation

and absence of *AT5G47260* and *AT5G47280* expression observed in Col-0 were also found in a set of publicly available data obtained in different laboratories from diverse plant tissues and conditions (Winter et al., 2007; Stroud et al., 2013; Klepikova et al., 2016; 1001 Genomes Consortium, 2016; Kawakatsu et al., 2016). It was also confirmed by our own data generated from inoculated roots, non-inoculated roots, and leaf samples. Finally, this methylation pattern was also robustly found in multiple replicates of individual plants. Thus, *Pb-At5.2* can be classified as a stable epiallele without reservation.

There has only been one report of a plant disease resistance caused by an inherited methylation variant that affects expression of a resistance-related gene (Nishimura et al., 2017). In that study, a stable expression polymorphism (between Ler-0 and Ag-0 accessions) on the TIR-only encoding gene *RBA1* (*AT1G47370*) affected effector-triggered immunity responses to the *Pseudomonas syringae* effector *hopBA1*. This expression polymorphism was linked to the nearby presence/absence of a TE sequence in the promoter region of the gene and to *MET1*-dependent DNA methylation variation. However, because DNA methylation was reversed when the TE sequence was





**Figure 6. Variation in clubroot symptoms among *Arabidopsis* accessions is linked to epivariation at *Pb-At5.2*.**

Effect of *Pb-At5.2* epiallele variation on clubroot susceptibility, evaluated in 126 *Arabidopsis* accessions with a similar Bur/Col-like genomic structure at the locus. Each open circle represents one accession. In total, 42 accessions had a Col-like epiallele (high percentage of methylation, High % Met), and 84 had a Bur-like epiallele (low percentage of methylation, Low % Met). For each accession, the mean GA/LA was obtained by modeling raw data of two biological replicates with two blocks (six individual plants in each block). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the IQR from the 25th and 75th percentiles; outliers are represented by dots; data points are plotted as open circles. The notches are defined as  $\pm 1.58 \times \text{IQR}/\sqrt{n}$  and represent the 95% confidence interval for each median. The  $p$  value (Wilcoxon test) is indicated.

segregated away (Supplemental Figure 2 in Nishimura et al., 2017), this DNA methylation variation is not “epigenetic,” as it is an obligate consequence of sequence variation (i.e., presence/absence of the TE sequence). In the present study, we showed that DNA methylation variation in the region between *AT5G47260* and *AT5G47280*, including the TE sequence *AT5TE69050*, is not linked to any nucleotide/structural variation at the locus or elsewhere in the genome. Thus, *Pb-At5.2<sub>COL</sub>* and *Pb-At5.2<sub>BUR</sub>* can be considered “pure epialleles” as defined by Richards (2006).

From available genomic and epigenomic data from the 1001 Genomes Project, it can be extrapolated that the Bur-like clubroot resistance epiallele is present in about half of the accessions from the “Relict,” “Spain,” and “Italy/Balkans/Caucasus” groups and 39% of the accessions from the “North Sweden” group (Supplemental Data 3). By contrast, the Bur-like epiallele is likely (taking into account missing methylation data) present at about 10% of the “Germany” group. On the other hand, the clubroot susceptibility of the Col-like epiallele was absent from the accessions in the “Relict” and “North Sweden” groups but reached at

least 16.8% in the “Central Europe” group. This geographic structure suggests that both epialleles can confer fitness gains, depending on the environmental context. However, it does not appear to be obviously related to the incidence of clubroot in *Brassica* culture (usually low in the warm southern European regions). Keeping in mind that NLRs can detect unrelated effectors from distinct microbial species (Narusaka et al., 2009) and echoing previous work (Karasov et al., 2014), we hypothesize that maintenance of this epivariation in natural populations may reflect additional roles played by *Pb-At5.2* against other plant pathogens (besides the control of clubroot infection).

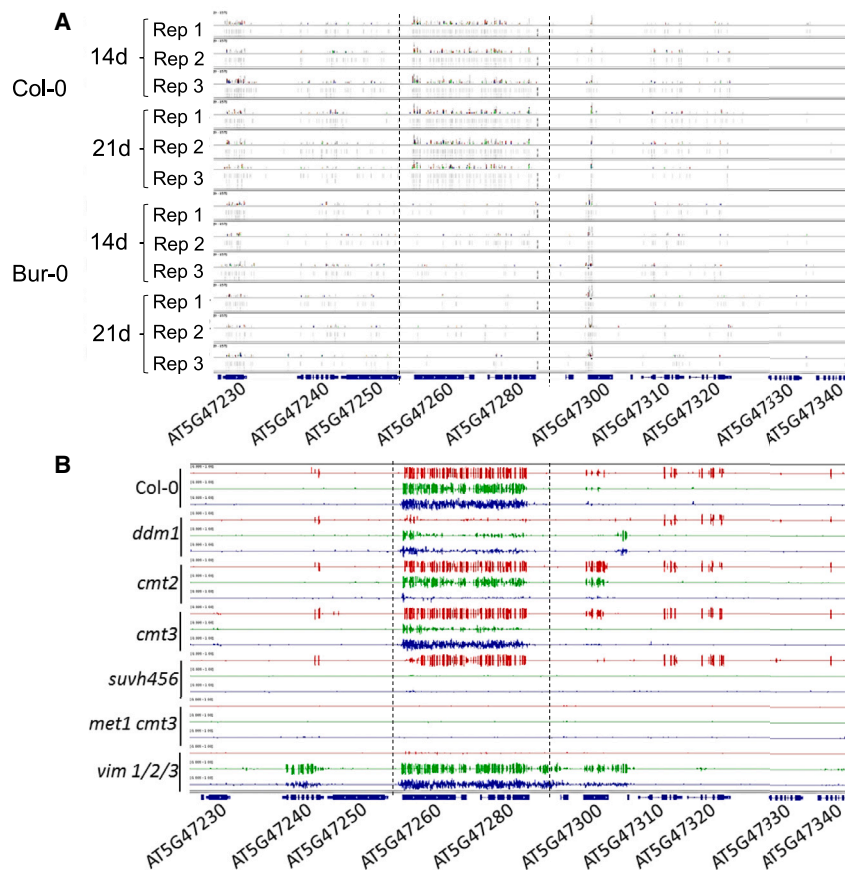
*AT5G47280* has been annotated as *ADR1-Like 3* on the basis of its phylogenetic relationship with the small family of helper *CC<sub>RPW8</sub>*-NLRs, including *ADR1*, *ADR1-L1*, and *ADR1-L2* (Saile et al., 2020, 2021). However, the absence of *RPW8* in *ADR1-L3* and the absence of *ADR1-L3* expression in Col-0 have raised questions about its actual role in plant immunity. *AT5G47260* and *AT5G47280* belong to a small heterogeneous cluster of three non-TIR-NLRs, which also includes *AT5G47250*. This small cluster is located on chromosome 5, not far from the largest NLR hotspot in the *Arabidopsis* genome (Meyers et al., 2003). None of these three genes has previously been shown to participate in plant–pathogen interactions. Here, we showed that expression of both *AT5G47260* and *AT5G47280* is necessary for partial resistance to *P. brassicae*. There are a few examples of tandem NLR genes encoding pairs of proteins that function as heterodimers (Cesari et al., 2014; Williams et al., 2014; Saucet et al., 2015). Similarly, the proteins encoded by these two jointly epigenetically regulated genes may function together in the control of cell defense responses during clubroot infection. Although the underlying molecular mechanisms are unknown, the canonical example of the TIR-NLR heterodimer *RRS1/RPS4* corresponds to a recessive resistance locus, similar to *Pb-At5.2*.

In *Arabidopsis*, DNA methylation is widely distributed in both the promoters and the bodies of most NB-LRR-encoding genes (Kong et al., 2018, 2020), predominantly in the CG sequence context. This suggests that plant genomes contain multiple functional resistance genes whose possible roles in biotic interactions are locked by epigenetic processes. This hypothesis is also supported by our previous study, in which we demonstrated that *ddm1*-triggered hypomethylation at different genomic loci resulted in the unlocking of genetic factors that ultimately exert significant control over clubroot symptom development (Liégard et al., 2019). It would now be interesting to carry out a careful genome-wide analysis of methylation profiles of all NLR genes among *Arabidopsis* accessions, which would take into account the structural diversity of all these individual genes (supported by additional targeted resequencing of NLR loci). The intraspecific diversity in methylation patterns of NLR and RLK/RLP genes in plants, their heritability, and their consequences for plant biotic interactions also deserve further attention in future studies.

## METHODS

### Plant materials and growth conditions

The HIF lines 10499 and 13499 and their parental accessions Col-0 (186AV) and Bur-0 (172AV) were provided by Versailles Arabidopsis Stock



**Figure 7. Epigenetic variation at *Pb-At5.2* correlates with the abundance of locus-targeted sRNA but is maintained by the RNA-independent methylation pathway.**

**(A)** Mapping of sRNA-seq reads. Reads were obtained from roots of Col-0 and Bur-0 accessions at two time points 14 and 21 days after sowing. For each condition, three bulks (numbered from Rep 1 to Rep 3) of six plants were used.

**(B)** Methylation state at the *Pb-At5.2* locus in knockout lines (Col-0 genomic background) defective for the RdDM or non-RdDM pathway (Stroud et al., 2013).

Red: methylation in CG context. Green: methylation in CHG context. Blue: methylation in CHH context.

### Phenotyping

HIF lines and *Arabidopsis* accessions were phenotyped 3 weeks after inoculation (21 days post inoculation) for their susceptibility to *P. brassicae*. Plants were thoroughly rinsed with water and photographed. Infected roots were removed and frozen in liquid nitrogen. Clubroot symptoms were evaluated by image analysis using the gall area/leaf area (GA/LA) index calculated according to Gravot et al. (2011).

### Fine mapping of the locus responsible for clubroot resistance

Fine mapping of *Pb-At5.2* was performed starting from crosses between HIF lines 10499 and 13499, followed by successive rounds of genotyping and clubroot phenotyping in subsequent plant generations (full details are given in Supplemental Text 1).

### RNA isolation, mRNA sequencing, and differential gene expression analysis

Total RNA was extracted from frozen and lyophilized roots (collected 14 days after inoculation) using the mirVana miRNA Isolation Kit (Invitrogen) according to the manufacturer's instructions. After extraction, the RNA samples were quantified using NanoDrop ND-1000 technology, and their quality was assessed using the RNA 6000 assay kit (Agilent). Samples with an RNA integrity number (RIN) greater or equal to 7 were used for sequencing. cDNA-sequencing (cDNA-seq) library construction and sequencing were performed by the NGS platform at the Marie Curie Institute of Paris. Each library was sequenced in paired-end mode using the Illumina HiSeq 2500 platform. Reads were aligned to the TAIR10 genome annotation and assembly of Col-0 *A. thaliana* concatenated with the *P. brassicae* genome using STAR software version 2.5.3.a (Dobin et al., 2013). Alignment conditions were selected according to the *Arabidopsis* genome. A maximum of five multiple read alignments was accepted, and no more than three mismatches were allowed for each alignment. The resulting BAM files were used to determine read counts using the counts function in featureCounts software (version 1.4.6) and the TAIR10 gff file of *Arabidopsis* concatenated with the gff file of *P. brassicae*. Differentially expressed genes were determined using the edgeR package (Robinson et al., 2010) in R software version 3.3.0 (R Core Team, 2013). Raw counts obtained as described previously were used as input data for edgeR. After CPM (counts per million) values were determined, only genes with at least one CPM in three samples were retained. Expression signals were normalized using the TMM method (trimmed mean of M values) with the CalcNormFactors function in edgeR. Finally, differentially expressed genes were identified using

Center (<http://publiclines.versailles.inrae.fr>). *Arabidopsis thaliana* accessions were all purchased from the Nottingham Stock Center. Individuals in the panel of 126 accessions were selected according to their methylation levels at the region of interest (Kawakatsu et al., 2016). All accessions and in-house-generated recombinant lines used in this study are listed in Supplemental Data 1 and Supplemental Data 3. Seed germination was synchronized by placing seeds on wet blotting paper in Petri dishes for 2 days at 4°C. Seeds were sown individually in pots (4-cm diameter) containing a sterilized mixture of two-thirds compost and one-third vermiculite. Growth chamber conditions of 16-h light ( $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 20°C and 8-h darkness at 18°C were used to grow plants. The 126 *Arabidopsis* accessions and HIF lines were challenged with *P. brassicae* in two biological replicates in a completely randomized block design (with two blocks per replicate, each block consisting of six plants per genotype). The *Arabidopsis* accessions Col-0 and Bur-0 and the HIFs 10499, 13499, 1381-2, and 2313-15 used in RNA-seq and sRNA-seq approaches were assessed when infected with *P. brassicae* or in the uninfected condition in three randomized blocks. The CRISPR-Cas9 edited lines and corresponding wild-type lines were challenged with *P. brassicae* in eight replicates in a completely randomized block design (each replicate consisting of 10–12 plants per genotype). Almost all clubroot tests were performed with the eH isolate of *P. brassicae* described by Föhling et al. (2003), which belongs to the most virulent pathotype, P1. The resistance spectrum of *Pb-At5.2* was also assessed using the additional isolates Pb137-522, Ms6, K92-16, and P1(+). For every isolate used in this study, the pathotype was validated in every experiment using the differential host set according to Some et al. (1996), also including two genotypes of *Brassica oleracea* ssp. *acephala* C10 and CB151. One milliliter of resting spore suspension ( $10^7$  spores  $\text{ml}^{-1}$ ) prepared according to Manzaneres-Dauleux et al. (2000) was used for pathogen inoculation 10 days after germination (stage 1.04; Boyes et al., 2001). This inoculum was applied to the crown of each seedling.

the `decideTests` function of `edgeR` with one minimum fold change between  $-1.5$  and  $1.5$ .

### CRISPR-Cas9 constructs and plant transformation

Two guide sequences were designed for each targeted gene (i.e., *AT5G47260* and *AT5G47280*) using CRISPOR software (Concordet and Haeussler, 2018), taking care to select sequences with very high specificity scores (Supplemental Data 5). Guide sequences were ordered as oligonucleotides (IDT) and cloned downstream of the *Arabidopsis* U6-26 promoter and upstream of an enhanced single guide RNA scaffold as reported previously (Chauvin et al., 2021) to produce individual guide modules. Assembly of guide modules for single genes was performed using PCR amplification with specific primers followed by classical restriction/ligation cloning (Supplemental Data 5, sheet 1). Guide assemblies were then cloned by a Gateway LR reaction (Thermo Fisher Scientific) into the pDe-Cas9 backbone (Fauser et al., 2014) harboring an *nptII* resistance cassette (Chauvin et al., 2021), resulting in two binary plasmids for CRISPR-mediated targeting of *AT5G47260* (pDe-Cas9\_T79-80) and *AT5G47280* (pDe-Cas9\_T81-82) (Supplemental Data 5, sheet 1). All constructs were checked by Sanger sequencing. The resulting plasmids were then transferred into *Agrobacterium tumefaciens* C58/GV3101pMP90 by heat shock and used to transform *Arabidopsis* plants via the floral dip method (Clough and Bent, 1998). Transgenic plants were screened on solid plates with half-strength Murashige-Skoog medium containing  $50 \text{ mg l}^{-1}$  kanamycin (Yeasen, cat. no. 60206ES10). A first screening was performed on the T1 generation using PCR and sequencing to identify plants with mutations in *AT5G47260* or *AT5G47280* (primer pairs are listed in Supplemental Data 5, sheet 2). A second round of screening enabled the identification of T2 plants homozygous for the mutations and free from CRISPR-Cas9 cassette T-DNA. T3 seedlings were used to evaluate *P. brassicae* resistance.

### GWAS analyses

A conventional GWAS on GA/LA data from 126 accessions was performed with easyGWAS (Grimm et al., 2017) (<https://easygwas.ethz.ch>). Association analysis was performed with EMMAX (Kang et al., 2010) using 1 806 554 SNPs with an MAF  $> 0.05$ , after correction for population structure by including the first three principal components in the additive model.

### Small RNA isolation, sequencing, clustering, and differential presence determination

sRNA was extracted from frozen and lyophilized roots (collected 14 days after inoculation) using the mirVana miRNA Isolation Kit (Invitrogen) according to the manufacturer's instructions. After extraction, the sRNAs were quantified using a NanoDrop ND-1000 and quality controlled using the Small RNA assay kit (Agilent). Samples with an RIN greater than or equal to 7 were used for sequencing. Construction and sequencing of cDNA-seq libraries were performed on the NGS platform of the Marie Curie Institute of Paris. For each sample, single-ended (50 bp) sequencing was performed using the Illumina HiSeq 2500 platform. Reads were aligned to the TAIR10 genome annotation and assembly of Col-0 *A. thaliana* concatenated with the *P. brassicae* genome using STAR version 2.5.3.a (Dobin et al., 2013), then counted and clustered using ShortStack software (Axtell, 2013). The presence of differentially expressed sRNAs was determined using `edgeR` (Robinson et al., 2010) in R version 3.3.0 (R Core Team, 2013). Raw counts obtained as described previously were used as input data to `edgeR`. After CPMs were determined, only genes with at least one CPM in three samples were retained. Expression signals were normalized using the TMM method with the `CalcNormFactors` function in `edgeR`. Finally, differentially expressed sRNAs were identified using the `decideTests` function in `edgeR` with one minimum fold change between  $-1.5$  and  $1.5$ .

### RNA isolation and RT-qPCR analysis

Total RNA was extracted from lyophilized roots of accessions and HIF lines 21 days after infection using the TRIzol extraction protocol. Samples with residual traces of DNA were treated with DNase (Promega ref. M6 10A). Before reverse transcription of RNA to cDNA with SuperScript II (Invitrogen), RNA quality was verified by agarose gel electrophoresis. RT-qPCR was performed in a LightCycler 480 thermocycler (Roche) with cDNA obtained as described above. Gene expression was normalized using as references two *Arabidopsis* genes defined as stable during infection using RNA-seq data (*AT1G54610*, *AT5G38470*) following Pfaffl's method (Pfaffl, 2001). Primer sets were designed for each gene and are listed in Supplemental Table 1.

### CHOP-PCR and qPCR assays

Gene methylation profiles were investigated using the enzyme McrBC (M0272L, BioLabs) (Zhang et al., 2014). Forty nanograms of DNA was incubated with  $0.5 \mu\text{l}$  of BSA (20 mg/ml),  $0.5 \mu\text{l}$  of guanosine triphosphate (20 mM),  $5 \mu\text{l}$  of NE Buffer (10 $\times$ ), and  $0.2 \mu\text{l}$  of McrBC (10 000 U/ml). For CHOP-PCR and qPCR, 2 ng of digested and undigested DNA was used. For CHOP-PCR, the temperature conditions were adjusted according to the primer design, and 35 amplification cycles were used. To determine the methylation state of the targeted region, each sample was digested or not (control) with McrBC before amplification. For CHOP-qPCR, the temperature conditions were adjusted according to the primer design, and 30 amplification cycles were used. Methylation levels of the target region were calculated as the percentage of molecules lost through McrBC digestion as described in Silveira et al. (2013) with the formula:  $(1 - (2 - (\text{Ct digested sample} - \text{Ct undigested sample}))) \times 100$ . The percentages of DNA methylation for *AT5G13440* and *AT5G47400* were calculated in all CHOP qPCRs as controls. *AT5G13440* and *AT5G47400* were selected from 1001 Genomes data as hypomethylated and hypermethylated, respectively, in most *Arabidopsis* accessions, and their expression did not vary during clubroot infection. The primer sets designed for each gene are listed in Supplemental Table 1.

### Published data

The DNA-seq data, RNA-seq data, variant sequences, and bisulfite data for the natural accessions studied here were obtained from previous studies (1001 Genomes Consortium, 2016; Kawakatsu et al., 2016) archived at the NCBI with SRA number SRP056687 and the NCBI Gene Expression Omnibus references GEO: GSE43857 and GSE80744. The bisulfite data and sRNA data for *Arabidopsis* mutants studied here were obtained from a previous report (Stroud et al., 2013) and are archived at the NCBI under accession GEO: GSE39901.

### Statistical analysis

Data were statistically analyzed using the R program (R Core Team, 2013).

## DATA AND CODE AVAILABILITY

The data supporting the findings of this study are available within the paper and its supplemental information files. All unique materials used are readily available from the authors.

### SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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of KASPAR markers. IGEPP colleagues are acknowledged for their technical support for clubroot phenotyping and sampling.

### AUTHOR CONTRIBUTIONS

B.L., A.G., M.J.M.-D., and M.J. designed and conducted the experiments. C.L. and A.G. carried out the fine mapping. C.L., J.L., J.B., T.B., B.L., A.G., and M.J. performed the phenotyping and sampling. B.L., A.G., J.B., J.L., and M.J. carried out epigenetic and gene expression studies. F.V. designed and performed the CRISPR-Cas9 constructs, and C.L. and T.B. carried out the transformation, selection, and handling of edited plants. B.L., Y.A., L.Q., and V.C. conducted bioinformatics analyses. A.G., B.L., L.Q., F.V., V.C., M.J.M.-D., and M.J. participated in drafting and revision of the manuscript.

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No conflict of interest is declared.

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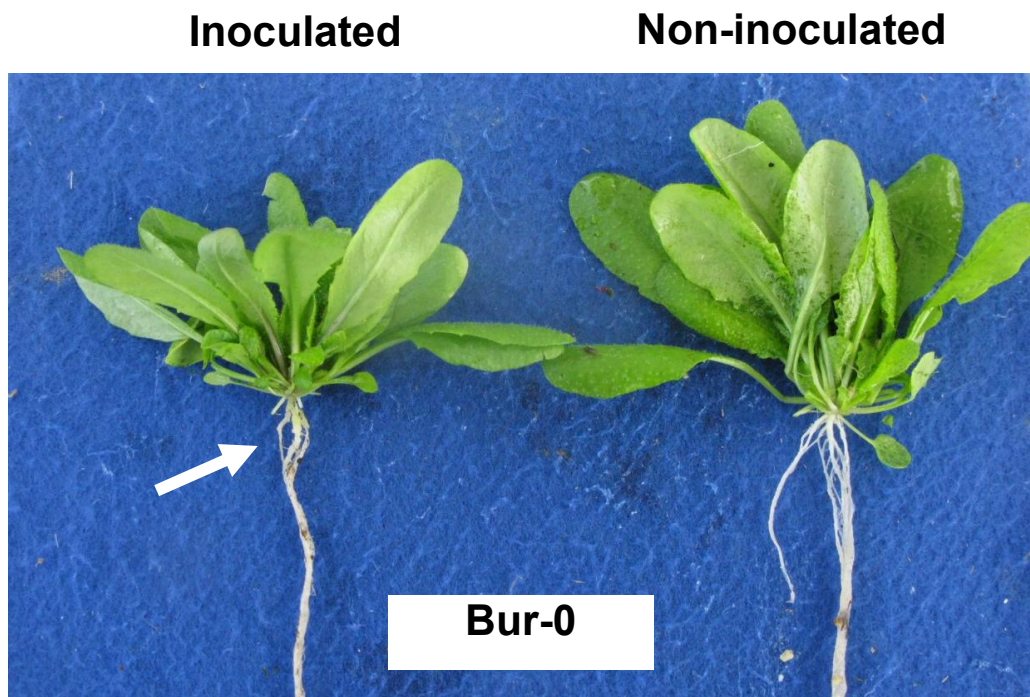
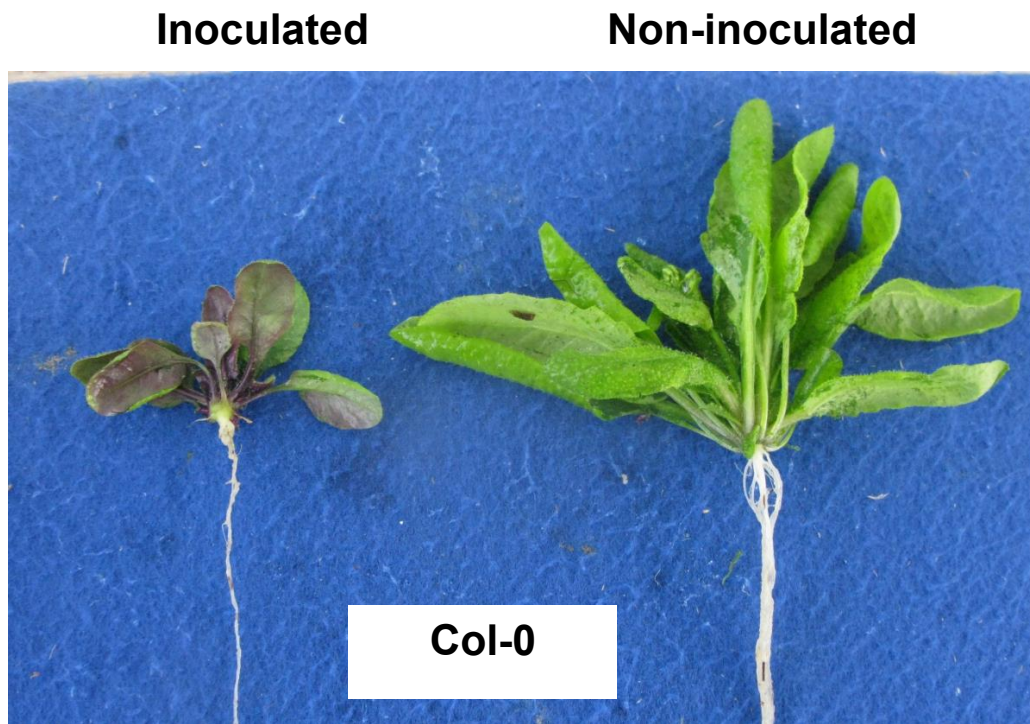
**Supplemental information**

**Two adjacent NLR genes conferring quantitative resistance to clubroot disease in *Arabidopsis* are regulated by a stably inherited epiallelic variation**

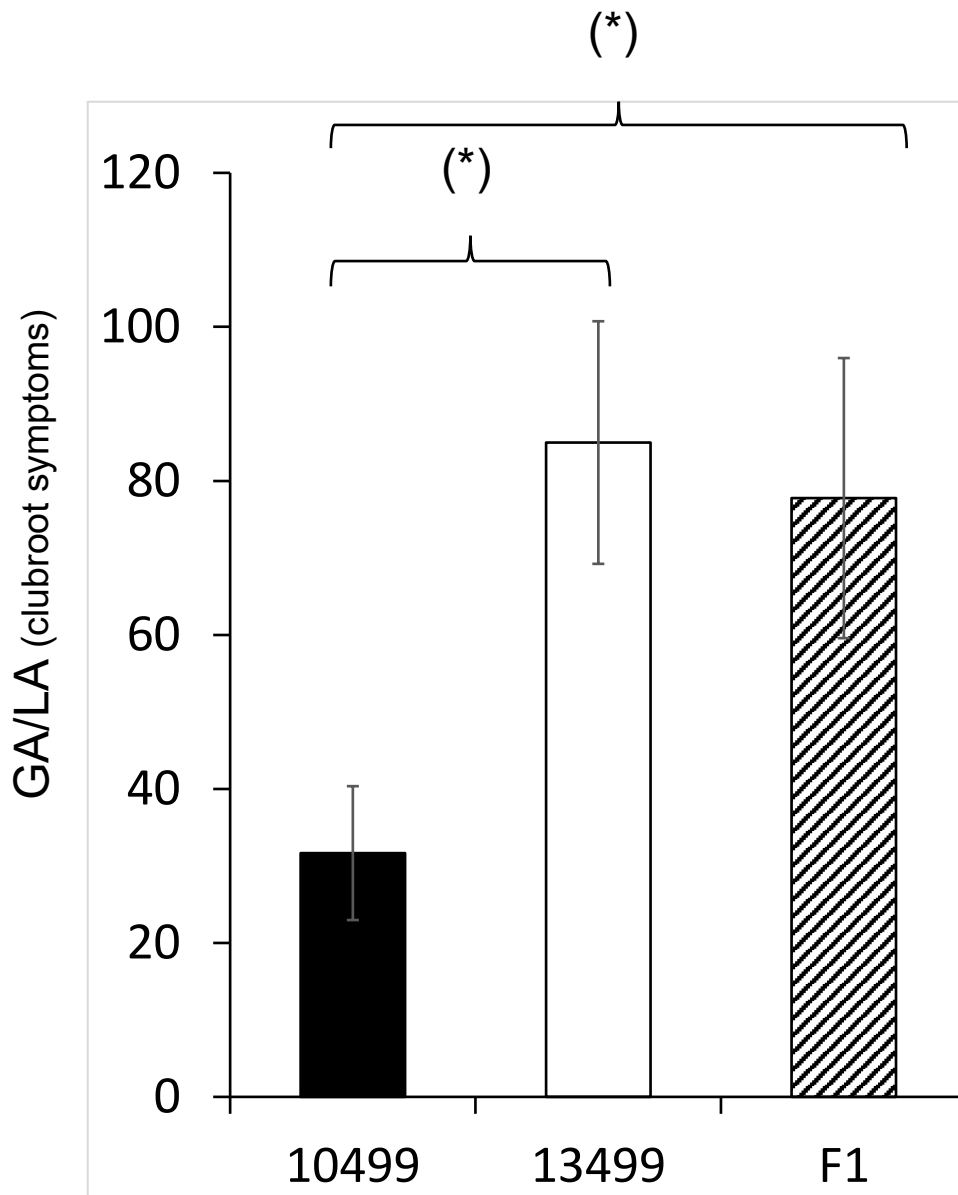
**Antoine Gravot, Benjamin Liégard, Leandro Quadrana, Florian Veillet, Yoann Aigu, Tristan Bargain, Juliette Bénéjam, Christine Lariagon, Jocelyne Lemoine, Vincent Colot, Maria J. Manzanares-Dauleux, and Mélanie Jubault**



## Supplementary Figures

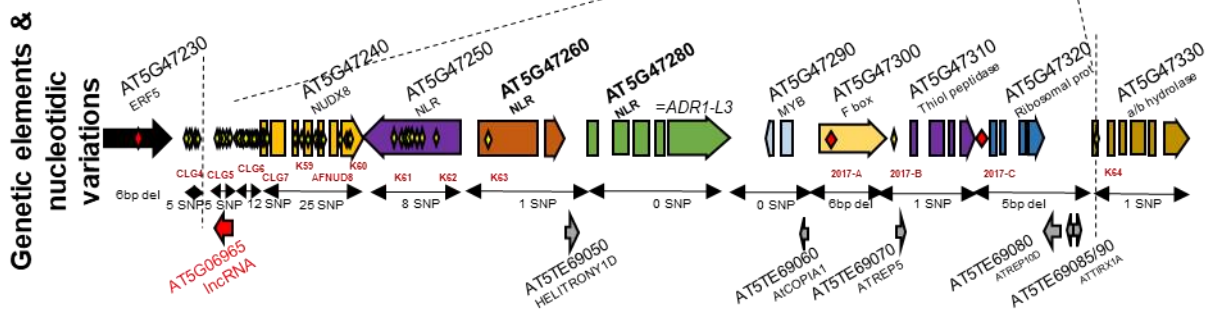
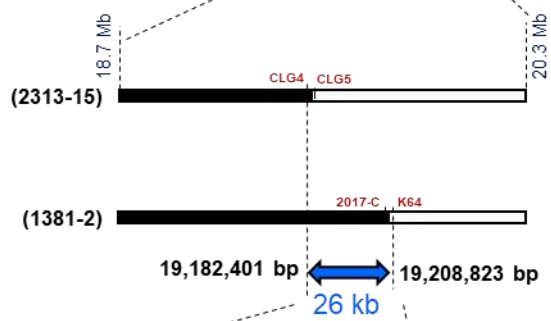
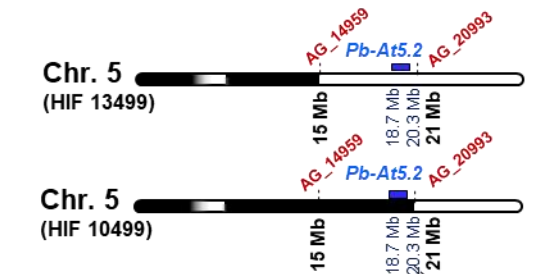
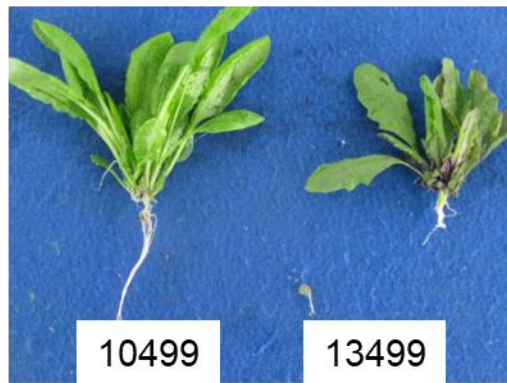
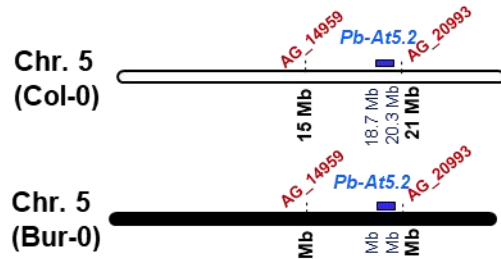
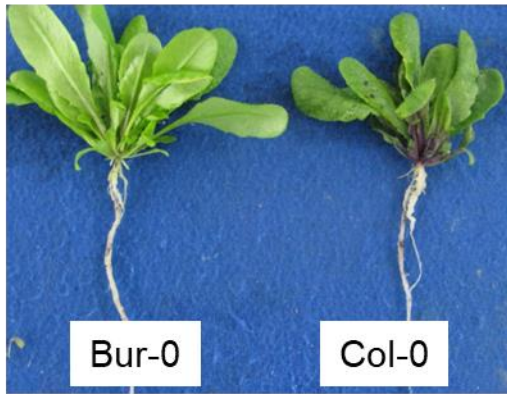


**Supplementary Figure S1 | Illustration of partial resistance to eH isolate in Bur-0, compared to the full susceptibility in Col-0.** Observations were done at 21 days post-inoculation. The white arrow indicates the presence of limited amount of galls in inoculated Bur-0. Plant individuals representative of standard observations made in our experimental conditions.



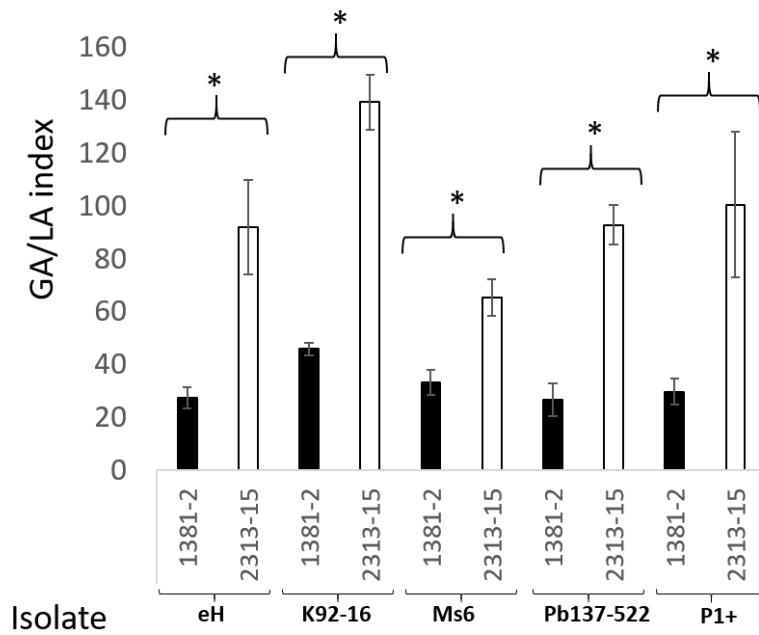
**Supplementary Figure S2 | Comparison of GA/LA disease index in the F1 progeny and in the parental lines 10499 and 13499.** GA/LA disease index is calculated through image analysis (details in the material and method part) from inoculated plants at 21 days post inoculation. Data are from 3 replicates (n=3). For each replicate, GA/LA disease index was calculated from 6 to 12 inoculated individual plants. Stars indicate statistical differences from the paired Student t-test ( $p=0.05$ ).

## Inoculated

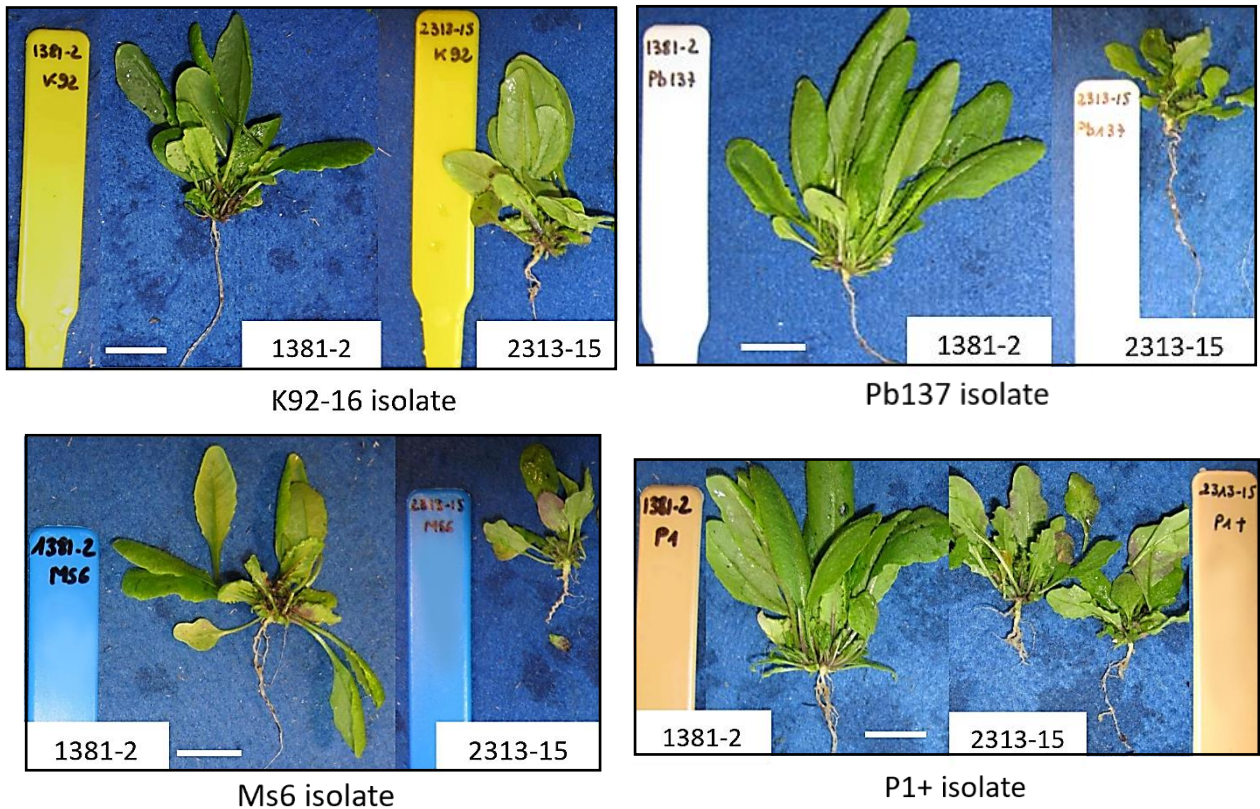


**Supplementary Figure S3 | Illustration of clubroot symptoms in a selection of genotypes used for the fine mapping.** Pictures of inoculated plants were taken at 21 days post inoculation. Genetic structure is indicated on the right side. Black=Bur-0 allele, white=Col-0 allele. Additional details in the legend of Figure 1.

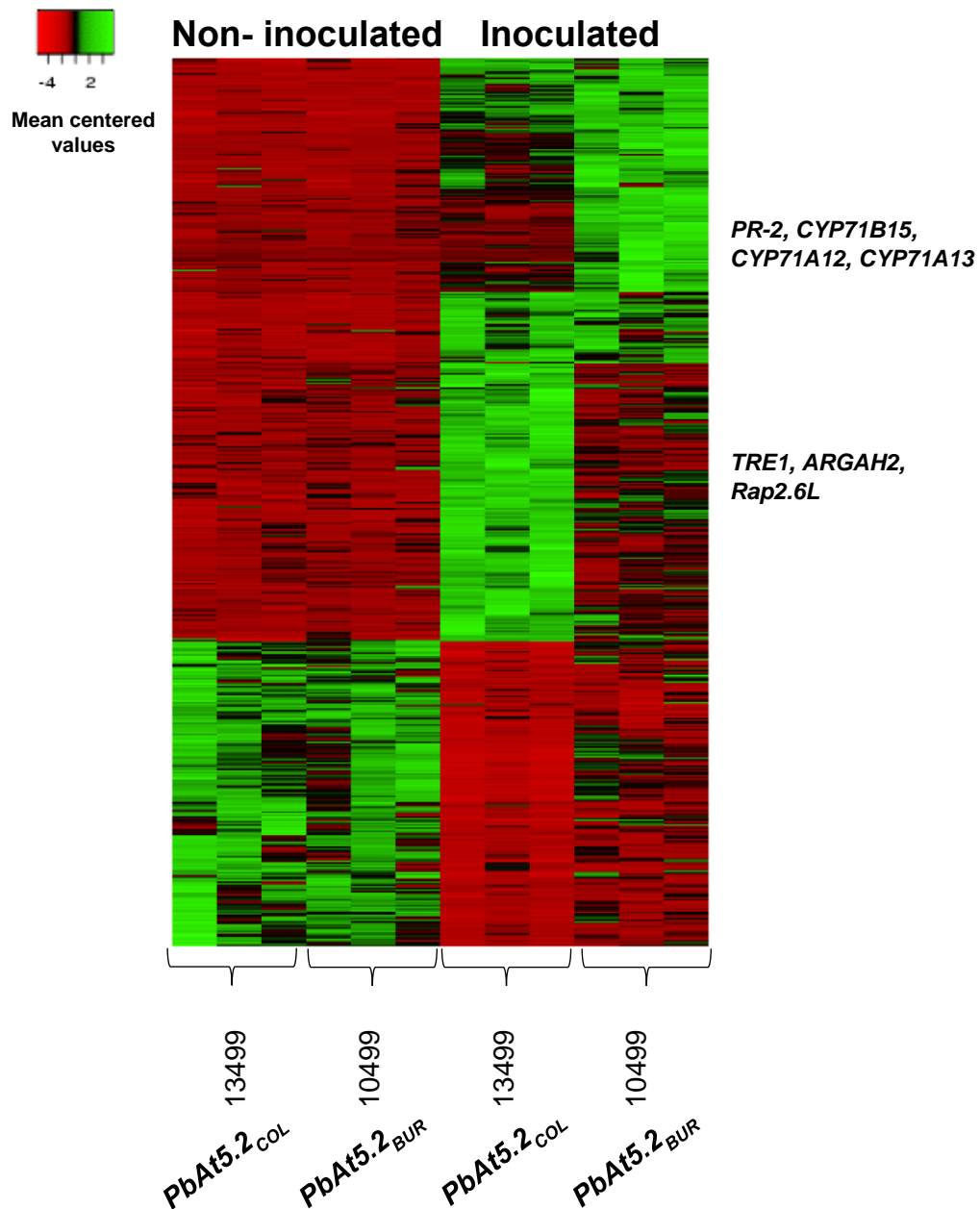
**A**



**B**



**Supplementary Figure S4 | Broad-spectrum resistance conferred by the Bur-0 allele of *Pb-At5.2*** **A** GA/LA disease index and **B** photos of RIL499-derived near-isogenic lines 1381-2 and 2313-15 challenged with a series of four European monospore *P. brassicae* isolates (eH, K92-16, Ms6 and Pb137-522), and with the field isolate P1+. This last isolate is representative of emerging European strains that are virulent on the variety ‘Mendel’ (a clubroot-resistant oilseed rape variety which has been used as a source of resistance for the creation of several modern clubroot-resistant varieties). Data are means of 4 independent replicates (n=4). For each replicate, GA/LA values are means of 6 to 12 individual plants. Error bars indicate standard errors. Statistically different values (from Student T-test) are indicated by stars. Bars, 1.6 cm



**Supplementary Figure S5 | Transcriptional regulations induced by isolate eH at 14 dpi in the recombinant HIF lines 10499 and 13499 harboring Bur-0 or Col-0 allele at *PbAt5.2*.** Data are mean-centered values of CPM (Counts Per Million). For each genotype, three columns are from three independent biological replicates. This set of 559 genes was selected as following: 1/Genes significantly induced ( $p$ -value $<0.05$  +  $\log(\text{FC})>2$  or  $<-2$ ) by eH isolate at 14 dpi in 10499 or 13499; 2/Mean gene expression $>1$ . 61 genes, including *PR-2*, *CYP71B15*, *CYP71A12* and *CYP71A13*, were induced at higher levels in 10499 ( $p$ -value $<0.05$ ). 58 genes, including *TRE1*, *ARGAH2* and *Rap2.6L* were induced at higher levels in 13499 ( $p$ -value $<0.05$ ).

<b>AT5G47260</b>	Bur-0 (WT)	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
	117-1	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
Bur-0 (Δ47260)	117-36	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe / 102nt / TAG STOP
	85-7	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
<b>AT5G47280</b>	Bur-0 (WT)	Ggtctaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
	21-20	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
Bur-0 (Δ47280)	160-2	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
	163-16	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
<b>AT5G47260</b>	HIF 10499 (WT)	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
	95-14	.....gaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt GluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
HIF 10499 (Δ47260)	98-7	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
	105-12	tggaagaagaaccacaagagcgaagcagcctgaataat tggcagctctaaaggaaagagattgtgtgttaactggagccatacag agggaaattggtattggaagaaattggagttcctttt TtpArgGluThrLysGluArgLysAlaAgluLlLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuaspolyllleGluInuGluLeuaspolyllleGlyValProphe
<b>AT5G47280</b>	HIF 10499 (WT)	Ggtctaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
	170-4	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
HIF 10499 (Δ47280)	172-9	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla
	176-15	ggctcaaccttggctctgaaagtcccttggcgcctctcattcaaacgatcgacctgaaacataatgggcaattgcag tggcaggggttacaaggttacaagcttggatgaacactcatgagagtaaggtttgct GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrrTpAlaAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAla

**Supplementary Figure S6 | AT5G47260 and AT5G47280 mutated sequences in CRISPR-Cas9 edited lines in Bur-0 and HIF 10499 backgrounds.** DNA and corresponding predicted protein sequences are detailed. Guide RNAs are highlighted in green and protospacer-adjacent motif (PAM) in blue. Mutations within AT5G47260 and AT5G47280 coding sequences are shown in red. aa, amino acids.

**At5g47260**

```

MGNNFSVESPSLAPFLCGKRKYLYLNRNLERLEALHKVMQDLNAMRNDLLKRLSKBEEIGLQGLQEVKWEISMVEEI -75 - Col-0
...T..... -75 - Bur-0
..... -75 - Bur-0 (Δ47260) 117-1
..... -75 - Bur-0 (Δ47260) 117-36
..... -75 - Bur-0 (Δ47260) 85-7
..... -75 - 10499 (Δ47260) 95-14
..... -75 - 10499 (Δ47260) 98-7
..... -75 - 10499 (Δ47260) 105-12

CC + linker
EPKANRLLEDSEVSEIQRLSRYGYCSLIPASTYRYSEKVLTTMEGVETLRSGVFEAVVHRALPPLVIKMPPIQL -150 - Col-0
..... -150 - Bur-0
..... -150 - Bur-0 (Δ47260) 117-1
..... -150 - Bur-0 (Δ47260) 117-36
..... -150 - Bur-0 (Δ47260) 85-7
..... -150 - 10499 (Δ47260) 95-14
..... -150 - 10499 (Δ47260) 98-7
..... -150 - 10499 (Δ47260) 105-12

VG-motif P-loop/Kin-1 RNBS-A
VSOAKLLDPTAWARLMDINVTGLGIYGRGGVGGKTTLLTKLRNKLVDAPGLVIFVVVGFEEVESIQDEIGKRLGLQ -225 - Col-0
..... -225 - Bur-0
..... -225 - Bur-0 (Δ47260) 117-1
..... -225 - Bur-0 (Δ47260) 117-36
..... -225 - Bur-0 (Δ47260) 85-7
..... -225 - 10499 (Δ47260) 95-14
..... -225 - 10499 (Δ47260) 98-7
..... -225 - 10499 (Δ47260) 105-12

WalkerB/Kin-2 RNBS-B
WRRETKERKAARILAVLKEKRFVLLLDGICRELDLEEIGVPPPSRDNGCKIVFTIQSLEACDESKWVDAKVEITC -300 - Col-0
..... -300 - Bur-0
..... -259 - Bur-0 (Δ47260) 117-1
..... -294 - Bur-0 (Δ47260) 117-36
..... -260 - Bur-0 (Δ47260) 85-7
..... -295 - 10499 (Δ47260) 95-14
..... -260 - 10499 (Δ47260) 98-7
..... -249 - 10499 (Δ47260) 105-12

RNBS-C GLPL
LSPEEAWDLFQETVGENTLRSHQDIPKLARVVASTCRGLPLALNLIIGEAMSGKRTVREWRYTIHVLASSTAEFPD -375 - Col-0
..... -375 - Bur-0

RNBS-D
MEDGTLPIKLSIYDNMSDEIIRLDFLYCALFENLDIGKEDLVNYYWICEGILAKEDREEAEIQGYEICDLVRMR -450 - Col-0
..... -375 - Bur-0

MHD LLR1
LLMESGNGNCVKVHGMVREMLWIASEHFVVVGGERIHQMLNVNDWRMIRRMSVTSTQIQNISDSPOCSLFTLL -525 - Col-0
..... -375 - Bur-0

LLR2 LLR3 LLR4
FRNRHLKVISGAFFQWMTGLVVLDSLFRNRELAELPEEVSSIVLLRFLNLSWTCIKGLPLGLKELKSLIHLDLDDY -600 - Col-0
..... -375 - Bur-0

LLR5 LLR6 LLR7
FSNLQEVVDVIASLNLQVLRFLFHSVSMDLKLMEDIQLKSLKELSLTVRGSSVLQRLLSIQRLASLIRRLHLET -675 - Col-0
..... -375 - Bur-0

LLR8 LLR9
TIVDGGILSLNAIFSLCELDLIGCNILEITIDWRCTIQREIIPQFQNI RTMTIHRCEYLRLDTWLLAPCLGELS -750 - Col-0
..... -375 - Bur-0

VSECPQMEEVISKDKAMAKLNTSEQPFQNLTKLVLDGLPKLESYWTPLPFPVLEYLVIRRCPELRRLPFNSES -825 - Col-0
..... -375 - Bur-0

TIGNQVETIIEEQVIKIVEWEDEATKQRFSHFNRRDFVQMAEDPKMDGLTSESHPICTIDLVTGTTGSGETATANN -900 - Col-0
..... -375 - Bur-0

AIG1-type Nucleotide Binding Domain
IQGKRVVQSGTHATVVTMECPYKVFPPDCPINNMIDTPGTNFLLCYT -948 - Col-0

```

**Supplementary Figure S7 | Full alignment of AT5G47260 protein sequences in CRISPR-Cas9 edited lines in Bur-0 and HIF 10499 backgrounds.**

**At5g47280-ADR1-L3**

P-loop/Kin-1 RNBS-A  
 MLFNLNDEARIIGISGMIGSCKTILAKELARDEEVRGHFANRVFLTVSQSPNLEELRSLIRDFLTGHGHEAGFGTA -75 - Col-0  
 ..... -75 - Bur-0  
 ..... -75 - Bur-0 (Δ47280) -21-20  
 ..... -75 - Bur-0 (Δ47280) -160-2  
 ..... -75 - Bur-0 (Δ47280) -163-16  
 ..... -75 - 10499 (Δ47280) -170-4  
 ..... -75 - 10499 (Δ47280) -172-9  
 ..... -75 - 10499 (Δ47280) -176-15

Walker B/Kin-2 RNBS-B RNBS-C  
 LPESVGHTRKLVILDVVRTRESLDQLMFNIPGTTTLVVSQSKLVDPRTTYDVELLNEHDATSLFCLSAFNQKSV -150 - Col-0  
 ..... -150 - Bur-0  
 ..... -150 - Bur-0 (Δ47280) -21-20  
 ..... -150 - Bur-0 (Δ47280) -160-2  
 ..... -150 - Bur-0 (Δ47280) -163-16  
 ..... -150 - 10499 (Δ47280) -170-4  
 ..... -150 - 10499 (Δ47280) -172-9  
 ..... -150 - 10499 (Δ47280) -176-15

GLPL ← 
GLPLSLKVL
GASLNDRPETYWAIA
VERLSRGE
PVDETHESKVFAQIEATLENLDPK
TKE
 →  
 SGFSKSLVKQVVGESKGLPLSLKVLGASLNDRPETYWAIAVERLSRGE PVDETHESKVFAQIEATLENLDPK TKE -225 - Col-0  
 ..... -225 - Bur-0  
 ..... -197 - Bur-0 (Δ47280) -21-20  
 SGFSKSLVKQVVGESKGLPLSLKVLG--TIALKHIGQL-SVERLSRGE PVDETHESKVFAQIEATLENLDPK TKE -222 - Bur-0 (Δ47280) -160-2  
 ..... -197 - Bur-0 (Δ47280) -163-16  
 ..... -178 - 10499 (Δ47280) -170-4  
 ..... -197 - 10499 (Δ47280) -172-9  
 SGFSKSLVKQVVGESKGLPLSLKVLGLDKHIGQLQWRGTOEVNLLMKLMRVKCLLKSQQL-STOP -210 - 10499 (Δ47280) -176-15

RNBS-D MHD  
 CFLDMGAFPEGKKIPVDVLIINMLVKIHDLEDAADFVLDVLANRNLLTLVKDPTFFVAMGTSYYDIFVTQHEVLRD -300 - Col-0  
 ..... -300 - Bur-0  
 ..... -297 - Bur-0 (Δ47280) -160-2

VALHLTNRGKVSRRDRLLMPKRETMPLSEWERSNDEPYNARVVSIHTGEMTEMDWFDMPKAEVLIVNFSSDNY -375 - Col-0  
 ..... -375 - Bur-0  
 ..... -372 - Bur-0 (Δ47280) -160-2

LLR1 LLR2 LLR3  
 VLPFFIAKMGM LRVFVIINNGTSPAHLHDFPIPTSLT NLRSLWLERVHVPELSSSMIPLKNLHKLYLIICKINNS -450 - Col-0  
 ..... -450 - Bur-0  
 ..... -447 - Bur-0 (Δ47280) -160-2

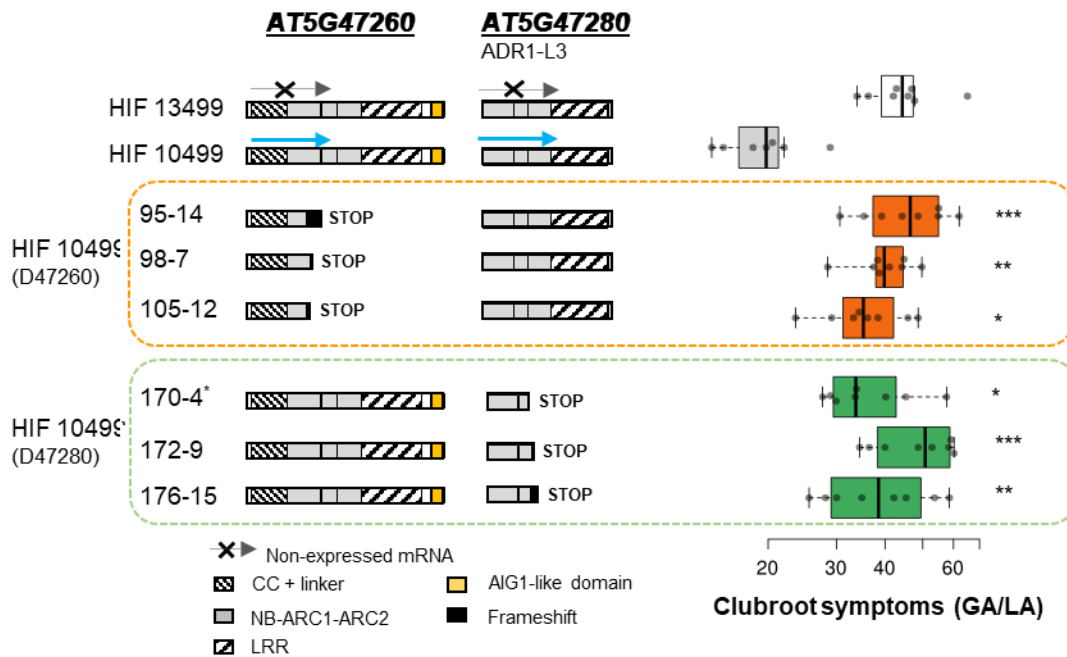
LLR4 LLR5 LLR6  
 FDQTAIDIAQIFPKLTDITIDYCDLAEPLSTICGITSLNSISITNCPNIKELPKNISKLQALQLLRLYACPELK -525 - Col-0  
 ..... -525 - Bur-0  
 ..... -522 - Bur-0 (Δ47280) -160-2

LLR7 LLR8 LLR9  
 SLPVEICELPRLVYVDISHCLSLSSLPEKIGNVRTLEKIDMREC SLSSIPSSAVSLTSLCYVTCYREALWMMWKEV -600 - Col-0  
 ..... -600 - Bur-0  
 ..... -597 - Bur-0 (Δ47280) -160-2

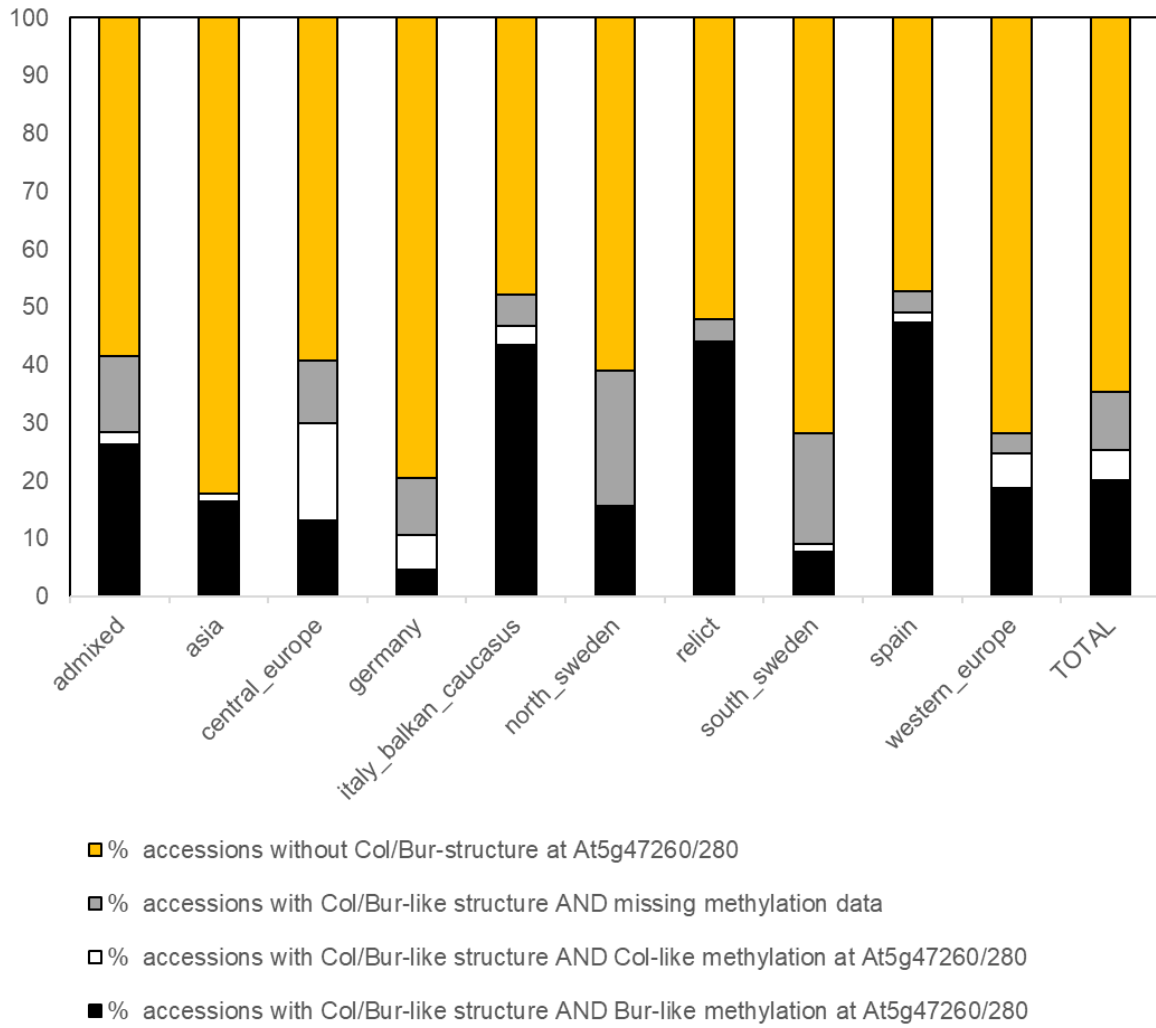
EKAVPGLRIEATEKWFNMTWPDE -623 - Col-0  
 ..... -623 - Bur-0  
 ..... -600 - Bur-0 (Δ47280) -160-2

**Supplementary Figure S8 | Full alignment of AT5G47280 protein sequences in CRISPR-Cas9 edited lines in Bur-0 and HIF 10499 backgrounds.**

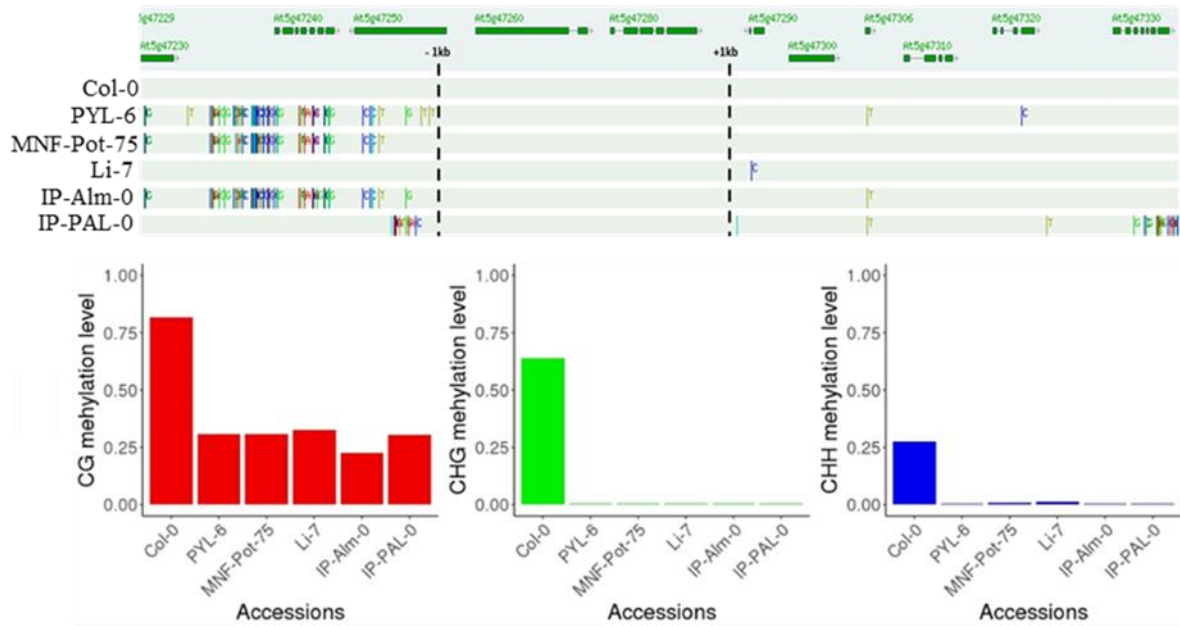




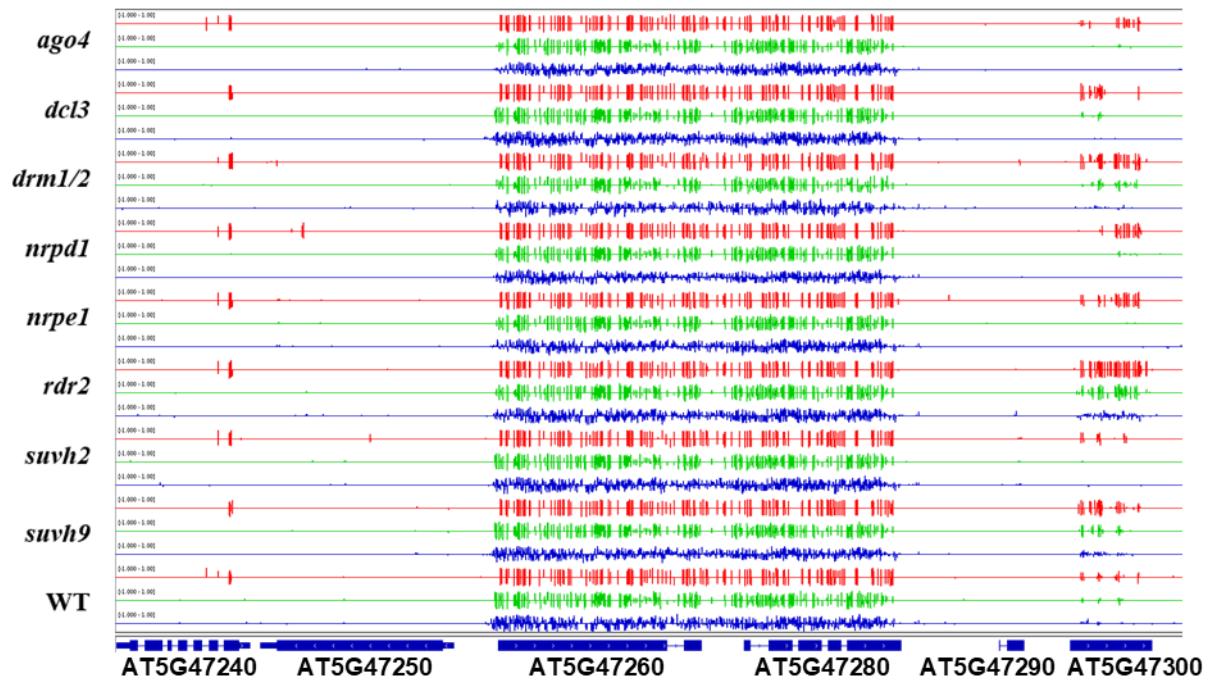
**Supplementary Figure S9 | Effect of *AT5G47260* or *AT5G47280* knock-out on GA/LA index (disease symptoms) in HIF 10499 background.** Cas9-mediated mutations were obtained in the HIF 10499 genetic background. For each targeted gene, three independent lines harbouring independent homozygous mutations were used. Line 170-4 no longer has the CRISPR-Cas9 cassette. For each line, the mean clubroot symptoms score (GA/LA) was obtained by modelling raw data of eight biological replicates (with 10 to 12 individual plants per replicate). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, data points are plotted as open circles. Edited line GA/LA values statistically different from 10499 GA/LA value are indicated by stars (from Dunnett 's test) with the following code: \*  $p$ -value <0.05; \*\*  $p$ -value <0.01; \*\*\*  $p$ -value <0.001.



**Supplementary Figure S10 | Proportion of structural and epigenetic variations on the locus *Pb-At5.2* among *Arabidopsis* accessions in each admixture group (details in Supplementary Data 2).**



**Supplementary Figure S11 | The epigenetic variation on *AT5G47260* and *AT5G47280* is not related to SNP variations on *AT5G47260*.** Sequence variant data were obtained from the signal SALK genome browser based on 1001 genome data. Average methylation level was calculated between 1 kb before the TSS site of *AT5G47260*, up to 1 kb after the TSE site of *AT5G47280*.



**Figure S12 | Methylation of *PbAt-5.2* region in Col-0 and in mutants in genes involved in RdDM methylation maintenance (Stroud et al., 2013). In red the methylation in CG context. In blue the methylation in CHG context; in green the methylation in CHH context. WT indicates the methylation profile of Col-0.**

## Supplementary Text S1: Details of *PbAt5.2* fine mapping

### Previous identification of *PbAt5.2*

A previous screen of *Arabidopsis thaliana* accessions found that Bur-0 is partially resistant to the *P. brassicae* eH isolate, whereas the canonical accession Col-0 is fully susceptible<sup>1</sup>. Two progenies were used to identify QTL controlling resistance to eH by linkage analysis: the first was derived from an initial Bur-0 (NASC accession N1028) x Col-0 (NASC accession N1092) cross followed by 6 generations of SSD (Recombinant Inbred Line set 20RV described in <sup>2</sup>). The second was derived from Col-0 x Bur-0 (described in <sup>3</sup>). These approaches led to the identification of a series of four additive QTL, including *PbAt5.2* ( $R^2=20\%$ , Bur-0 allele at this locus confers partial resistance). The QTL peak of *PbAt5.2* was at the marker C5\_19316 (around 19.3 Mb), with a confidence interval of 4.3 cM, between positions 18.7 Mb and 20.3 Mb (157 annotated genes between *At5g46260* and *At5g47690*).

### HIF499 for *PbAt5.2* validation

Among the RIL set 20RV from Simon et al. (2008), one RIL line (RIL499) displayed a single residual heterozygous region including markers c5\_17570, c5\_19313 and c5\_20318, framed by the homozygous loci c5\_14766 and c5\_21319. Heterogeneous Inbred Family (HIF) lines 10499 and 13499 were derived from RIL499 to obtain homozygosity at this locus (Institut Jean Pierre Bourgin, INRAE Versailles, France). These lines are near-isogenic, with identical combinations of Bur-0 and Col-0 homozygous genome sequences at every locus, except in the region between c5\_14766 and c5\_21319. The use of PCR-based markers (**Supplementary Data 1 Sheet 1**) allowed us to reduce this interval between markers CL5\_15283 and CL5\_20983 (excluded). HIF line 10499 displayed a higher level of partial resistance to eH isolate compared to 13499, thus confirming the position of *PbAt5.2* in this interval (<sup>4</sup>, see also the Figure 1 in the present work).

### Generation and phenotyping of F1 individuals derived from crosses between 10499 and 13499

Fine mapping of *PbAt5.2* started from the cross between HIF-13499 (allele Col-0) and HIF-10499 (allele Bur-0) lines. Crosses were made in both directions, *i.e.* using one or the other parent as female. Heterozygosity in the *PbAt5.2* region was checked in several F1 individuals using a series of PCR-based markers (**Supplementary Data 1 Sheet 1**). Clubroot index was evaluated in a series of F1 plants using the eH isolate, and was statistically identical to 13499, and higher than for 10499, thus suggesting that the Bur-0 resistant allele at *PbAt5.2* was recessive (**Supplementary Fig. 2**).

### Screening of recombinant individuals in the segregating F2 progeny

One validated F1 plant was chosen from each of the two crosses. Those two plants were self-pollinized and approximately 3200 F2 plants were sown (about 1600 from each cross). Individual F2 from number 1 to 1581 were from a 10499 x 13499 cross. Individual F2 plants from 1582 to 3153 were from a 13499 x 10499 cross. DNA was extracted from young leaves sampled from these 3152 plants, and then subjected to a first round of genotyping using a series of 10 KASPAR SNP markers (list in **Supplementary Data 1 Sheet 2**). Analyses were performed on the GENTYANE platform using a LightCycler 480 device (UMR INRAE 1095, Clermont-Ferrand France). Due to low DNA concentrations in some samples, about 6% of the

genotyping points were 'negative'. Good or average-quality (i.e. genotyping ambiguity at maximum one marker) data were obtained for 2751 F2 individuals. Among those, 563 plants displayed at least one recombination in the region between *At5g37660* and *At5g51670*, which represented about 20.5 % of the F2 plants, and was consistent with the distance of about 22 cM between those two marker genes (**Supplementary Data 1 Sheet 3**). This mean value of 20.5 however masked a clear disparity between genotypes derived from the 10499 x 13499 cross (32% of individuals with one recombination in the region) from the 13499 x 10499 cross (18% of individuals with one recombination in the chromosomal region). Nevertheless, most of this disequilibrium was focused in the region between the markers on *At5g42520* and *At5g44630* (i.e. outside the confidence interval of the clubroot resistance *PbAt5.2* QTL), and the male x female direction of the initial hybridization step did not affect the recombination rate in the region corresponding to the peak of the QTL (between *At5g46910*, *At5g47120* and *At5g47510*).

### **Phenotyping of recombinant F3 lines**

One hundred and seven recombinant F2 lines were selected based on the presence of a recombination event near the closest markers to the QTL peak (19.3 Mb). Using the seeds derived from the selfing of those individual lines, the clubroot symptoms were estimated from 18 inoculated F3 plants (6 plants x 3 biological replicates). Those phenotyping data confirmed the presence of a segregating resistance locus in the region (**Supplementary Data 1 Sheet 4**). Lines with Bur or Col homozygous alleles at markers *At5g47120/At5g47510* displayed a mean GA/LA (clubroot symptoms) of 37.1 (SE=7.3) and 87.2 (SE=14.1), respectively. Lines with heterozygosity at those two markers displayed a mean GA/LA of 76.7 (SE=16), which was consistent with the above conclusion that the resistance Bur-0 allele was apparently recessive.

### **High-density genotyping of recombinant F2/F3 lines**

A subset of 69 F2 recombinant lines was selected, based on the presence of a recombination event near the closest markers to the QTL peak (19.3 Mb). For each of these, 12 to 18 F3 progeny individuals were grown and their leaves were bulk-sampled, for subsequent analysis of 93 SNP (**Supplementary Table 1 Sheet 5**). All leaf samples were analyzed at the GENTYANE platform. SNP genotyping was performed with the KASPAR genotyping chemistry and Dynamic Array™ IFC 96\*96 (UMR INRAE 1095, Clermont-Ferrand France). Genotyping data obtained from bulked leaves of F3 individuals represents the genotypes of the parental F2 individuals. This genotyping workflow was also applied to a series of non-recombinant F2 lines and parental HIF 10499 and 13499 lines, which were used as controls. The comparison of the 93 SNP genotyping data and clubroot GA/LA index for all those 69 lines (**Supplementary Table 1 Sheet 6**) finally led to the identification a small region between the markers K58=*At5g47230*prom (position 19,175,831 bp) and K65=*At5g47360* (position 19,214,446 bp). Due to the recessive status of the resistance allele, some recombinant lines with possibly interesting recombination events (especially in the line 1381) were not useful at this stage.

### **F4 lines with fixed alleles in the region of the resistance locus allowed further reduction of the *PbAt5.2* resistance locus interval**

DNA was extracted from 12 to 18 individual F3 plants derived from selfing a series of F2 recombinant lines. Among them, F3 lines with homozygosity in the region of the resistance locus were screened using the following PCR-based markers: CL5\_16921=*At5g42320*;

CL5\_17802=*At5g44200*; CL5\_18135=*At5g44900*; AF-NUD8; CL5\_19601=*At5g48375* (details in **Supplementary Data 1 Sheet 7**). Homozygous F4 seed stocks were then obtained from the selfing of the selected homozygous F3 lines, and thereafter used for additional clubroot phenotyping assays. From the resulting data the interval could be reduced to a region between markers K58=*At5g47230*prom (position 19,175,831 bp) and K64 (position 19,208,823 bp), as shown in the **Figure 1e** (detailed phenotyping data also in **Supplementary Data 1 Sheet 8**). Finally, every other SNP and indel in the region was analyzed by sequencing the PCR-amplified fragments from 2313-15, 1381-2, 2509-11, and 1600-5 (details of primers are given in the **Supplementary Data 1 Sheet 1**). This allowed a final confidence interval of 26 kb between the marker CLG4 (19,182,401, in the promoter region of *At5g47240*), and the marker K64 (on SNP at position 19,208,823 bp, in *At5g47330*) to be identified.

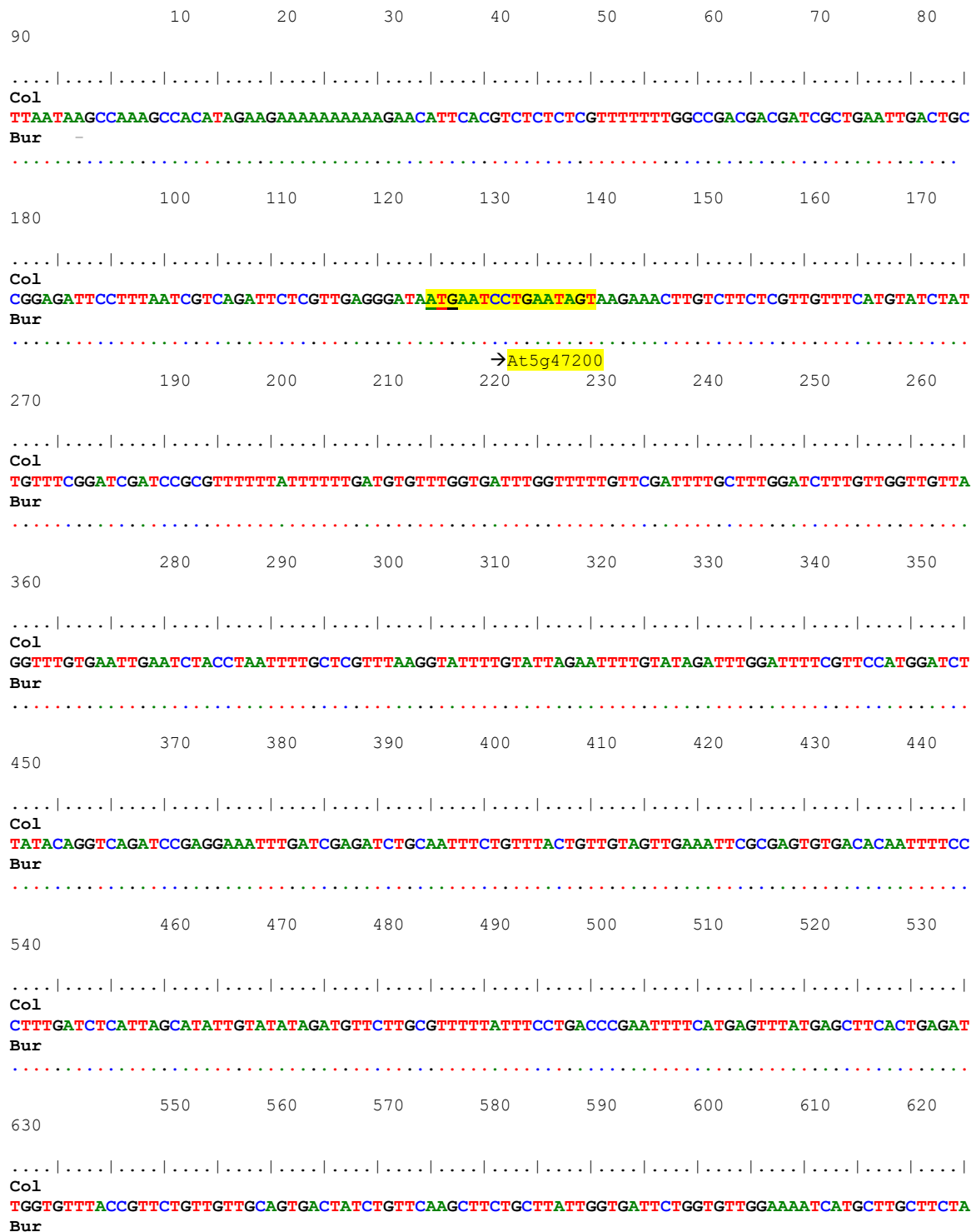
## **Supplementary Text S2: Influence of *PbAt5.2* on transcriptomic responses to clubroot infection**

The analysis of transcriptome responses to isolate eH in 10499 and 13499 highlighted a series of 61 genes that were induced by infection only (or with higher range) in the presence of the resistance allele *PbAt5.2<sub>BUR</sub>* (**Supplementary Figure S5**). This series was enriched in genes associated with innate immunity, systemic acquired resistance, and notably included the SA-responsive gene *PR2*, and the set of genes *CYP71B15*, *CYP71A12/CYP71A13* involved in camalexin biosynthesis, confirming our previous studies on the cellular functions involved in clubroot resistance QTL *PbAt5.2*<sup>5,6</sup>. In contrast, a series of 58 genes was found to be induced by infection specifically (or with higher range) in the presence of the susceptibility allele *PbAt5.2<sub>COL</sub>*. This set of genes included *ARGAH2*, a JA-regulated arginase encoding a protein involved in the biosynthesis of N-delta-acetylornithine, previously shown to play a role in basal resistance toward the eH isolate in genotypes harbouring the susceptible allele Col-0 on QTL *PbAt5.2*<sup>6,7</sup>. This list also included the trehalase encoding gene *TRE1*, involved in resistance to massive amounts of trehalose synthesized by *P. brassicae* during clubroot infection<sup>8,9</sup>.



## Supplementary Text S3: Aligned genomic sequences of Bur-0 and Col-at the *PbAt5.2* locus

Position of sequence polymorphisms, genetic markers and primers used in this study for the fine mapping in this region. The 26 kb region identified by fine mapping is between the marker CLG4 (sequenced using the two primers clonage\_4for ATGTGTTTTGCGTTCGACCTC and clonage\_4rev AGCTCTCCGTGTCTACGACT) and the SNP marker K64. Additional details about primers and markers are given in Supplementary Data 1 SHEET1.



```

.....
720          640      650      660      670      680      690      700      710
Col
AGATTTGCTGTAAGTATCCACAAATTCGGATTCAATCTCTGGTAGTTACTTTTAAACATTGTGTACACACAAAAATTTGAGGATCA
Bur
.....
Snp_K53_At5g47200
.....
810          730      740      750      760      770      780      790      800
Col
CATCTTGGAGTTC AATACCTGTTGCTCAAGACTCAAAACTCAAAGTCATTACTCCATTGGATTAGCTTAGTTCTCACTATGGTATCATT
Bur
.....
900          820      830      840      850      860      870      880      890
Col
GTTACTCCTTTGTGTTCTTATTTCTTGATATCTTGAATTTTATGTGGACAGGATGATTCCTTACCTGGATAGCTACATAAGCACCATTGGT
Bur
.....
990          910      920      930      940      950      960      970      980
Col
GTTGACTTTGTAAGCACCTTCATTTGCTCATCACTCAATTTATATACAGGAATCAGAATAATAAGTGTAACTTTACTAATGATATCATG
Bur
.....
1080         1000     1010     1020     1030     1040     1050     1060     1070
Col
CAGAAAAATTCGCACAGTTGAGCAGGACGGAAGACCATCAAACCTCCAGATCGTAAGTGTCTTCAGCTAGATATGCAATCATAAATCTGTT
Bur
.....
1170         1090     1100     1110     1120     1130     1140     1150     1160
Col
AAAAATTTTGGAAAGAGCAGATAGTTACTCTTGTGTTTGGTAATCGCCTGTGTATACAGTGGGACACAGCAGGCCAAGAACGTTTCAGGACA
Bur
.....
1260         1180     1190     1200     1210     1220     1230     1240     1250
Col
ATCACTAGCAGCTACTACAGAGGAGCTCATGGGATCATTGTATGTACTCTTACTCTAACCAACCAATCATCTTCTTGTAAATAACACAT
Bur
.....
1350         1270     1280     1290     1300     1310     1320     1330     1340
Col
CCTATACTCTTGCTCACAATTGCCTATCTTTGCAGGTCACCTTATGATGTACAGACCTAGAGAGCTTCAACAACGTCAAAACAATGGCTG
Bur
.....
1440         1360     1370     1380     1390     1400     1410     1420     1430

```





2890 2900 2910 2920 2930 2940 2950 2960  
2970

Col  
ATATGACAAAACCATATCAGTTCAAATGTTAGCAAAACATTCTACTTCAAACCTAACTAAACCTCAAAGATGTTTCAAGAGCTGTAAT  
Bur  
.....

2980 2990 3000 3010 3020 3030 3040 3050  
3060

Col  
GATTGTACCCTGGCTTCAGCTTCTCAGCTTCTTCTGTGCTTCTCTCCGCAGTGAGTCTTCTTTGCTTCAGGGTTTCATCCTCA  
Bur  
.....

3070 3080 3090 3100 3110 3120 3130 3140  
3150

Col  
CCTCCTTGCTTCTCAGCAACAGGGCTTCTCCTAACCTCTGTGGTAGGTTCTCAGACGTTCTAATAAAGAACAACAAAAGTTTATAAAC  
Bur  
.....

3160 3170 3180 3190 3200 3210 3220 3230  
3240

Col  
AACCTAAAGTTAAAGTCATATGGATAATTAAGTGAAGACAAGGATGCTTACGGAGGGATATCATCTTCAGTAGTGCCCCAGTTTCCACG  
Bur  
.....

3250 3260 3270 3280 3290 3300 3310 3320  
3330

Col  
ACCTCCACCATTACGTTTCATGCCAGTACTATCAAACATCAAGTTAACATAAATGAATCAAAACAGAAGAAGTCAAACAAAGTAGTAAC  
Bur  
.....

3340 3350 3360 3370 3380 3390 3400 3410  
3420

Col  
TAAAGTAGTGGACAGAACAAAAAGAAAGCTTACCCATGACCTGTCTGCTATGGCGTCAATAATCTCGGTGGGCGTTCAACATCACCA  
Bur  
.....

3430 3440 3450 3460 3470 3480 3490 3500  
3510

Col  
GATTTCCCGTTGGCAACTCCACCACGGCGAGGTCTTTCACGACCACCGCCAACACGGTATCCACCAACAGACCCACACGGCTTGCTCCA  
Bur  
.....

3520 3530 3540 3550 3560 3570 3580 3590  
3600

Col  
TCAGCATCTTCAGAAGGTCTCCTGTATCCTCCAGAAAATCCATTCTCATTTCAGGAGCATCATTGTTCTGTTCCCGTTGTATCCA  
Bur  
.....

3610 3620 3630 3640 3650 3660 3670 3680  
3690

Col

```

CCATTACCCCTGCCACGGGAAAATCCACCACGTCCACCAGTCCCACCACGACCTCCTTGAGGAGCATTCTCGACTCCCTCACTACATTC
Bur
.....
3780          3700      3710      3720      3730      3740      3750      3760      3770
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ATAACAAAAGCTTTCATATATATACTTATCCTAATCTAACAAAGTATTAAGCTTACCCTAATCTAACAAAGCAGTAGAGAAACAAA
Bur
.....
3870          3790      3800      3810      3820      3830      3840      3850      3860
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TAAGATACGGAAGAAACTAACAAAGCAGCAAATGAAAAAGCACACACTTTGACTATGGATCTTTCATGACAACCATAAACAAACTTCA
Bur
.....
3960          3880      3890      3900      3910      3920      3930      3940      3950
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CTAGAACTAATCAATCAAACTAGACAAAACACAGCTGAATCTAAATCTGTTAAGTATCAACATAAAGGAAAGAACTTTTACCTGCTTG
Bur
.....
4050          3970      3980      3990      4000      4010      4020      4030      4040
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AGAAGGAGGAGCCGGCTTGGTTGGGAAGTTAGCGGCCTTAGGAGGCTGAACAGCAGCAGCAGCTTTCCTCGACTTTCCTGAGACAAAGCCAC
Bur
.....
4140          4060      4070      4080      4090      4100      4110      4120      4130
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AGCGAGCTGGCTTGGATCCTCAGCATCATCTCCTAGAAGATCGAAAGGTTCAAAGAGCCCATCACCAGTCTGGTAAGATCGAGTTAGGT
Bur
.....
                                     Start At5g47210←
4230          4150      4160      4170      4180      4190      4200      4210      4220
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ACAAGTCAAGGAGAAATTGGTTACTTTGATGGGTTATAGAGAGGGAAATCAGTAAATTCATGGCGATATACAGAATCAGAAAAAGAG
Bur
.....
4320          4240      4250      4260      4270      4280      4290      4300      4310
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GTACTTGAAGAGAGATTATTCGGACAGAGCTGTTTGTCTTAAGAGATAGCGAAACAAGAACCCTAAGAAAAGATCGGCAGTGAGAGAG
Bur
.....
4410          4330      4340      4350      4360      4370      4380      4390      4400
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AGAGGTGCGACTTAAAAACCTAATCTATAAACCTTCCCTGAGTTATTATTCTCTCGGCGGGTGCTATTTAACGTGTTCCTGGTCTCTC

```



5220

Col  
ACCAATAACTCATCAACACGTGCTCTCATCACCAACCTCACACTTCACCTGCACCACCTCGACGTCAGATTCTCTGCTTTTCTCCFTTCGT  
Bur

5230 5240 5250 5260 5270 5280 5290 5300

5310

Col  
TTC AAC TTCCCGTTTTCAGACGACGACGTAGAAGAGGACGACGACGACGACGAAGAAGATCTCTTAGACGTGATCCGAACCGGGTCA  
Bur

A

Snp\_K55

5320 5330 5340 5350 5360 5370 5380 5390

5400

Col  
GGTTCACCGGAATTAACCCCTCAACGGAATAATCAATAAGCGGGAAACCACGCATCCATAAAGCAGCTATATCGTAAGCTAAAGCCGCA  
Bur

A

Snp\_K56

5410 5420 5430 5440 5450 5460 5470 5480

5490

Col  
TCTTCGCGCGTCTCAAACGTCCCTAACCAACCCCTAGCTCCATTCTTCGCGGATCACGTATCTCCGCGCGAATTTCCCCACGGTCTC  
Bur

5500 5510 5520 5530 5540 5550 5560 5570

5580

Col  
TGCCCTCACTCCTCTGTAATGCTTCGCCCTTCACTGCCGTCTCCGTAAACGGTATCGCTTCTTTGGTTTCTCCTCCATCGCGTAAAGTTC  
Bur

5590 5600 5610 5620 5630 5640 5650 5660

5670

Col  
TCAGTTGGCTCGACTTTAACCGCCGAAAAATCAAAAAGACAGCTCAAGTCCGATGATGACGTGTCAAAATGGAAGGCATCTTTGAGGAGT  
Bur

5680 5690 5700 5710 5720 5730 5740 5750

5760

Col  
CCGTACCAACATGTCCTCTGAATCATTTCTTTCAATGGCAAACCTCCCAACTCTCTGTGAAACACGAACCTCGGTGTTGACTCATTC  
Bur

T

Snp\_K57

5770 5780 5790 5800 5810 5820 5830 5840

5850

Col  
AGTCGCAGCTCGTCTCTCCTCCTCCTAGCAAGTGACGTGTTATCGACTCCAACAAAGCGTAGTCGGATTCTATATTGCACTGTCCG  
Bur

5860 5870 5880 5890 5900 5910 5920 5930

5940

Col  
TACATTTTCAGAGAACTAACTATAGAGTTTTTTTTGTTTTGTTGATTGATTTATCAAAATATCTGAACTTTTTCTATGTGGAAGGTGGT





Col  
ACAAGATTTCATGATTAAAGAAATTCATTAGTGCCACTTTAGTCAATAAATATGGAGTAGTGAATCATAGTGATAAAGTTTCATGCAA  
Bur

6840                     6760             6770             6780             6790             6800             6810             6820             6830

Col  
GCTCAATTACAAACCAGTTTAAATCCATATTTGTTGCATTTCTTTCTTTCTTTTGAATAAATACAGCCTTAGATCTTTTGATC  
Bur

.....T.....C.....G.....  
6930                     6850             6860             6870             6880             6890             6900             6910             6920

Col  
ACCAATGATTTGGAATTTTGTGGATAATTATTGAATTTCCATCTTTCTATTGGCTAAAAGTCAATAACAACAATAAGGCTCAAAGACA  
Bur

7020                     6940             6950             6960             6970             6980             6990             7000             7010

Col  
AATTAATGGTGGGTCTCTCACGTATGTCACCTTGCAAAGGGACCTCCTTCACGGAAGCATAGTTTAGTCAATAGTCACTCAAACCTCTC  
Bur

.....G.....  
7110                     7030             7040             7050             7060             7070             7080             7090             7100

Col  
AAAAATTAGTTTACTATATATTTTAACTATAATTCGATTCCAAGAGTAGTTTTGGTTCAAACACATCATTCTGGTTCAGATGGTTATTT  
Bur

7200                     7120             7130             7140             7150             7160             7170             7180             7190

Col  
AATTTTGCCTGTTTTGAGTGTATATATGATGTTATGTTGTAAACAAATGAACTTTTAAATTGATTACAAGAACATGAATAGCTCTAAAT  
Bur

.....A.....  
7290                     7210             7220             7230             7240             7250             7260             7270             7280

Col  
ATGATTAACCAACGAATTTATTTATTTCTTTGGTAAAAGAAATAAGATAATATACTTATGGATCCAATCAGGTAAAGTTCGGTTTTGCAAT  
Bur

.....A.....  
7380                     7300             7310             7320             7330             7340             7350             7360             7370

Col  
TAAATACTTTGATTAAATTAATAATGAAAGTAGTAGTTGAACTTTGTTTTATGTTTGAACCTCCGAGCTAATCCTCAAATTATCTTTT  
Bur

7470                     7390             7400             7410             7420             7430             7440             7450             7460

Col  
GGACGTTTTACATACATCCACTTTTGTGTATCTTAGGAAATATATTGTTTTCATATTGTTCTTTGTTCTTTATGATTTGGGTTTATATA  
Bur

.....  
7560            7480            7490            7500            7510            7520            7530            7540            7550  
.....  
Col  
A T T T T C A A A T G T C A T G A T G A T C A T C A T T T A A T C T T A G T T G T T T T A G T C A C A T C T T A T T A T G C T T A T T A T T A G T C G G T G A T A G T T T A A T T T  
Bur  
.....  
7650            7570            7580            7590            7600            7610            7620            7630            7640  
.....  
Col  
T A A G A C G T A A A T C A T C T A T T C T C A T A T T A T G C T A G A A C A A A C T T T T T C T T T G T G C A A C C T C C T A G A A C A T A T A G T C G C C T A T T A T C C A T G  
Bur  
.....  
7740            7660            7670            7680            7690            7700            7710            7720            7730  
.....  
Col  
G A T C C C A G A T A C T C C T C C A A G A C C A C C G A A A G G T T T A A T T A T G G A T A G G A A C C C T T T G G T C T A C G T T A A C A C C T T G A T T G A C T T C C A G A C  
Bur  
..... G .....

7830            7750            7760            7770            7780            7790            7800            7810            7820  
.....  
Col  
G A A G A G G T G G A A A G T A G A T C G T C T A C G G A G T T G T T C C C C C C C C C C C C C C C C G A G G A T A T T A C T T T G A T T T T A G G G A T A A A A C C G A G G C  
Bur  
.....

7920            7840            7850            7860            7870            7880            7890            7900            7910  
.....  
Col  
T A A A T G T C T C G C G A G A T G G A T A T A G T T G G A C A T T G A C T A A G T T C G G T A A T T A T A C T G T C A A G A C A A G A T A T G A A G C T G C G A G A G C C C T C T  
Bur  
.....

8010            7930            7940            7950            7960            7970            7980            7990            8000  
.....  
Col  
C T C G C C C G T C T T G C G A C C A C C C T C T T C A G G G A C C T A G T G T T A C G G C A C T A A A G G C G C A A G C G T G G A A A T T A A A A C T A C A C G A A A G C T A A  
Bur  
.....

8100            8020            8030            8040            8050            8060            8070            8080            8090  
.....  
Col  
A G C A T T T T G T G T A G C A A T G T G T C A G A G T G T T T A G C A A C T T G T C A A C G C C T A T A T T T T C G C C A T A T T G G T A G A G A T A A A A A A T G T C C T A  
Bur  
.....

8190            8110            8120            8130            8140            8150            8160            8170            8180  
.....  
Col  
G A T G T G G G G C G G A T G A A G A A C C A T C A A T C A T T T A A T A T T T G A A T G T C C C C C G G C A A G A C A A G T C T G G G C C T A T C C G G T A T C C C T C C T  
Bur  
.....

8280            8200            8210            8220            8230            8240            8250            8260            8270  
.....

Col C T C C A T C T A G G T T T C T T T C G T C T T C T A T A T A C A A T A A T C T C G A T T A T C T G T A T T G G A G A G C G A A T G A G A T T G G A G C T T C T G A G G A G A G C T  
Bur .....  
8290 8300 8310 8320 8330 8340 8350 8360  
Col T A C G G G T C T T T C C A T G G A T A A T G T G G T A T A T T T G G A A G C G C G A A C C G A A A A A A T T T C G A A G T A T T T G C G T G C A A C C T C A A G A C A C T T  
Bur .....  
8380 8390 8400 8410 8420 8430 8440 8450  
Col T A G A C T T A G C A A T A C A T G A G G A A G A G T A T G G A G G C G A G C C A A T A G G A G A G A A G C A A C C A G A A G G T A C C A A G C C A A G T T T G G A A G G C  
Bur .....  
8470 8480 8490 8500 8510 8520 8530 8540  
Col A A C A T A T A G A T A T G G C T T C C C C A A T C T G C T T C A T T G A T G G G T C T T G G C A T A T A A C T G A T T C G C G G A G C G G T C A T G G G T G G A T T T T G A C C C  
Bur .....  
8560 8570 8580 8590 8600 8610 8620 8630  
Col G T G G G A A A G A T T G C T T C A T T T A G G A T T G A A G G G T T C A C G T C G T T G T T T A T C A C C G C T T C A T G C A G A A C T A G A C A C A T T A G T T T G G G C T T  
Bur .....  
8650 8660 8670 8680 8690 8700 8710 8720  
Col T A A A G T G C T T A G T A G A C T T A C A A T C A A G G A A G T C C T T G T T A A G A C G G A T T G C T C T G A T C T T C A C T A T G G T T A A T A C C C G G A G G A G T  
Bur .....  
8740 8750 8760 8770 8780 8790 8800 8810  
Col G G C C C A T T T T T G C A T C A G A G T T A A A A G A T T T C G A G T A T T T T A A G A A T C A A C T T G T A T C T T T T A A T A T T A T G C A T G T T C C C C G T A C T A G T A  
Bur .....  
8830 8840 8850 8860 8870 8880 8890 8900  
Col A T A T C C G A G A G A T T A T C T T G C G A A A T G T G C A A G A A C T C G C G G A T T C T A T T T T T C C C A T G T A A G T T C A A C G G T T C T C G A T T G G C T C T C T T  
Bur .....  
8920 8930 8940 8950 8960 8970 8980 8990  
Col T A A A C C G A G A G C G C T T A T C C A T A G A A T A A T A T A T A G A G T T T T A A C C C G A A A A A A A A A A T A C T C G C C T A T A G T T T G C T T T A A A A A A A A A G T T  
Bur .....



Col  
CAGTTTAGTGTAATTTGTTAACTTTAAATCGATTCCTAATTCATTTTAAATCAACACATCTATATGTAGGCAATTGGATGCAAAGGTACAC  
Bur

9900 9820 9830 9840 9850 9860 9870 9880 9890

Col  
AAAACAAATGCAAAGGAAGGATCAAAGGAAAGATGTTATATGACACGTCAGCAGTATTCAAAGTTTGAAGTTGAACAAATCTAGTCATG  
Bur

9990 9910 9920 9930 9940 9950 9960 9970 9980

Col  
TTTGACTTTGACCCTGACTTTTCAAACCTATCTTTTATTAGTAGCAAACCTCAGTTCCATACGTGCAAACAACCTCACACGTGCGTTCCC  
Bur

10080 10000 10010 10020 10030 10040 10050 10060 10070

Col  
ACCGCAATGTTTGAGGTTTCTTCAACAGAAGACGATATTTCTTATTATTATAACGTTAGATTGAGAATTCAAAGATTCTCGACAAATGA  
Bur

10170 10090 10100 10110 10120 10130 10140 10150 10160

Col  
ATGGTAACTTTTTCTATAAGAAATCACAAATAAGTTTATAGTTATACAAATTTATAAATGTTGGAGTAATTGTCACATAGATGATTGGTT  
Bur

10260 10180 10190 10200 10210 10220 10230 10240 10250

Col  
GGTGATAATAGTTGTTTATGTATGATTGATCAATTAACCTTAATAATTTCCATTGGAATAATATTTCTTGACCTTAAATTTGGAATGT  
Bur

10350 10270 10280 10290 10300 10310 10320 10330 10340

Col  
AGATTTATATGGAAAAGTAGATAATCATTTTTGTGACGCTAATTAATAATGTTGTCCCATAACGACAGAAAAAAAAAATGAAACAAA  
Bur

10440 10360 10370 10380 10390 10400 10410 10420 10430

Col  
ATAGAGACCAGATTGGTTCAAGAAAACGACACACAGTTTAGGATGCTAAAAAGCTTTGTTAGTACCATATGATTGTTATATTGTTATT  
Bur

10530 10450 10460 10470 10480 10490 10500 10510 10520

Col  
GGTCCCTAATAATGTAATTTAGACGTCAAAAATGTTTTTGTATTGATATGTGACCTCATAACTGGTTAGCTGCTTAGGGCCATTAGATT  
Bur

10620            10540          10550          10560          10570          10580          10590          10600          10610

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**AGAGTTTGGATTGTTTCAAATGTCAAATCTTACAGTCCGTCAAATTTTATTTGATGTGAAGTAAGTGTGATAAATGGGACTACTTTC**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

10710            10630          10640          10650          10660          10670          10680          10690          10700

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**TCCAGTTGGCTAGGGTCGACTGCTAATAACATTCTCCAAAGGAATTAATCTCAACGGCTTGTTGCTCAGGCAACAATTTATAGGAATAG**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

10800            10720          10730          10740          10750          10760          10770          10780          10790

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**AGATTGAACGAAATAATTACAGATTACACAACAATGTTCCCTCTACTTTAAGCAAGCAATCTTCAAGGCTATATTGATCGACTGATTCG**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

10890            10810          10820          10830          10840          10850          10860          10870          10880

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**AGACGCAATCGTCTTGTCAAGAAGAACCACAAAACATGATGATATGTAATGCAAACTTGACTCGTCTTTGAGTAACACTTCTTCATCT**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

10980            10900          10910          10920          10930          10940          10950          10960          10970

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**AGTCCAAATCGTCGCAGGGTTTCTTTTAAATTATTATTATTATTATTATTCTTCTCTTTTGGTAAGAAAGAGCTTCTTTAATTTGGTTAAACTTTT**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

11070            10990          11000          11010          11020          11030          11040          11050          11060

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**TTTTTTTGGTTAAGTACAAAACCTCTCATTCCTTAATAAAATGATATCTATATTATAAAATATCAAATTTGTAAAACAAAAATAATTA**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

11160            11080          11090          11100          11110          11120          11130          11140          11150

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**AGAGTAGTTTTCACAACAACAACAATAAAAAAGACTACTATATAAAATAAAAAAGACTACTTGGCTTCTAGAAAATTAATAAATAA**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

11250            11170          11180          11190          11200          11210          11220          11230          11240

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

**TTTTTATATTAAATCTCTGATTTGCTATATCATTATTAAAGTGTGAACTTTATTTTCTTTCATTGTAAACAAGTAAACAACATGACTTTC**

Bur

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

11340            11260          11270          11280          11290          11300          11310          11320          11330

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Col

```

TAGAATCTAGTGAGTGAGTCGTTGATTCATTCTACAAACCAGTTAATGCACCGGTAAATCTTTTCACGTGCATGGTGCAGTGCATGAA
Bur
.....
11430      11350      11360      11370      11380      11390      11400      11410      11420
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GATGGATCAAATTAATACGAAACCAACATAAATTCAAATAGTATATCATCTTTTAAAAATTTGTATGATTAATAATCTCTGTCAATAAAG
Bur
.....
11520      11440      11450      11460      11470      11480      11490      11500      11510
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GCCGCATGCATTGACTTGACTCTTTCAGTCTTGCTTGTCTTACTAATTAAACCTCCATAATGCATCAACCTAATCATAACCGAAAAATT
Bur
.....
11610      11530      11540      11550      11560      11570      11580      11590      11600
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TCTGAGTTGTATTGGTTAGACTTAAATGCTTTACTATCATTTTAGTTACGTTTGTGTTTACCFCGCAAAAAATCTTCTAGAAGGATAAT
Bur
.....
11700      11620      11630      11640      11650      11660      11670      11680      11690
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ATAATACTACAATACAATGTTGGCATTATCCATTACTGAGCGGTGTGAAATTTGGTTTTGATTGTTTAAACATATGAGTTAAAAATTTGTTCC
Bur
.....
11790      11710      11720      11730      11740      11750      11760      11770      11780
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CAATATTGGCAAAATAGCATCGGATATGCTATTGTATAAGAAAACGCTTATTTTTGGAGTGGCGTCTACCTGTAACTGTATAAACTACG
Bur
.....
11880      11800      11810      11820      11830      11840      11850      11860      11870
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TAACGAACTTTGAACGCAGAGTGAATGTGAGTCTCTGTGACACGACTTAAAGCTTAATCAGAAGCAGATTATTGGACCTTATGGAGACT
Bur
.....
11970      11890      11900      11910      11920      11930      11940      11950      11960
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TTATCAAGATTAGCTAATGAAGGTTACTATATATGAAACTTTGAAGTAAATGTTTGCAGCCTTTGGGATCAATAAACCATAAAAGCATT
Bur
.....
12060      11980      11990      12000      12010      12020      12030      12040      12050
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GGTTTTGTTTTCTATCTATATCCAATTCGGAAAGTAGAAAACCTGGATTAGTAAAAATCATATTCAACATTGCGAAGAAAAACTATG
Bur
.....
12070      12080      12090      12100      12110      12120      12130      12140
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GGTTTTGTTTTCTATCTATATCCAATTCGGAAAGTAGAAAACCTGGATTAGTAAAAATCATATTCAACATTGCGAAGAAAAACTATG
Bur
.....

```



12150

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
TGC~~CGCGGATA~~~~CGTTGGGAAAA~~CTGCGCATGGTTTAAAGTTTGGCTTTCAGTCAATTATAAATTCGTTTTTTATACTCCCTCTGTCCA  
Bur

12240          12160          12170          12180          12190          12200          12210          12220          12230

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
AGATAC~~TTGATA~~~~TTTTGGG~~~~TTTTGCAC~~AAGAA~~TTAAG~~AAAGTAAC~~TTTTATA~~~~TTTTTA~~ATTATTCTTTAGTTAGTTTAAATAATTA  
Bur

12330          12250          12260          12270          12280          12290          12300          12310          12320

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
A~~TTTTACTTCTC~~~~CATTT~~~~CATTAT~~~~TGGTT~~CAAA~~CAAAAA~~TAA~~TAA~~TGATAG~~TTTTT~~CAAAACATCAATTTGGTGGAA~~CAAA~~TAAAAA  
Bur

12420          12340          12350          12360          12370          12380          12390          12400          12410

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
A~~CTCAAAA~~~~TATCAAA~~~~TACTT~~GAACGAGGGAGTAG~~TAA~~TTAAAAAAGATA~~TTT~~CACACTTTGACTTGGCGAAGCCTCATAACAATG  
Bur

12510          12430          12440          12450          12460          12470          12480          12490          12500

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
AAGTTA~~TGTAT~~GAAC~~TATATAT~~GAA~~TTAG~~AAACAATGGAAACAGCTTGTAAATATTCATTTGTTGATATATGTTTTTTGGG~~CAAT~~  
Bur

12600          12520          12530          12540          12550          12560          12570          12580          12590

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
TGGTGCATGAACAAAAATAAAA~~CGTAGAT~~GAAAA~~CCGGATA~~TTTTGGTGTAA~~CA~~TTTGCATTTGAAC~~TT~~CGTGAAGAC~~CGGATA~~AAAG  
Bur

12690          12610          12620          12630          12640          12650          12660          12670          12680

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
CTCA~~TTTTTT~~GTTC~~TTTATT~~ATATGG~~CTGCTAT~~TAGTACAGAGTTGAACTTTAGA~~A~~ACTAAAACTCGACATCTTTTATTTTATTT  
Bur

12780          12700          12710          12720          12730          12740          12750          12760          12770

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
TGTCAAGCATCGACATCTTT~~CTGTTCAAGAAAACGACCGCA~~ATAGTCGAATAATATAA~~CTTTGGACTAGTT~~AATATATATTTGCGATA  
Bur

12870          12790          12800          12810          12820          12830          12840          12850          12860

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
Col  
GA~~TTTT~~CGATCTCAGTTATATCTTATAACCAAGAGACAAAA~~CA~~AATATTGCAGTCAAGTACAAAA~~CG~~AAAA~~CAAT~~CACAATGTCGACTAT

CLG1: Clonage 1F

Bur

.....

12960               12880       12890       12900       12910       12920       12930       12940       12950

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
AGATGAGTCGGTCATTTCGATCCAACGGCTCTGAGTCCACGAAACCGCAACCAAGTGGTGCTCTCTTTTACACCAAAATCATATTATAAAA  
Bur

.....T.....

13050               12970       12980       12990               CLG2: Clonage 2E               13010       13020       13030       13040

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
AACTTAAAGAAAGAGAGGATGGTTTCGTTGGCTCCTTCCTTGTTCCTTAATTAATTCAAAATATATTTCATCACCTCCATTGAATAAGTCCA  
Bur

.....

13140               13060       13070       13080       13090       13100       13110       13120       13130

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
TTTCACGACAAAGTCACCAATGCTTCTTTTACATGTATATATACTTCCTTCCACTCCTCCTTCTACTCAAATCAAATCTTCTTCCTTC  
Bur

.....

13230               13150       13160       13170       13180       13190       13200       13210       13220

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
TCTGTTTTCTTAAGCTTTTGGAAAATTTTATCAATGGCGACTCCTAACGAAGTATCTGCACCTTTGGTTTCATCGAGAAACATCTACTCGAC  
Bur

.....

13320                → Start At5g47230  
                    13240       13250       13260       13270       13280       13290       13300       13310

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
GAGGCTTCTCCTGTTGGCTACAGATCCATGGATGAAGCAGCAATCATCATCAGCAACAGAACTAGCTCTGACTCTTCTTCTATCATCTTTC  
Bur

.....C.....G.....

13410               13330       13340       13350       13360       13370       13380       13390       13400

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
GGATCATCGTCCCTCTTCCTTCGCCCCAAATTGATTTCTCTGAATCCGTATGCAAACCTGAAATCATCGATCTCGATACTCCAGATCTATG  
Bur

.....

13500               13420       13430               CLG2: Clonage 1R               13440       13450       13460       13470       13480       13490

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
GAAATTTCTATCGATTCCATTTGAAATTTGACTCAGAAGTTCTGTTCCTGATTTGATTTTAAACCTTCTAATCAAATCAAATCAGTTT  
Bur

.....

13590               13510       13520       13530       13540       13550       13560       13570       13580

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|  
Col  
GAACCCGGAGCTTAAATCTCAAATTCGTAAACCCGCCATTGAAGATTTTCGCTTCCAGCTAAACAGAGTGGATTCAAATTCGCGAGCTGAAAC  
Bur

.....

13670               13600       13610       13620       13630       13640       13650       13660       13670

13680

Col  
ACCAAAACCGGAAGTTACTAAACCGGTTTCGGAAGAAGAGAAGCAATTACAGAGGAGTAAACAAAGACCGTGGGGGAAATTCGCGGCG  
Bur

CLG2 Clonage 2R

13690 13700 13710 13720 13730 13740 13750 13760

13770

Col  
GAGATTTCGTGACCCGAATAAACCGGATCTCGCGTTTGGCTTGGGACGTTTGATACAGCGATTGAAGCGGCTAGAGCTTATGACGAAGCA  
Bur

CLG3 Clonage

3E

13780 13790 13800 13810 13820 13830 13840 13850

13860

Col  
GCGTTTAGACTACGAGGATCGAAAGCGATTTTGAATTTCCCTCTTGAAGTTGGGAAGTGGAAACACCGCCCGATGAAGGTGAGAAGAAA  
Bur

13870 13880 13890 13900 13910 13920 13930 13940

13950

Col  
CGGAAGAGAGACGATGATGAGAAAGTACTGTGGTTGAGAAAGTGTGGAAGACGGAACAGAGCGTTGACGTTAACGGTGGAGAGACGTTT  
Bur

13960 13970 13980 13990 14000 14010 14020 14030

14040

Col  
CCGTTTGTAACTCGAATTAACCGAATTAATGACTGGGATTTAACGGGGTTTCTTAACCTTTCCGCTTCTGTCGCCGTTATCTCCTCAT  
Bur

14050 14060 14070 14080 14090 14100 14110 14120

14130

Col  
CCACCGTTTGGTTATTCACAGTTGACCGTTGTTGATTAGTTTTTTTTGAGTTTTTGAACGATGTGATGCTGACGTGGACGTACACGTA  
Bur

End At5g47230 →

14140 14150 14160 14170 14180 14190 14200 14210

14220

Col  
GGTGCATGCGATGAAAAAACATCTATTTGTTCAATTTTTGCGTTTTTCTATTTGTTCAATTTTTTCACAATCACAAATACATATTT  
Bur

14230 14240 14250 14260 14270 14280 14290 14300

14310

Col  
CAGTTAATGATTACGGATAATTTAGCTTACGTTAATTTATTATGAGTACTAGAAGAAATCGGAGTAAATCAACATATAGATTATACTAG  
Bur

14320 14330 14340 14350 14360 14370 14380 14390

14400

Col



15210 15130 15140 15150 15160 15170 15180 15190 15200

Col  
CGTCATCATACGACTATTCAATATCCAACATATTTATTTGGAAAACCCGACCACGAATAATGGTTAGCTTACATCAATTATCCACACAT  
Bur  
.....G.....A.....

15300 15220 15230 15240 15250 15260 15270 15280 15290

Col  
ACTCAAATGGTTAAATTTCTAGAATTGTAATGGAAGACACAATGTTATCACGAATGAGGATCATCATGCACCTTCAGGTTACCTAATTAT  
Bur  
.....

15390 15310 15320 15330 15340 15350 15360 15370 15380

Col  
CCTACGTGGACAAATACCTAATCCCTCCATGATTTTCAATTATTGCAGATAATTTTCTGAACGTCATCATTTCCCATAACGAATAATCAT  
Bur  
.....G.....

15480 15400 15410 15420 15430 15440 15450 15460 15470

Col  
TTCCTCAATCAACGCAACCTAACTAT-  
CTATGTCATTTACATGTTTATGTGGTAAAAATCTGCTTAGAATAAGTAAACCTTCACATATA  
Bur  
.....A.....

15570 15490 15500 15510 15520 15530 15540 15550 15560

Col  
TATTTGTAACAAAATAATTGAGGTTAATGTTATTCAATGCGAAAAAATGTAAACACTTGCTAATGAGTCGTAATAACCCATATATTATTTT  
Bur  
.....G.....

15660 15580 15590 15600 15610 15620 15630 15640 15650

Col  
CCAAAACATGTTTCTATTGAAGTTACAAATTAAGTTTCCACTCTATTTGGATGGAGCGTACTAGTCGTAGACACGGAGACTTCCAAGTC  
Bur  
.....

15750 15670 15680 15690 15700 15710 15720 15730 15740

Col  
CATCGGATCAACTTATCAAACCTTATCGACCTTTTGGTCCACAACAAGATATCTTTTGTCCATCTTCATCATGGCTCTCGCAAGAAGGATGG  
Bur  
.....

CLG5: clonage 5 CLG4: clonage  
4R  
End At5g06965 (lncRNA) ←

15840 15760 15770 15780 15790 15800 15810 15820 15830

Col  
GTTACATATATGGTTTGTCTTATTGCTTAATAGGGATTAATTTCTCTAATACTTTTGAATTAGCTTTTCGCAAACATTTTTCAGCATTAAGG  
Bur  
.....G.....

15930 15850 15860 15870 15880 15890 15900 15910 15920





17460 17380 17390 17400 17410 17420 17430 17440 17450

Col  
CGCTTTCATGCTTCGAGCTTCTCTATCCGATGGAGAAGAAGGTAAAAACGATAAACCCCTATAAAAGTCTTAAACCCTTTTGTTTTGT  
Bur

17550 17470 17480 17490 17500 17510 17520 17530 17540

Col  
ACATATGTAAATTTGGGGGAAATTTTTGTAGGGAAAGAAGGAGTTTGGTAAAGTTACCTGTGGAACAATCAGAAATAGTCCC AATAGC  
Bur

17640 17560 17570 17580 17590 17600 17610 17620 17630

Col TATAAAGGTAAG-  
CAATAAGAAATGGTTCCTTGTATCTTGGTGAATAAGCAGAGATTGTGTTATGCAAGTTAATGATTTTTTGTGGATTG  
Bur

17730 17650 17660 17670 17680 17690 17700 17710 17720

Col  
ATTTTTGCAGGAAGGTTTTGAGTATCATCATGCAGAGAAAGGATATGTAATGTTAACATATTGGATACCAGAGAGGAACCTAGTATGC  
Bur

17820 17740 17750 17760 17770 17780 17790 17800 17810

Col  
CCTGCAATGCCTCACATCAAGTTGGTGTGGAGGTTTTGTATTAAATCAACATAAAGAGGTATCAATATATGAATGATTATTCTCTCAA  
Bur

17910 17830 17840 17850 17860 17870 17880 17890 17900

Col  
GTCTCAACACTTAAAGTAGAGTAGGTAAAAAGAAGGTTACCTGAATTTTTTTTAAATCTCATTAGGTGCTTGTTGGTACAAGAAAAGTA  
Bur

18000 17920 17930 17940 17950 17960 17970 17980 17990

Col  
TTGTGCTCCTTCGATTACTGGTCTATGGAAGTTACCAACAGGGTTATTAAATGAATCTGAAGAGATTTTCTCTGGTCTGTAAGAGAAGT  
Bur

18090 18010 18020 18030 18040 18050 18060 18070 18080

Col  
CAAGGAAGAAACTGGGGTAATTAATCCGAGAAGATTAGTATATAGTATAAATCTTGATTCTGTTAAAAAATTCGCAAGATCATAACCAT  
Bur

18180 18100 18110 18120 18130 18140 18150 18160 18170

Col  
Bur

Snp\_K60

Snp\_K59

CLG8clonage 8R

V





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.....
18990      18910      18920      18930      18940      18950      18960      18970      18980
Col
CCAAGCTAGCCAAACATGTCGAAGATCCGTGCAAAATATCTGCTTCTCTCTCAAACCCCTGAGCACAATAGATAACATACAATTGTGGTGG
Bur
.....

19080      19000      19010      19020      19030      19040      19050      19060      19070
Col
AACATGGAAACGTTTTTCAGGAATTTGACAAGACAAAACAAATGACAAGTTGTGGAAACATACAAGAAATAGAACGTTACGGGACAAAGTG
Bur
.....

19170      19090      19100      19110      19120      19130      19140      19150      19160
Col
ACAGGAAAAAATAGCTCTTGCCCTCCTCCACTATCCCATTTCAAAGCCTTATATATGAGATCATCATCGATTTTCTTATAATGGACGTTGA
Bur
.....
End At5g47250 ←
.....

19260      19180      19190      19200      19210      19220      19230      19240      19250
Col
TGAAGATTCGGGCAGTTTTCTATATCGACTTTGTTTCAGCTTCAGTTTTGGAAAAGAGACTTGACTCCCATAGATGCTTCCCTAGTTCCCTTC
Bur
.....

19350      19270      19280      19290      19300      19310      19320      19330      19340
Col
AAATAATGTAGACGAAGGACTTGTAGCTCTTGAAAAGGATCAACCCCAACACCTTGAGCTTTCCTTTGTTTATTAATCTGTCACTTTA
Bur
.....

19440      19360      19370      19380      19390      19400      19410      19420      19430
Col
GGCGAGGATTCACGCTTAGAGACTCGAGATTTGCAGCATACATCAGCCATGTCAAATCCTTTAGATGTATGCATGAGTTTATTACCACA
Bur
.....
C
  Snp_K61
.....

19530      19450      19460      19470      19480      19490      19500      19510      19520
Col
GCTGAGAGATCCTTGAACCATGGATTGCTTGGAGTGATTTCACTGGATGATGTGGATGGAGAATACTGGTCTCTTCTTACCTTCCCAT
Bur
.....

19620      19540      19550      19560      19570      19580      19590      19600      19610
Col
TCTGTTCCCGACTCTGTGATATCGCAGTTTACCATTTCAAGTTTGTGGAGACTACTCAACGTACCAATGGCTGCAAAATGATACCTTAAGT
Bur
.....
C.....T...G.....

19710      19630      19640      19650      19660      19670      19680      19690      19700

```



.....  
20520            20440        20450        20460        20470        20480        20490        20500        20510  
.....  
Col  
TAAATATGCCTTGGGAAATAAAGCACAATACAGAAAACACTTGGCATTTTTGTTTTCAAATAATCATAGCTCAACTTCAAAACTTGAAAT  
Bur  
.....  
20610            20530        20540        20550        20560        20570        20580        20590        20600  
.....  
Col  
ATTCCTTCTCTGTACCTTTCATCTCACTCCGATAAGACTCCAAGTATCGAGTGCACGACGCCATTGAATCACAGTAGATTAGATGCC  
Bur  
.....  
20700            20620        20630        20640        20650        20660        20670        20680        20690  
.....  
Col  
ATAGCTTTCTTATAACTTCAAGTGCAAGGGGTAAGCCACAACACTTAGCCACAATCTTTTTGCAATATCAGAAATTCATTTAACCCG  
Bur  
.....  
20790            20710        20720        20730        20740        20750        20760        20770        20780  
.....  
Col  
TCGCAATGGACCTTCATATCGAACAAATCCCATGCGTCATTCTCGACAAACATTGAACCTTCTATGTCCTCATTTGCCCTCATAACTGAA  
Bur  
.....  
20880            20800        20810        20820        20830        20840        20850        20860        20870  
.....  
Col  
CAGACATCCTTAGAACGAGTAGTAAACACGACTTTGTATTTTTTACCGAGCACTGGGATGCCTATTGCTGTTAAACTCACATCCTCCCAT  
Bur  
.....  
20970            20890        20900        20910        20920        20930        20940        20950        20960  
.....  
Col  
AAGTCATCTAATAACAGCACGAACCGGGGCTTCATATCTCTTAGTACCTGCTTATTTTCACTAGCTTTCTCCCTCTTGAGTATGTAGAC  
Bur  
.....  
21060            20980        20990        21000        21010        21020        21030        21040        21050  
.....  
Col  
CAATTAATGTCACAGATGTGTAATCTTTCCCCGATGGCATCTTGAATCTTCCCGACATCTGCATCTTTAGACGATTCAACCCAAATAACA  
Bur  
.....  
21150            21070        21080        21090        21100        21110        21120        21130        21140  
.....  
Col  
ACATCATAATCATCACTGACTTCAACGAACTTGTGTTAATTAGAGTGAGGAGGGTAGTTTTGCCTACGCCTCCCATACCGAAGATTCCC  
Bur  
.....  
21240            21160        21170        21180        21190        21200        21210        21220        21230  
.....

G  
Snp K62

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AACATTCGTTTTTCATCTTTCTAAGACTTTCCCAAGCTTTTCAAGCGTCGTATCAAGACCGACTGTTTGGCAAAGTCTCACTTCT
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21330      21250      21260      21270      21280      21290      21300      21310      21320
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ACCACAGGAGGAGGAGGTTGCTCAGTCACCTTCTTGAAATCTTTACCGGAGAGACTTTTAACTTCAGTCAACTTCTTGAATACCTTCTCG
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21420      21340      21350      21360      21370      21380      21390      21400      21410
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CCTAGGTTGCAGGTTGAGAACCAGCACCCAGATGTTGACAGACGTCGACGTACAGCTGAAGCATCTGAGAAGAAGCATCCCAGCGGAC
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21510      21430      21440      21450      21460      21470      21480      21490      21500
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GCAACATCCATTAACCTTTGGTGTCTTTCCTCAATGATTTTCGACTTGTGAAAGCCACGTAGCCACTATAGCTAGCCGTTGACCACCCCTTA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21600      21520      21530      21540      21550      21560      21570      21580      21590
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AGCTCACCAGCATTGACTCTGTTTACAACATCTCTTTTTCTGCTTTAAGCTCATCAAAGCACTCTTCAACAAGACAAGATTTTCTTCTC
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21690      21610      21620      21630      21640      21650      21660      21670      21680
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AACATGCAAATGTTACCCACCTTACACACAAGTAAGACAAAGCAGACTTATAACATGGCTCAACTACCTGCCAACAGCAATTCATTTGTG
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21780      21700      21710      21720      21730      21740      21750      21760      21770
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CTCAAGATTGAGAAAGAGAACTAAGATGAGAGTAGCAAAATTTAAACAGATCTTTGTACAGAACAGGATGCTGAAATCGGGGAGTAGAA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21870      21790      21800      21810      21820      21830      21840      21850      21860
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ATAGGGAAACAGAACTTTTGTGCAGGACGAAGTAGAGAGACCAGAGAAAAGGAAGAGACCAGAGAAATGGAGAGATGATGAAAGAAGAAA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
21960      21880      21890      21900      21910      21920      21930      21940      21950

```

Start At5g47250 ←



Col  
**ACAACTGGAGAGAAATCTAGAGGCTTTGCATAAAGTAATGCAAGACCTCAACGCAATGAGAAACGATCTGTTGAAAGAGGCTGTCGAAAG**  
 Bur

.....  
 22690      22700      22710      22720      22730      22740      22750      22760  
 22770

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**AGGAGGAGATAGGTCTACAAAGGCTACAAGAAGTCAAAGAGTGGATTTCAATGGTGAAGAGATTGAACCTAAAGCCAATCGGCTGCTTG**  
 Bur

.....  
 22780      22790      22800      22810      22820      22830      22840      22850  
 22860

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**ATGAAAGTGTCTCTGAAATTCAGAGACTATCAAGGTACGGCTATTGTTCTCTGATCCCTGCGTCGACCTATCGTTACAGTAAAAGGTAC**  
 Bur

.....  
 22870      22880      22890      22900      22910      22920      22930      22940  
 22950

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**TTACGACTATGGAAGGAGTTGAAACTCTGAGATCTAAGGGAGTCTTCGAAGCTGTCTGTTACAGAGCTCTTCGGCCTTTGTGATAAAGA**  
 Bur

.....  
 22960      22970      22980      22990      23000      23010      23020      23030  
 23040

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**TGCTCCAATTCAACTTACTGTTCTCAGCAAAAGTTGCTTGATACGGCATGGGCTCGTCTAATGGACATAAATGTTGGGACTTTGGGTA**  
 Bur

.....  
 23050      23060      23070      23080      23090      23100      23110      23120  
 23130

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**TTATGGTAGGGTGGAGTAGGCAAAACACCCTTCTTACTAACTCAGAAACAAGTTACTTGTAGATGCATTTGGTCTTGTGATCTTTG**  
 Bur

.....  
 23140      23150      23160      23170      23180      23190      23200      23210  
 23220

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**TTGTTGTGGGTTTGAAGAGGTGAGAGCATACAGGATGAAAATGGTAAAAGATTAGGCCTCCAATGGAGAAGAGAAACCAAGAGCGCA**  
 Bur

.....  
 23230      23240      23250      23260      23270      23280      23290      23300  
 23310

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**AGGCAGCTGAAATATTGGCAGTCTTAAAGGAGAAGAGATTGTGTTGTACTGGATGGCATACAGAGGAATTGGATCTTGGGAAATTC**  
 Bur

.....  
 23320      23330      23340      23350      23360      23370      23380      23390  
 23400

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|  
 Col  
**GAGTTCTTTTCCAGCCGAGATAATGGATGCAAAATGTATTCACTCAATCTCTGGAAGCATGTGACGAAGCAAGTGGGTGTATG**  
 Bur

.....







25020

Col  
TTCTGGAAATATTTAGTGATAAGGCGTTGTCCAGAGCTGAGAAGACTTCCATTCAACTCTGAGAGCACTATAGGAAATCAAGTTGAAACGA  
Bur

25030 25040 25050 25060 25070 25080 25090 25100

25110

Col  
TAAATTGAGGAGCAAGTGATAAAAAATAGTTGAATGGGAGGATGAAGCTACAAAACAACGTTTCTCCATTTCAATAACAGGTATCTTCTTC  
Bur

25120 25130 25140 25150 25160 25170 25180 25190

25200

Col  
CTTATCCACATTTCTTCTCTATTTTTTTTCGATAAGGTTTCTTAAATCTATAAAAGCTTGGCCATGAATAACTAGCATCTCCACGGG  
Bur

25210 25220 25230 25240 25250 25260 25270 25280

25290

Col  
AATGTCACCTACCATCTCTTAATTTTTTATATATTTCAATGTCACCTTATTATTTCATAGAATCTGGAAAGCTGATTTGATAAGATTTT  
Bur

25300 25310 25320 25330 25340 25350 25360 25370

25380

Col  
GCAATGGTGATCCCTTATTTTGATTGATCATTTGTTTTCGAATTATGTAAACAAACGAACGGAGTGCAGAGACTTTGTACAGATGGCTGAA  
Bur

25390 25400 25410 25420 25430 25440 25450 25460

25470

Col  
GATCCGAAGATGGATGGTTTGACATCGGAGTCACATCCAAATTCAAACCATAGACCTGGTCGGGACTACAGGAAGTGGAGAAACTGCCACT  
Bur

25480 25490 25500 25510 25520 25530 25540 25550

25560

Col  
GCAACCAACATCCAAGGAAGAAGGTGGTCCAAATCGGGAACACACGCAACTGTTGTTACCATGGAATGCCAGACATATAAAGTTTTCACA  
Bur

25570 25580 25590 25600 25610 25620 25630 25640

25650

Col  
CCAGATTGCCCATCAACAATATGATTGACACTCCTGGTACGAATTTCCTTTTATGTTATACCTAACTAAATTATCATGCGTGGGAAGAA  
Bur

25660 25670 25680 25690 25700 25710 25720 25730

25740

Col  
AAAAACAAATTTTCTAATAAGTAAGGTTTGTACTTTACGTATACAATTAGAATAGGATCCACGTAAAAATGTGTATTTCTAATTTTCTAT

Start AT5TE69050→

End At5g47260→

**Bur**

```

.....
25830        25750      25760      25770      25780      25790      25800      25810      25820

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

ATAGTTTAAAAATAAAAAGTGACAAAACTAATAATGCTGAACCAAATAATATAATGTATATAACTATCAATACTATTTTCAATTATATAA

**Bur**

```

.....
25920        25840      25850      25860      25870      25880      25890      25900      25910

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

TTGATATGATTTTGTGTCACCTACCATACGCATATGACATATATATTTATTATTATATGAACCAAACCTCATTTCATTAAACCTAGTGA

**Bur**

```

.....
26010        25930      25940      25950      25960      25970      25980      25990      26000

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

FAAGTTTACTTTGCTCACAAAAGAGTTGATTTAAACGTTTTCACAAACCCATCCGGACGTAAAATGTGTAATGGAACATACATAGAGA

**Bur**

→ End At5TE69050

```

.....
26100        26020      26030      26040      26050      26060      26070      26080      26090

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

CCAAATAATTATAAAATTATAATAGATAATGCTTCTATGTATATGTATGTTGTATGTAAAGATTACGTCATCTCAGGTGAACATATGTTG

**Bur**

```

.....
26190        26110      26120      26130      26140      26150      26160      26170      26180

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

AGTTTTTGATATTGAACACTGGTTAAAAGTCATTGAGACTGTGTCCTCTGATGCTAGAAAAGTCCATTCATTGATGCTAAAAGACTTTGGG

**Bur**

```

.....
26280        26200      26210      26220      26230      26240      26250      26260      26270

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

ATCCGAGTTTCTTTCTGACTGTGTCAATTTTTCTGACTTTGGAACTGGATTTAGGCCAAGAGGAAGGTGAAGGAGATGCTTTTAAATT

**Bur**

Start At5g47280 →

```

.....
26370        26290      26300      26310      26320      26330      26340      26350      26360

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

GAACGATGAGGCAAGAAATTATTGGGATCTCAGGGATGATCGGTTTCAGGGAAAACCATTTCTTGCCAAGGAGCTTGC CCGGACGAGGAGGT

**Bur**

```

.....
26460        26380      26390      26400      26410      26420      26430      26440      26450

```

```

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Col

```

CCGAGGTAAATCAGTTTTCGTTGTTATGCTCTGAAACTATCCATTGTTAATAATGCTTGGGCCATCTTTGAAGTCTTTTGAGCAGTTTAT

**Bur**

```

.....
26550        26470      26480      26490      26500      26510      26520      26530      26540

```

.....  
Col  
GTTGTTGCTCAGTGGCATGTTTACTGGTTTATTGGATGATCATGCATTATCTCTGTATGTTCCATTGTTGTCATGTTTCATCTCCGGTGA  
Bur  
.....

26640            26560          26570          26580          26590          26600          26610          26620          26630

.....  
Col  
ACTGTTGATGAGTCGTATAGTTGAGTTCCTTGATATTAGAATCTGTTAAGAGTCGGAGAGACTGTTCCCTTGATGCTAAAAAGCTTTAAT  
Bur  
.....

26730            26650          26660          26670          26680          26690          26700          26710          26720

.....  
Col  
ACAGGCCATTTTCGGAACCGAGTTTGTCTGACTGTGTCACAATCTCCCAATCTTGAGGAGCTGAGATCCCTTATACGGGATTTTCTT  
Bur  
.....

26820            26740          26750          26760          26770          26780          26790          26800          26810

.....  
Col  
ACTGGTCATGAGGCTGGCCTTGGTACCGCTCTCCGGAATCCGTTGGTCATACCGGAAGCTAGTGATCCTTGATGATGTTAGGACAAGG  
Bur  
.....

26910            26830          26840          26850          26860          26870          26880          26890          26900

.....  
Col  
GAATCTCTAGACCAGCTGATGTTCAATATTCCGGAACCAACCGCTTGTGGTCTCACAGTCTAAACTCGTAGATCCTAGAACCACCTAT  
Bur  
.....

27000            26920          26930          26940          26950          26960          26970          26980          26990

.....  
Col  
GATGTAGAGTTATTAATGAACATGACGCAACATCTCTGTTCTGTCTCTGCTTCAACCAGAAATCAGTTCCTTCAGGGTTCAGCAA  
Bur  
.....

27090            27010          27020          27030          27040          27050          27060          27070          27080

.....  
Col  
AGTTTGGTCAAGCAGGTAAATGGGCTGCTACAAGTGTACATGCATAGTAGTAATATTCTTTGTACTTTCAGTACTCATCTTGACTCTAT  
Bur  
.....

27180            27100          27110          27120          27130          27140          27150          27160          27170

.....  
Col  
TTGTTAGGTTGTTGGGAGTCTAAAGTCTACCTTTGCTCTGAAAGTCTTTGGCGCTTCATTAACCGATCGACCTGAAACATATTGGGC  
Bur  
.....

27270            27190          27200          27210          27220          27230          27240          27250          27260

.....  
Col  
AATTGCAGTGGAGAGGTTATCAAGAGGTGAACCTGTTGATGAAACTCATGAGAGTAAAGTGTTCCTCAAATCGAAGCAACTCTAGAAAA  
Bur  
.....

.....  
27360 27280 27290 27300 27310 27320 27330 27340 27350  
.....  
Col  
TCTCGATCCAAAAACCAAGAGTGTTCCTTGGATATGGGTGCTTCCCTGAAGGCAAGAAAATCCCTGTTGATGTTCTCATCAACATGTT  
Bur  
.....  
27450 27370 27380 27390 27400 27410 27420 27430 27440  
.....  
Col  
GGTCAAGATACATGATCTTGAGGACGCAGCCGCTTGTATGTTCTTGTGATCTAGCAAATAGGAATCTCTTACTCTCGTAAAAGATCC  
Bur  
.....  
27540 27460 27470 27480 27490 27500 27510 27520 27530  
.....  
Col  
AACGTACGGTTATAGAACTCTTATGTTCTCATCTCTTGTAGCCACTTTTATAATTTTAAACCATTCTTAACATAATTACCCTGGATAATG  
Bur  
.....  
27630 27550 27560 27570 27580 27590 27600 27610 27620  
.....  
Col  
TTGCAGGTTTGTGCTATGGGCCTAGCTACTATGATATATTCGTGACGCAGCAGCATGTTTTAAGAGATGTAGCACTTCATCTTACCAA  
Bur  
.....  
27720 27640 27650 27660 27670 27680 27690 27700 27710  
.....  
Col  
TCGTGGAAAAGTAAGTAGAAGAGACCGCTTATTGATGCCAAAAAGAGAGACCATGCTTCCCAGCGAATGGGAGAGGAGCAATGATGAGCC  
Bur  
.....  
27810 27730 27740 27750 27760 27770 27780 27790 27800  
.....  
Col  
ATACAATGCACGAGTGGTTCCATTCACACAGGCAAGAATTTGTTATGCAACGATCTTCTAATGAATTAATTCCGGTTCCTCACTAGAATC  
Bur  
.....  
27900 27820 27830 27840 27850 27860 27870 27880 27890  
.....  
Col  
ATAAGGTATTAATATGGATTTCCTTACAGGAGAAAAGACTGAGATGGACTGGTTTGACATGGAATTTCCCAAGGCAGAAGTCTGTAGT  
Bur  
.....  
27990 27910 27920 27930 27940 27950 27960 27970 27980  
.....  
Col  
AAACTTCTCTCAGACAACATATGATTTGCCCTCCTTTCATTGCTAAGATGGGAATGCTTAGGGTCTTCGTGATTATAAACCAACGGTACCTC  
Bur  
.....  
28080 28000 28010 28020 28030 28040 28050 28060 28070



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TGTATTATTATTATTAACTCGATTAGGACCCCTGTATGATATACGATTTTATTAATACATGTTTGTCTTATAACGTCAATATATAAA
Bur
.....
28890      28810      28820      28830      28840      28850      28860      28870      28880
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TTATATGTTGATTTAAGTATTAAAAGTTTCTATTTGGAATCTCAAAGATATGTTTTAAAGATTCACTTATAAGTAATAACAAACAAC
Bur
.....
28980      28900      28910      28920      28930      28940      28950      28960      28970
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AAAAACTATTTAGCTTAATGGTAAAAGCATGAGTCTATATAGAGAAGGGTTCATAATTTAAAATTAGTTGAATGTTGTTTGTATTAA
Bur
.....
29070      28990      29000      29010      29020      29030      29040      29050      29060
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GTGAGATACATTTTAAAATAATTTAGTGAGATAAATATATCGTTAATATTATGCATGTGCTGATTATTATATGACCAATTATATGACCCA
Bur
.....
29160      29080      29090      29100      29110      29120      29130      29140      29150
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TCAATAGTTGTCAACATTTTCCTGGTGGATCGACGAGGACGAACCCAATGATTTAGAAACAGGGATGATATATAACAAGTAAGTATAGTC
Bur
.....
29250      29170      29180      29190      29200      29210      29220      29230      29240
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GCAAGTGTCCCTGATCTATAGTCATACAGGTAAAGGCCCACTGCTGAAAAGAGAAGTGGCGGTGGATTTAAAACAATACAAGTGAAGTA
Bur
.....
29340      29260      29270      29280      29290      29300      29310      29320      29330
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GTGTATCGAGCCCTTTGTAACATGAGATTGTATAGATCCAGTAAGAGATGGTAGATTTTAACTCTGAGATAAGAACTATCTCTATTTG
Bur
.....
29430      29350      29360      29370      29380      29390      29400      29410      29420
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GAAATCAGAGAAATACTTTAGCAGGGGAGAGGGATGGGAGCTACCTCGAGGGCATCAAGTTCCTTCAAACCTGGAGAAATCATCTTTGATTTG
Bur
.....
29520      29440      29450      29460      29470      29480      29490      29500      29510
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
GATATCAGAAAGAGAAGATATATCTATGGTTATAGTGGCTGAGACAAACGATTCCTCTCTCCCAAGGATCATTTGGGACTGCCTTGGCCATC
Bur
.....
29530      29540      29550      29560      29570      29580      29590      29600

```

29610

Col  
TGGACTGCAAGGTATAAACAGGAAGAAATAAAAACAACAAAAATATCAAGGATATAGCACTACCAATATGATAAATGCATTGACAAAATC  
Bur

29620 29630 29640 29650 29660 29670 29680 29690

29700

Col  
AGTCTTTTAAGTACAAAAATACTTTGGTGAAGGGAAAATAAATAAGGAACTTGTGCTATTGAAGCGGACAAACAGCTACCCACACAC  
Bur

29710 29720 29730 29740 29750 29760 29770 29780

29790

Col  
CATTTGGGGGAATTTTGAGAAACAGATTTGAGACTTTTATTGTTGATAGTAACATATACATAATGCTCTAGCTTCTTCTTCTATGCC  
Bur

29800 29810 29820 29830 29840 29850 29860 29870

29880

Col  
ACGAATCAACAATATTGTCCATGCTATCAACAATACAAAATAATAGCTAAAACCCCAAAAATCATAAAACCTAAGCAACAAGCTAATCTTCT  
Bur

29890 29900 29910 29920 29930 29940 29950 29960

29970

Col  
TCAGTTTCAGAGCAAGAATCAAAATGTACAGTATTTAAAGAGCTAGACAGACTTTAACGTAGAGAAAGAATCAATCTCCAGAGCCTT  
Bur

29980 29990 30000 30010 30020 30030 30040 30050

30060

Col  
CCTTAGTCATCTCTGCTTCCTTTTTTTGCATCTTCACGTAATGTTGGTTATGCGATTTAACCTGTTATGGATTCCTAGTCTTTACAAATT  
Bur

30070 30080 30090 30100 30110 30120 30130 30140

30150

Col  
CTCTAGATATGACCTCCCAAGCTCCTGGCCCTTCGACTTTAGTCCCTCAAGAAAGAGTCTGAAAACGTACACCAAAGTAAATGGATAAA  
Bur

30160 30170 30180 30190 30200 30210 30220 30230

30240

Col  
CAGATGAGAGATTTCATCATATGATCTATATTTACCAAAATAGCAACTTTAAACAATTAATGCCTAAGCAAAGCCAAAGCCTCCATCTAAC  
Bur

← End At5g47290

30250 30260 30270 30280 30290 30300 30310 30320

30330

Col  
ATTCATCATCTTAAATTAGCAAGAAGACAACCTTACTCGTTCCTTGGGCTGACCAACGAGTTCCTTCTTCGCCACTATCGGCGTCGCTG





```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TATTTCAAACATCATTGTTTCATTGACTTTTTCATGTTTTCAAATATTTACTTTAATACAAAATAAACAAAATACTAGTGGCGTATAAAA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

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31230          31150       31160       31170       31180       31190       31200       31210       31220

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.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TATCATAAAACACGAGCAAATATCGGAGGATAC TAGAAAACATATAAAGTTGAAAAATAACTAACTCCGTGTATAAAGAGGAAGAAGTTA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

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31320          31240       31250       31260       31270       31280       31290       31300       31310

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```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TCTTATGACTAGAAAATATATATATATATATATATATATAATATATAATATTTAAAAGTGAGAGCACGGGTCCGAGCAAACAATAAACCCCTAATTA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

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```

M T R K Y I Y I Y I I L K V R A R
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAATTTAAAA
M T R K Y I Y I Y I I L K V R A R

```

→ Start At5g47300 polymorphism:2017-A:deletion 6bp Bur → 2 AA, no frameshift

```

31410          31330       31340       31350       31360       31370       31380       31390       31400

```

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CAATCAAAGCCACCTGATAGAGATGAGAAACACATTGATGTTGTCGGACCTTCCAGGAGATTTGTTAGAGGAGATACTTTGTCGGCTTCC
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

```

31500          31420       31430       31440       31450       31460       31470       31480       31490

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.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
TGCCACATCTCTGAAGCAGTTACGATCTACTTGCAAACAATTGGAACAATTTATTCAACAATGGGAGATTCACAAGAAAACACTTGGATAA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

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```

31590          31510       31520       31530       31540       31550       31560       31570       31580

```

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
AGCCCCAAGGATTTTCAGAACTCATGTTGAGCGACTCTAGGGTATTTTCGATGAGTGTGAGTTTCCATGGAATCCATCTGTAGAGGC
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

```

31680          31600       31610       31620       31630       31640       31650       31660       31670

```

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
CACATGTGAACCTTAGCCTAATCGACTCTTTTTCTAGTTTTGAAGATAAATTCGAGATTTCTCAAGTCTTTCACGTGACGGCTTATTGTT
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

```

31770          31690       31700       31710       31720       31730       31740       31750       31760

```

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Col
ATGCACCGACGCAGACAACACTAGAATCGTGGTTTTGGAACCCCGTACTGGTAAAAC TAGGTGGAATTGAACCCCAATAATCGTTGCTACTA
Bur
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

```

31780          31780       31790       31800       31810       31820       31830       31840       31850

```

31860

Col  
CTATGC TTTTGGAT CCTACT TGGACAAATCCTACGGTAA TAGCTACAAAATATTGAGCTATAGTGGTTATGGCTACGAGAACCAAGAACT  
Bur

31870 31880 31890 31900 31910 31920 31930 31940

31950

Col  
CGCAATCTATGAGATTAACTCTCAATCATGGAGGTTCTTGATGTCACTCGTGACTGCATCCTCGAAAGATATACTGATTACGGTGTGTC  
Bur

31960 31970 31980 31990 32000 32010 32020 32030

32040

Col  
TTTGAAGGGACATACTTACTGGTTTGGCTCAGATGAGAAAGAGAAAAATCTCAGCGTATTCTAGTCAGTTTTGATTATCAACTGAAAG  
Bur

32050 32060 32070 32080 32090 32100 32110 32120

32130

Col  
ATTTAGACGTCTACGCTC CCGTATCAGTGCCCTGATTATAACACTGCGTCTTTATCCGTTGTTAGAGAAGAGAACTTGCGGTGTGTT  
Bur

32140 32150 32160 32170 32180 32190 32200 32210

32220

Col  
ACAACGCGAAAAATACATCAAGGACAGAGATATGGGTGACAAGTAGGATTGGTGAGACC AAAGTGGTGTCTGGAGCATGGTCTTAGCAGT  
Bur

32230 32240 32250 32260 32270 32280 32290 32300

32310

Col  
GGATTTCCCGTCCGAAC TATTCATTTTGCTGGCATAAGTTTCTTGGTCGATGCGGAGAAAAAATTCGTCGTATGTTGTGATAATTACTT  
Bur

32320 32330 32340 32350 32360 32370 32380 32390

32400

Col  
CGGAGAGGATGAATACGATACCAAAAAC TTGGTTCACATTGTTGGAGAGAACAACAAAGT GAGAGAAGTTAATTT CCGAGTATCCGAATC  
Bur

32410 32420 32430 32440 32450 32460 32470 32480

32490

Col  
ATCTTGGCCATTTTGT TTAATTATGTTCCAAGTTTGATTCAAACTCTGGGAAGGTGTAGGAGCCAAAAGAAAGAGAGTCGAATAAGTAAG  
Bur

32500 32510 32520 32530 32540 32550 32560 32570

32580

Col  
CTTCACGCAAACTGTTTTTTTTTTTCTTCTCACCGTTATCTTTGGTCATATCTAGTTATGTTTTGACTAACAAATGTTTCATACATA  
Bur

End At5g47300 ->





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.....
34200      34120      34130      34140      34150      34160      34170      34180      34190
Col
GGTTTTTCTTCGTAATAATTAGGGCTCCGATTTGTTACCCTTAGTTCATTTTCTCTAAATTTGGTTTTTGATTGATGGGTTTCT
Bur
.....
34290      34210      34220      34230      34240      34250      34260      34270      34280
Col
TGATTTAGCAATTTTAAGATTTACTTGAAAGATTCCGTAATTTGTTCTGATTCGGATTCCTTGATCTGTGCTCACTAGCTCTCTACCA
Bur
.....
34380      34300      34310      34320      34330      34340      34350      34360      34370
Col
AAGCAGCTTCCTTTTCTGATCCCTAGTGTCAATTAATGTGAACAGATTGTTACTTTAGCTTCTCTCCTTTTCACTTAGTTTTGCAGA
Bur
.....
34470      34390      34400      34410      34420      34430      34440      34450      34460
Col
GATTTTAGGAGGACATGATTGTTGAATTGGGAGAGACATTGGTTCGTAAAATGCGGGTGCCTGGTTTGAGCTCAAGCTTATGCTCT
Bur
.....
                                                → Start At5g47310
34560      34480      34490      34500      34510      34520      34530      34540      34550
Col
GGTGAGGATAAGGAAGAAGAAGAGATTAATGGAGAAGGCTCTCTCACGCCTGTTTATCTCAACGCTATGATCTAACTCCTGTCACAAAT
Bur
.....
34650      34570      34580      34590      34600      34610      34620      34630      34640
Col
TATCTCTATTGGTTCGGCCTTGGCATAATTCACCTCTGGCATTGAGGGTAATTGTGGCTTCTTCTCTATTACTTACTTGACCTAACTAAAT
Bur
.....
34740      34660      34670      34680      34690      34700      34710      34720      34730
Col
TTTTCTTTGTCATTGCTTTCTATTGCTTTCAATCCATATCTGGTTTATCATGTTATCAAAGTACTTCTAGCCTTGAATAGGAACAATGAT
Bur
.....
34830      34750      34760      34770      34780      34790      34800      34810      34820
Col
ATTCCTCAATTGTTCTCCACAAGTTGTGATTTATTATTATTAGGTGAAAATGGTGTTTATTATATTAGCTATGGAATACATTATTAGAA
Bur
.....
34920      34840      34850      34860      34870      34880      34890      34900      34910

```







36450 36370 36380 36390 36400 36410 36420 36430 36440

Col  
TGGTCTCTGTACTATCATTCTGCGAAATGGGTCTTCTCTTTGGAGGCTAATCGACGTTGTGCTTAATCTAATGCTCTTTTAGGTTG  
Bur

36540 36460 36470 36480 36490 36500 36510 36520 36530

Col  
TTTAGAGGAATATATCACAGGTTTTCAATTAGATTGATCTGCTTAGTTTTGTAATCAGTGTAGGTTACCAGTGAATCATGAACCTCCGTT  
Bur

36630 36550 36560 36570 36580 36590 36600 36610 36620

Col  
AGGGTTATAAGCTCTCTGTTTCTTTCCGACTTATCAATGTCACATAATTAGATTGATGCGTAGTTTAGCTTTGAAGATCAGGGAAC  
Bur

36720 36640 36650 36660 36670 36680 36690 36700 36710

Col  
TGAATATATCTGGTTTAAGGAAGCTTTGAATTTCTGCTTGCCATGGACATTCGACTTATCAGTATTTTTGTACATTTGGTTAATGTTT  
Bur

36810 36730 36740 36750 36760 36770 36780 36790 36800

Col  
ATCGAATTGGTTTGATCAGTCTGTGGATTTTAACTTTACAAGTTAAGATGCTATCTTTTAGGTCTAGTTGATAGGTCATGGATTGTTG  
Bur

36900 36820 36830 36840 36850 36860 36870 36880 36890

Col  
TTATATGGAGTCTAGACATTTTGCCACTGCTTTAGTGTCTTAGCTTCTAAGGGTTTCGTTGATATATTGACTGGGTTTATCGTTCCA  
Bur

36990 36910 36920 36930 36940 36950 36960 36970 36980

Col  
GGCATCTAGTATGGCTTTCTGCACTAAACTTGGCGGTCACCTGGAAACAAGGGTAAATGTTCCAGTGTTCATCAATGCTCGGTTCTCTTCG  
Bur

→Start\_At5g47320

37080 37000 37010 37020 37030 37040 37050 37060 37070

Col  
CTACATGTCCACAAAACTTTATATTGGTGGTAAGTATAACTTCCATCAGATTGTTTCATTTCTTACTCTTCGCAGCTGTGGATGTTTT  
Bur

37170 37090 37100 37110 37120 37130 37140 37150 37160





Col  
ATGGATTTGATATGTTTACTCAGAACTGTCGTCTAGGCTTGAAAGGATTTATTTATTCAGTTCCACATTTTCATGCAATGGAGCCCTCACA  
Bur

38790 38710 38720 38730 38740 38750 38760 38770 38780

Col  
AGACCTGTAATAAGACGAGTTCTATTTGTAAGTTTTTATGATTTATAAAAAGCTCAGAAATCATTATAGTCTATAGCTTTTATGGTT  
Bur

38880 38800 38810 38820 38830 38840 38850 38860 38870

Col  
TACATCTTGAGCTGAAAAATAAAACCTTTGCTGTAGATATTATAACTTACATAAGCTATGAATTATATATATAAATTTGTATTTCCGA  
Bur

38970 38890 38900 38910 38920 38930 38940 38950 38960

Col  
GCTAAAAATGTTATAATTTTATTATAGATTTCTACTTCTATGTTGCTTACCTAAAATGTTAATTCACCATATATTTTGAAGTGAAG  
Bur

39060 38980 38990 39000 39010 39020 39030 39040 39050

Col  
GGGGTGTAAGCATATGGAATTATTTGTGTTAAAATGACAAATCCTCTGTTATTCAATCATCAATATTTAAAAGTTTTATAAAAATCCA  
Bur

39150 39070 39080 39090 39100 39110 39120 39130 39140

Col  
TTGTTATTGAAATAATGATTCCAAAAAGTACCATCAAATCCAGTATTATGGAATATTATAACCACCTGGATTTTGAATGACTTTAAGG  
Bur

39240 39160 39170 39180 39190 39200 39210 39220 39230

Col  
TGGTTTATAAGATTTTAACATGTTTGTGTTTGGAAFTTGGAGAACTTTAGAACAAATCATAACTTTAATCTAAAATCACCCAAAAA  
Bur

39330 39250 39260 39270 39280 39290 39300 39310 39320

Col  
TTCATCTAAAACCTCATTAAAATTCGAATCATTCAAATATATGGTTGGATAACATAGAAATTTGTCATTTCAACACCAATCATTCAATG  
Bur

39420 39340 39350 39360 39370 39380 39390 39400 39410

Col  
TGATTTATTGATTAATATTTTTTATCATAGAGAGAGCAAAAATCTCTGTCAATGGCAAGTAGGTACCAAGTAGTGGCATGAGAGAGTGATG  
Bur

.....  
39430 39440 39450 39460 39470 39480 39490 39500  
39510  
.....  
Col  
TCTGGATTATTGATTTCTTTGGTAGCTTATACCTCCATATGTTTCCCTATTTTAAATTTTTCATTCCAATAATGGGACAAAGTTATAACAA  
Bur  
.....  
39520 39530 39540 39550 39560 39570 39580 39590  
39600  
.....  
Col  
ATAGTATTCGATATCAAACGTCAAAACATGTACTCCCAAATATATTTTATTGGAAATATAGTTTGGAGATATTTTACCTGGGGGAGAT  
Bur  
.....  
39610 39620 39630 39640 39650 39660 39670 39680  
39690  
.....  
Col  
GGAACCAAAATAGATCTAGAAATGTGATTGGCTAACGAAAGAAGCACAAATAAGTTAACCAAGTGACAGTGACAAATTTATCCCTCGACAAC  
Bur  
.....  
39700 39710 39720 39730 39740 39750 39760 39770  
39780  
.....  
Col  
AACACACATTCACATATATCTTTTATTATTATTTTCTGTGTCTTTAAAGTTTACAGCACAAAGTAGTTTCTAAATCTTATAATCA  
Bur  
.....  
39790 39800 39810 39820 39830 39840 39850 39860  
39870  
.....  
Col  
ATTTTCAATTGATAAACAGAAATTTAAATATTTAAATAGACAAATAAATGATCAAATCTATATTTCTATACAAGATTAAATTCACAAAA  
Bur  
.....  
39880 39890 39900 39910 39920 39930 39940 39950  
39960  
.....  
Col  
TTTGTGTGAAACAAACTCTTCTATATTTCTATACAAGAGTTAACATATTTCTATATAAGTTATTGTAAAGATCAAAATATGAAAATTA  
Bur  
.....  
39970 39980 39990 40000 40010 40020 40030 40040  
40050  
.....  
Col  
TGGTATAAAATGCATAGACACATATATACGTGCCCTATTAAAAGAGGCAGCCGAGAAGATAATATAGGAGGAAGGAAGAGAAGAGATG  
Bur  
.....  
40060 40070 40080 40090 40100 40110 40120 40130  
40140  
.....  
Col  
GTGAAGAAGAGAGTTAAATGCAACTGCAAGAAGATAGTAACATAATCAGCACCGTCCATTTTGTATCTAATTCCTTCTACTTGGCCGCA  
Bur  
.....  
40150 40160 40170 40180 40190 40200 40210 40220  
40230



.....  
41040            40960            40970            40980            40990            41000            41010            41020            41030  
.....  
Col  
AAGTTTTTTTATAAAATCTTTTTCTCTAAATATTTTTCTATGATTTTAATTAATCAATCAATCACAGTAACAACATTGTCTTTTTCT  
Bur  
.....  
41130            41050            41060            41070            41080            41090            41100            41110            41120  
.....  
Col  
TGTTAAAGTCTGGTTTATCTGTAAAGTTAGCAGATGAGCTAATCAAGGGAGATATCTATAGCGACTTCATTCAAGTATAAAATCTCTTCT  
Bur  
.....  
41220            41140            41150            41160            41170            41180            41190            41200            41210  
.....  
Col  
GTCTTTTTTAGGATTAATCCTCAGTAGTTACAAATTAATTAACATATATCTTTAATAACCCGTCAGGATCATCTTGCTCCTAGTGGTTA  
Bur  
.....  
41310            41230            41240            41250            41260            41270            41280            41290            41300  
.....  
Col  
TCTCAAAATTCCTACTGTATGTCTACTACCAAAATATATTTTGATGATAAAAAAATCTTCAAAATTTGAGATATCACAAAATCTTTGT  
Bur  
.....  
41400            41320            41330            41340            41350            41360            41370            41380            41390  
.....  
Col  
TTTTGATTTATCAGGATATGACAAAGTACTTGGGAAGCTCTAAGTATTTACCTAAGCTTAACAAATGAGATACCAGACCAAGAAACCAA  
Bur  
.....  
41490            41410            41420            41430            41440            41450            41460            41470            41480  
.....  
Col  
CTTACAAAGACCGTTTCACCAGTTTACATAACTTGGTTCTTATCAAGGTTTGGTCTCCTAAAAACCATCTAGACTTTTCAATTATCTTTT  
Bur  
.....  
41580            41500            41510            41520            41530            41540            41550            41560            41570  
.....  
Col  
GATTCCCTCTTTGATGATTCGGTTTTGTTTTGTTTACAGTTTCAGGGCGACAAGGTTATAGTTCCAAAAGATTCACTCTGGTTCCGGTT  
Bur  
.....  
41670            41590            41600            41610            41620            41630            41640            41650            41660  
.....  
Col  
TTATCCGGATGGTGAATTCGAACCTCTTCTCTCTGCTCAACAGACAAAGCTCTATACAGAGGATTGGATCCGGTCTGAAAACATTGGATGA  
Bur  
.....  
41760            41680            41690            41700            41710            41720            41730            41740            41750





# Supplementary Text S4: *AT5G47260* and *AT5G47280* sequences in corresponding CRISPR-edited lines in Bur-0 and HIF10499 backgrounds.

## *ΔAT5G47260* : CRISPR-edited lines for *AT5G47260*

### WT

ggcctccaatggagaagagaaccaaagagcgcaaggcagctgaaatatggcagctcttaaaggagaagagatttgggttggttactggatggcacaagagggaattg  
GlyLeuGlnTrpArgArgGluThrLysGluArgLysAlaAlaGluIleLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuAspGlyIleGlnArgGluLeu  
gatcttgaggaaattggagttcctttccagccogagataaatggatgcaaaattgtaccactcaatctctggaagcatgtgacgaaagcaagtgggttgat  
AspLeuGluGluIleGlyValProPheProSerArgAspAsnGlyCysLysIleValPheThrThrGlnSerLeuGluAlaCysAspGluSerLysTrpValAsp

### in Bur-0 background

#### 117-1

tggagaagagaaaccaaagagcgcaaggcagctgaaatatggcagctcttaaaggagaagagatttgggttggttactggatggca---cagagggaattggatcttgaggaaattggagtcc  
TrpArgArgGluThrLysGluArgLysAlaAlaGluIleLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuAspGlyT---hrGluGlyIleGlySer .

#### 117-36

tggagaagagaaaccaaagagcgcaaggcagctgaaaatattggcagctcttaaaggagaagagatttgggttggttactggatggca--cagagggaattggatcttgagg / 102nt / TAG  
TrpArgArgGluThrLysGluArgLysAlaAlaGluAsnIleGlySerLeuLysGlyGluGluIleCysValValThrGlyTrpHi---sArgGlyAsnTrpIleLeuArg

#### 85-7

tggagaagagaaaccaaagagcgcaaggcagctgaaatatggcagctcttaaaggagaagagatttgggttggttactggatggcaca-cagagggaattggatcttgaggaaattggagtt  
TrpArgArgGluThrLysGluArgLysAlaAlaGluIleLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuAspGlyIleThrGluGlyIleGlySer .

### in HIF 10499 background

#### 95-14

gaaaccaaagagcgcaaggcagctgaaaatattggcagctcttaaaggagaagagatttgggttggttactggatggcaca-cagagggaattggatcttgagg / 102nt / TAG  
GluThrLysGluArgLysAlaAlaGluAsnIleGlySerLeuLysGlyGluGluIleCysValValThrGlyTrpHisIleArgGlyAsnTrpIleLeuArg

#### 98-7

tggagaagagaaaccaaagagcgcaaggcagctgaaatatggcagctcttaaaggagaagagatttgggttggttactggatggcaca-cagagggaattggatcttgaggaaattggagtccctt  
TrpArgArgGluThrLysGluArgLysAlaAlaGluIleLeuAlaValLeuLysGluLysArgPheValLeuLeuLeuAspGlyIleThrGluGlyIleGlySer .

#### 105-12

tggagaagagaaaccaaagagcgcaaggcagctgaaa-----aaaggagaagagatttgggttggttactggatggcaca-cagagggaattggatcttgaggaaattggagtccctt  
TrpArgArgGluThrLysGluArgLysAlaAlaGluL-----ysArgArgArgAspLeuCysCysTyrTrpMetAla .

## *ΔAt5g47280* : CRISPR-edited lines for *AT5G47280*

### WT

Ggtctacctttgtctctgaaagtcccttggcgcttcattaaacgatcgacctgaaacatattgggcaattggcagtgagaggttatcaagagtgaaacctgttgatgaaactcatgagagtaaaagt  
gtttgtcacaatcgaagca  
GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrTrpAlaIleAlaValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysVa  
lPheAlaGlnIleGluAla actctagaaaaatctcgatccaaaaaccaaagaggtttcttggatattgggtgctttccctgaaaggcaagaaa  
ThrLeuGluAsnLeuAspProLysThrLysGluCysPheLeuAspMetGlyAlaPheProGluGlyLysLys

### in Bur-0 background

#### 21-20

ggtctacctttgtctctgaaagtcccttggcgcttcattaaacgatcgacctgaaacatattgggcaattg-cagtgagaggttatcaagagtgaaacctgttgatgaaactcatgag  
GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrTrpAlaIleValSerGlyGluValIleLysArg .

#### 160-2

Ggtctacctttgtctctgaaagt**ccttgg**-----  
aacgatcgacctgaaacatattgggcaattgt**cagtgg**agaggttatcaagaggtgaacctgttgatgaaactcatgagagtaaagtgtttgctcaaatcgaagca  
GlyLeuProLeuSerLeuLysValLeuGly-----  
yThrIleAspLeuLysHisIleGlyGlnLeuSerValGluArgLeuSerArgGlyGluProValAspGluThrHisGluSerLysValPheAlaGlnIleGluAla

## 163-16

ggtctacctttgtctctgaaagt**ccttggcgcttcattaacgat**cgacctgaaacatattgggcaattg**cagtgg**agaggttatcaagaggtgaacctgttgatgaaactcatga  
GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrTrpAlaIleAspSerGlyGluValIleLysArg .

## in HIF 10499 background

### 170-4

ggtctacctttgtctctgaaagt**ccttgg**-**gcttcattaacgat**cgacctgaaacatattgggcaattg**cagtgg**agaggttatcaagaggtga  
GlyLeuProLeuSerLeuLysValLeuGlyLeuHis .

### 172-9

ggtctacctttgtctctgaaagt**ccttggcgcttcattaacgat**cgacctgaaacatattgggcaattg**cagtgg**agaggttatcaagaggtgaacctgttgatgaaactcat  
GlyLeuProLeuSerLeuLysValLeuGlyAlaSerLeuAsnAspArgProGluThrTyrTrpAlaIleAspSerGlyGluValIleLysArg .

## 176-15

ggtctacctttgtctctgaaagt**ccttgg**-----  
cgacctgaaacatattgggcaattg**cagtgg**agaggttatcaagaggtgaacctgttgatgaaactcatgagagtaaagtgtttgctcaaatcgaagcaactctag  
GlyLeuProLeuSerLeuLysValLeuGly-----  
yAspLeuLysHisIleGlyGlnLeuGlnTrpArgGlyTyrGlnGluValAsnLeuLeuMetLysLeuMetArgValLysCysLeuLeuLysSerLysGlnLeu

**Table S1: List of primers used for qPCR and CHOP qPCR**

<b>Gene</b>	<b>Experimentation</b>	<b>LP primer (5' &gt; 3')</b>	<b>RP primer (5' &gt; 3')</b>
At1g47550	qPCR	CTCGCTCTTTCCGTCAAATC	CCCCAGTGTGAAAAGTGCATC
At1g54610	qPCR	GGTCGGACAGAGGTAGAGCAG	GTATGGTTCACGGGGTTTGT
At5g38470	qPCR	TGIACTCGGGTATCCCTGCT	CTGGAGCTGCTGCTTGTTG
At5g47260	qPCR	AAGGTGGTCCAATCGGGAAC	GATGGGGCAATCTGGIGTGA
At5g47280	qPCR	AGTCTCTGGCTTGAGAGGGT	ATGGTCGAAGGTAGTTCCGC
At5g47260	CHOP qPCR	TGCGTCGACCTATCGTTACA	CCATGCCGTATCAAGCAAC
At5g13440	CHOP qPCR	ACAAGCCAATTTTTGCTGAGC	ACAACAGTCCGAGTGTGATGGT
At5g47400	CHOP qPCR	GAAGCCGAAC TGCAAAC TGT	ATGGTCCGGCTCTAGGAAAA

## Supplementary references

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