

SUPPORTING INFORMATION

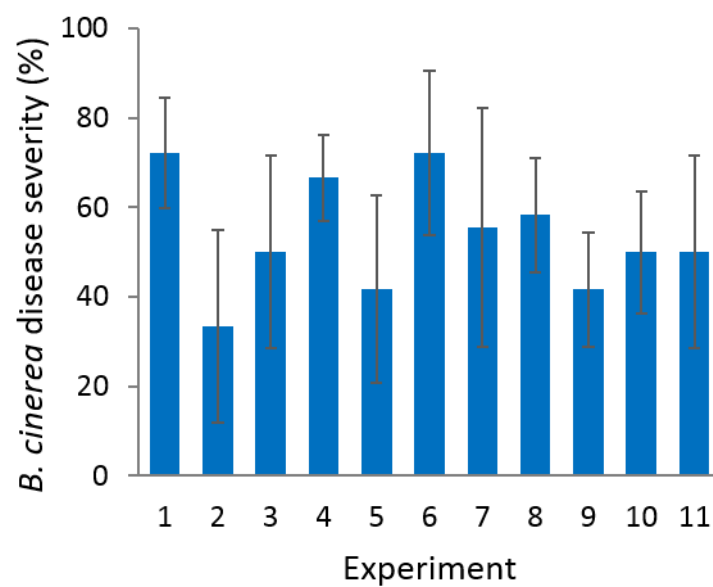
Mining the natural genetic variation in *Arabidopsis thaliana* for adaptation to sequential abiotic and biotic stresses

Silvia Coolen, Johan A. Van Pelt, Saskia C.M. Van Wees and Corné M.J. Pieterse

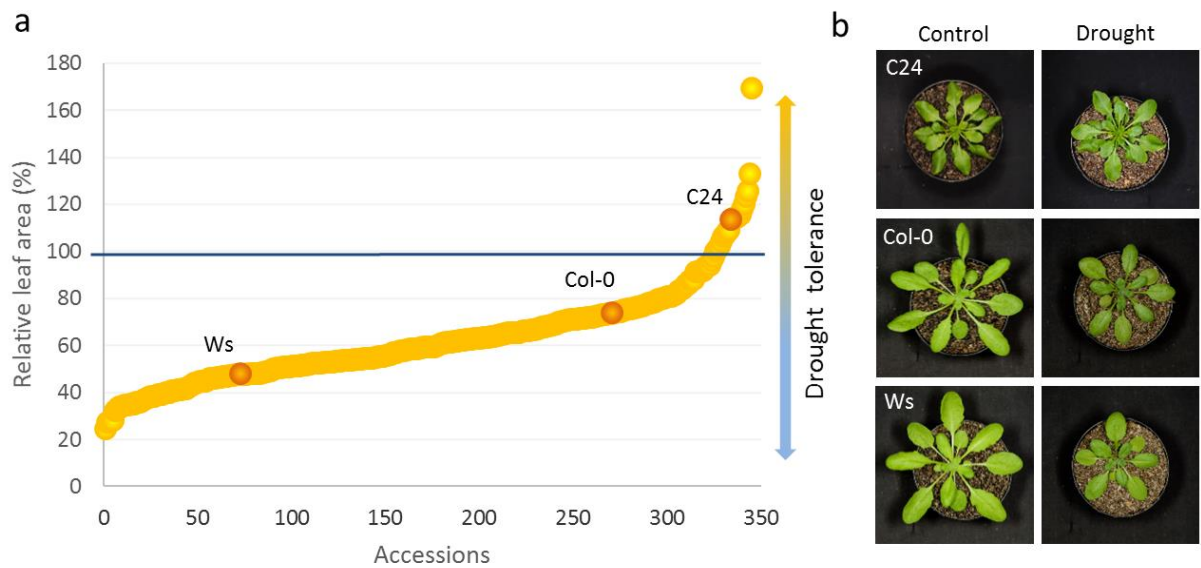
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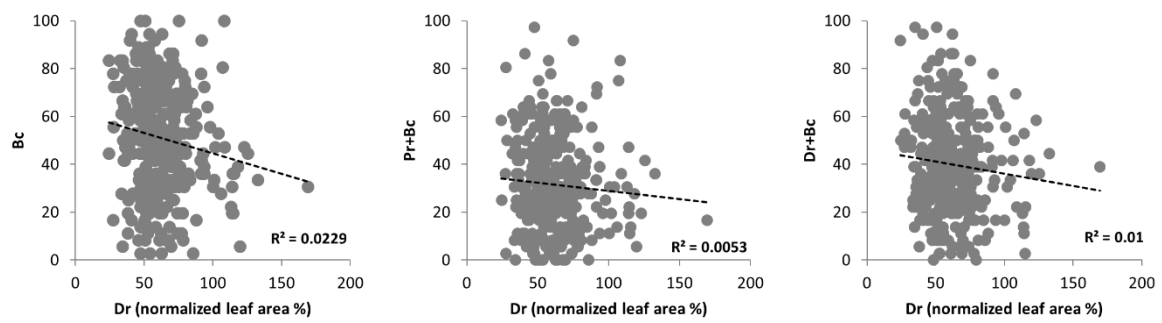
SUPPLEMENTAL FIGURES S1 – S9



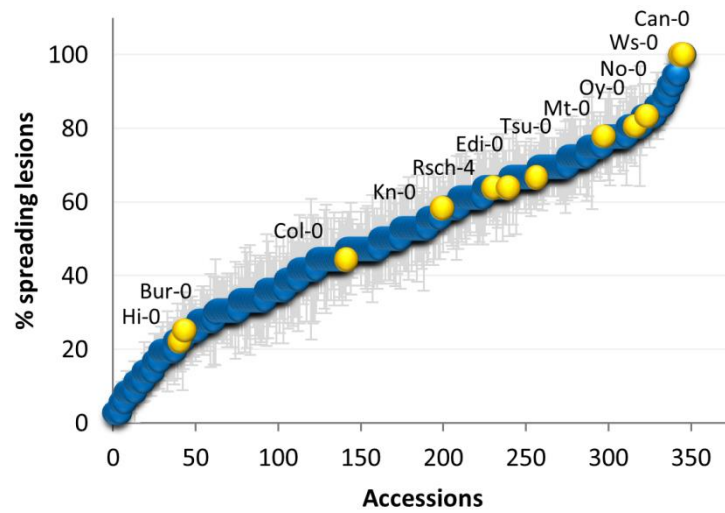
Suppl. Fig. S1 *B. cinerea* disease severity during 11 rounds of experiments in average percentage of spreading lesions per plant ($n = 6$) at 3 d after pathogen inoculation. Error bars represent the standard error



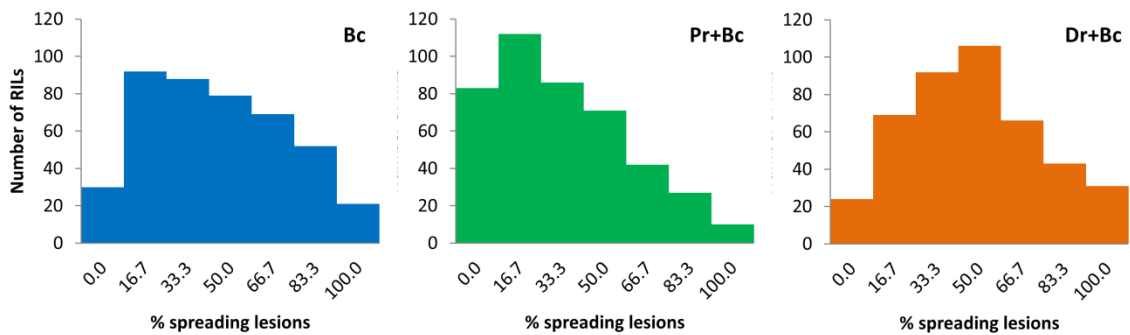
Suppl. Fig. S2 Natural variation of drought stress tolerance amongst 346 *A. thaliana* accessions. **a** Level of drought tolerance of all 346 tested *A. thaliana* HapMap accessions. Of each accession, photographs were taken from representative 4-week-old plants that were normally-watered or withheld from water for 7 days. Subsequently, total leaf area per plant was measured using the Adobe Design and Web Premium (CS6) Analysis tool. The leaf area of normally-watered plants is set at 100% for each accession (blue horizontal line), the leaf area of drought-stressed plants of each accession are plotted relative to that. **b** Visual effects of drought stress on the drought-tolerant accession C24 (CS76106), and the drought-sensitive accessions Col-0 (CS76113) and Ws (CS28823), 7 d after water withheld



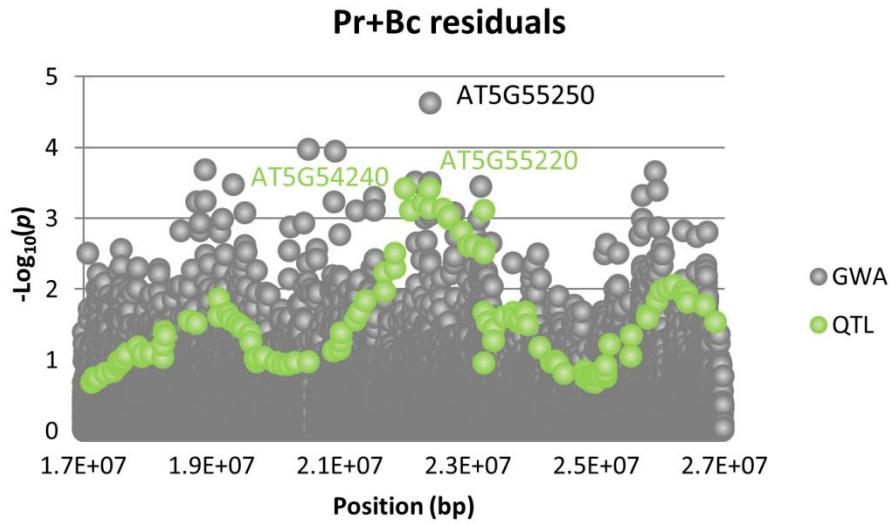
Suppl. Fig. S3 Relation between *Botrytis* resistance and drought tolerance. Correlation between the level of *B. cinerea* resistance in the 346 *A. thaliana* accessions for the single and the sequential double stresses (PrBc and DrBc) versus drought (normalized leaf area %). In the plots a linear regression line was fitted with corresponding Pearson correlation (R^2) values



Suppl. Fig. S4 *B. cinerea* disease severity in 12 MAGIC parental accessions within the population of 346 *A. thaliana* accessions of the HapMap collection. Dotplot showing the level of *B. cinerea* disease severity in average percentage of spreading lesions per accession ($n = 6$) at 3 d after pathogen inoculation. The 346 accessions of the *A. thaliana* HapMap collection are ordered on the x-axis according to their level of *B. cinerea* disease severity in the single stress condition. The parental accessions of the MAGIC RILs are indicated in yellow. Error bars represent the Standard Error (SE)



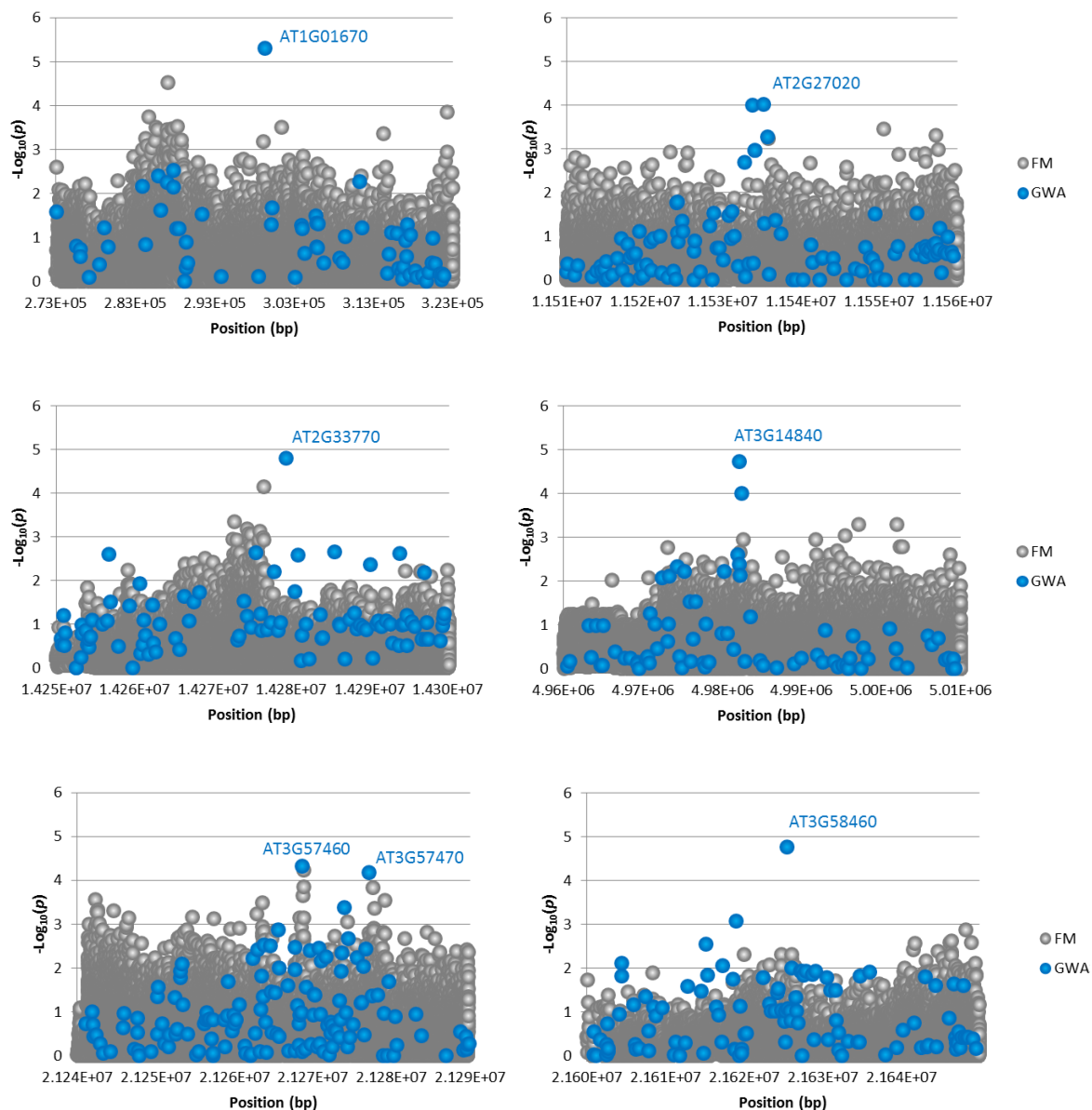
Suppl. Fig. S5 Phenotypic variation in the level of *B. cinerea* resistance in the 431 RILs of the MAGIC population under conditions of single and sequential stress. The graphs show the frequency distribution of the disease severity scores of the 431 RILs ($n=1$) over the indicated disease severity classes. Disease severity classes reflect the average percentage of spreading lesions scored on 6 leaves per plant at 3 d after inoculation with *B. cinerea*. Bc, *B. cinerea* single stress (blue); Pr+Bc, prior herbivory by *P. rapae* followed by *B. cinerea* inoculation (green); and Dr+Bc, prior drought stress followed by *B. cinerea* inoculation (orange)

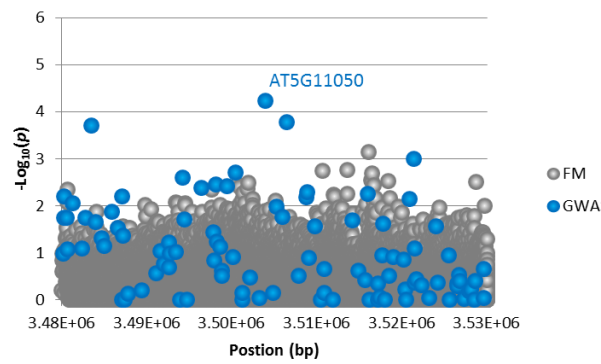
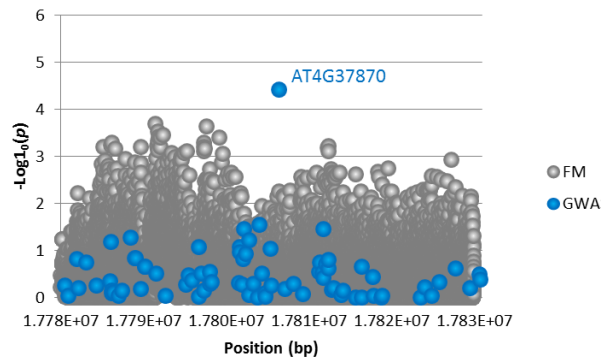
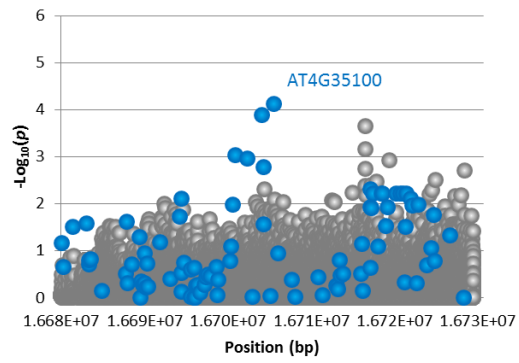


Suppl. Fig. S6. GWA and QTL mapping results of the Pr+Bc residuals dataset around locus AT5G55250. Manhattan plots (grey dots) showing the $-\log_{10}(p)$ values of the SNP marker-trait associations from the GWA mapping results of the Pr+Bc residual data around the GWA $\text{LOD} \geq 4.0$ SNP-trait association at position AT5G55250 on *A. thaliana* chromosome 5 (x-axis; position in base pairs (bp)). QTL mapping results with the *A. thaliana* MAGIC lines are indicated with green dots. Genes indicated in green are significant SNP-trait associations obtained with QTL mapping

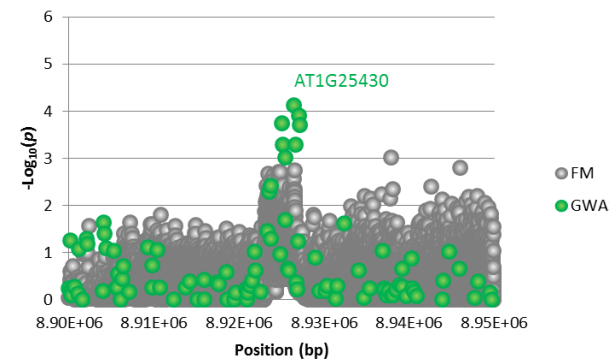
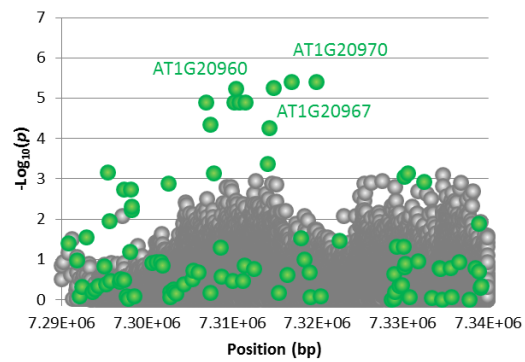
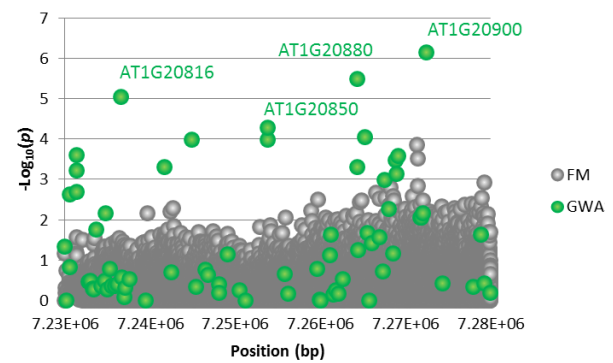
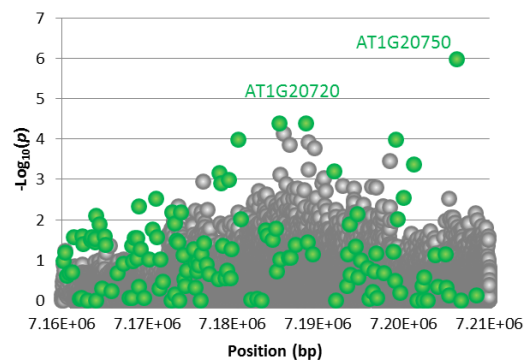
Suppl. Fig. S7 (9 pages below) Non-significant fine mapping results of GWA SNP-trait associations. Manhattan plots for the fine mapping results (grey dots) showing the $-\log_{10}(p)$ values of the SNP marker-trait associations of the GWA loci for the Bc single stress (blue dots), Pr+Bc sequential double stress (green dots), Pr+Bc residual (light green dots), Dr+Bc sequential double stress (orange dots) and Dr+Bc residuals (yellow dots). On the x-axis the position in base pairs (bp) is shown. Genes indicated in blue, green, light green, orange and yellow show genes obtained from the GWA mapping SNP trait associations

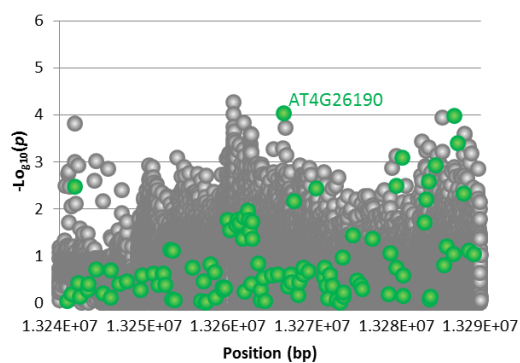
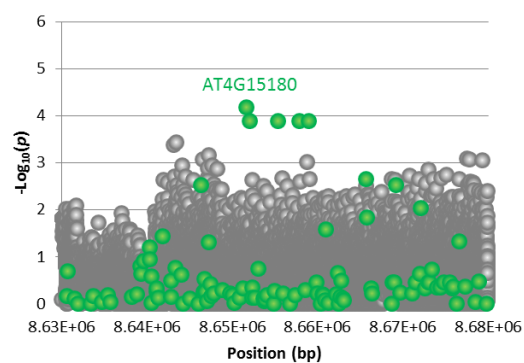
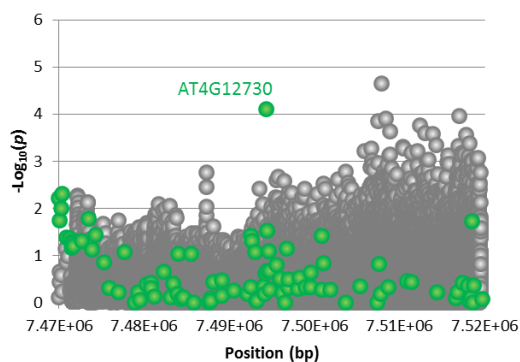
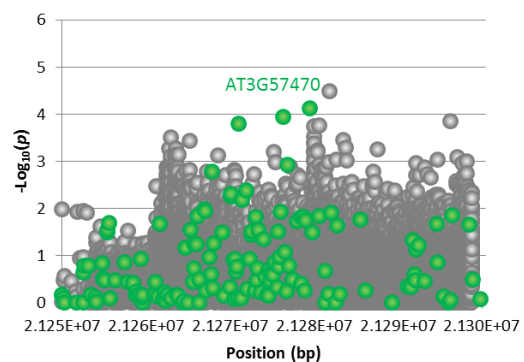
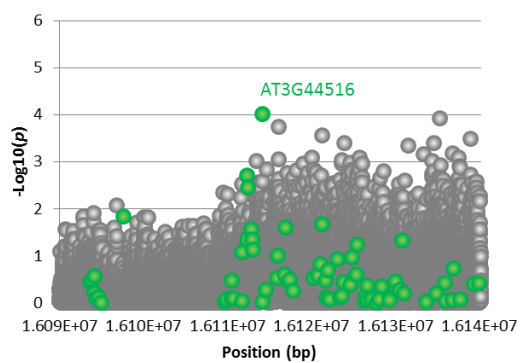
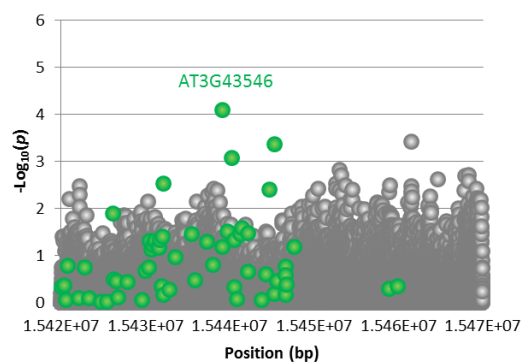
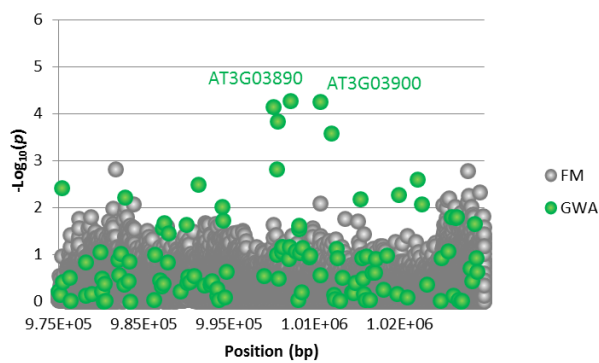
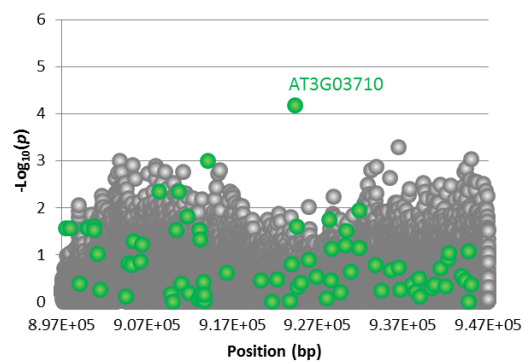
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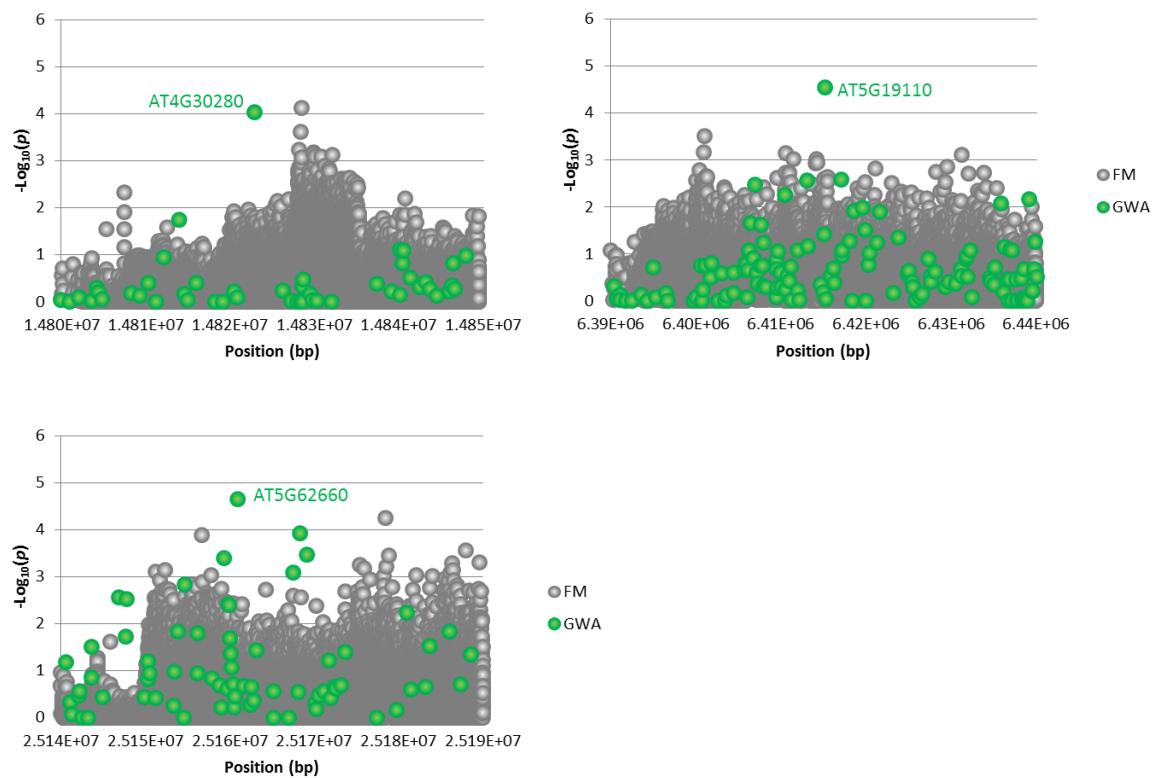




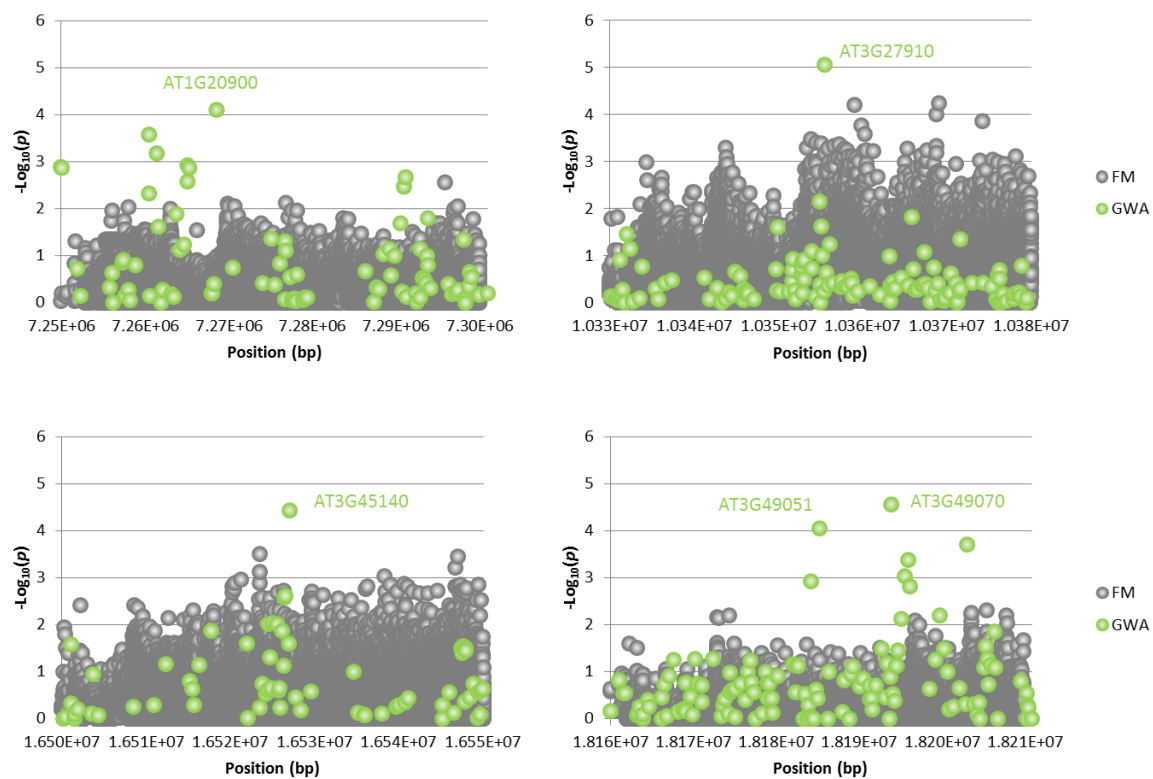
Pr+Bc

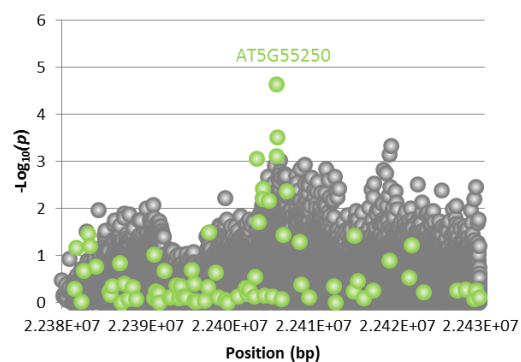
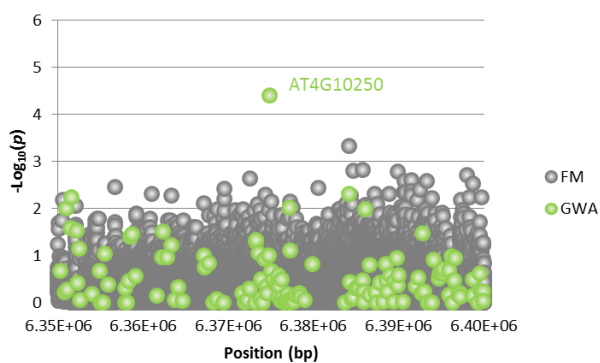
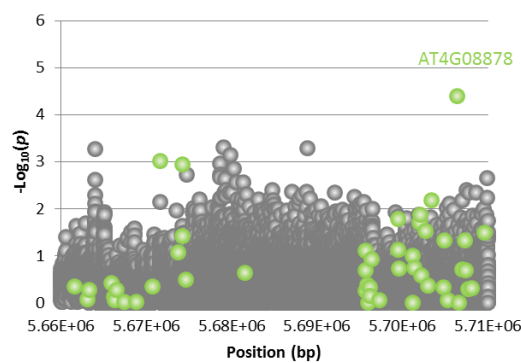
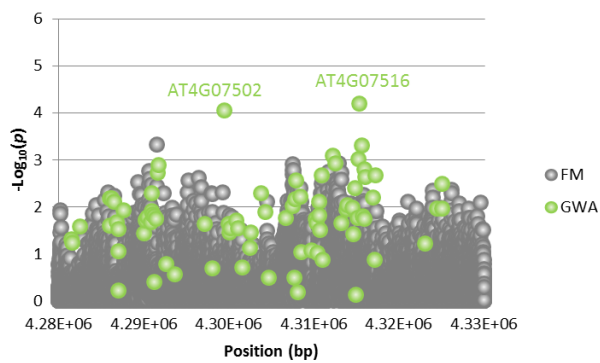
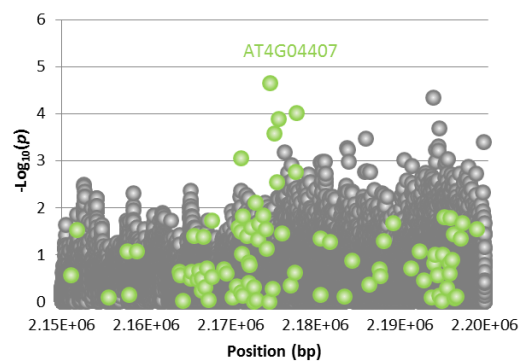




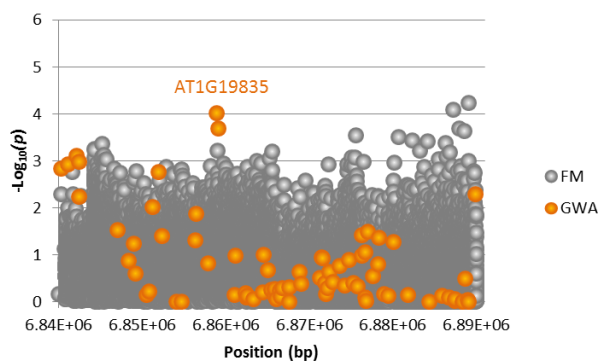
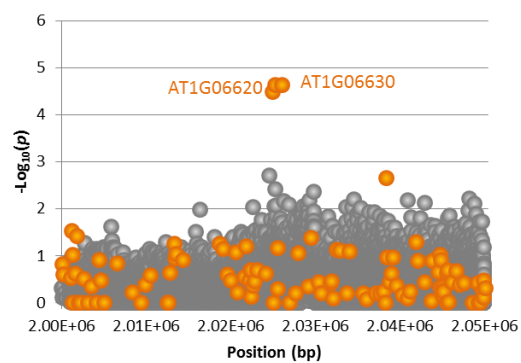


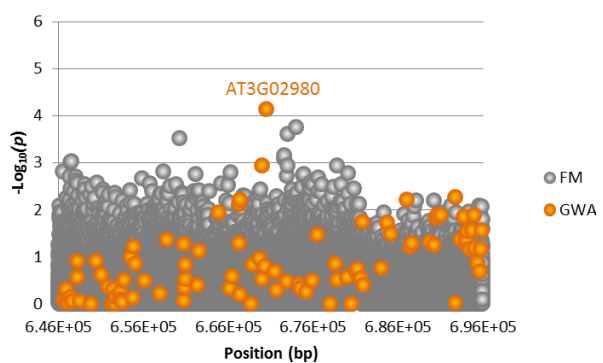
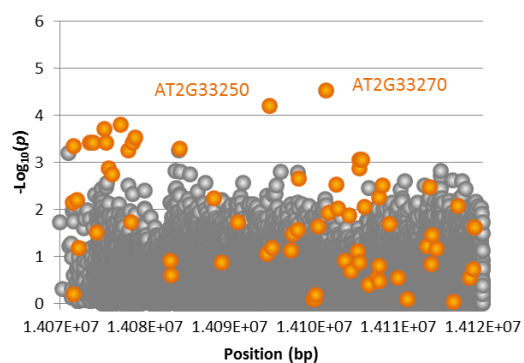
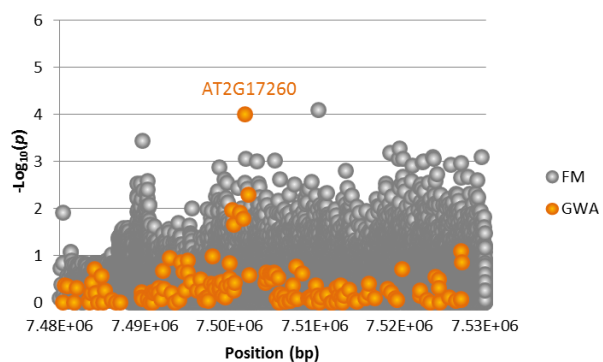
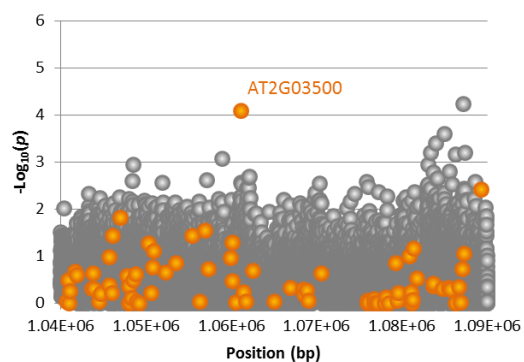
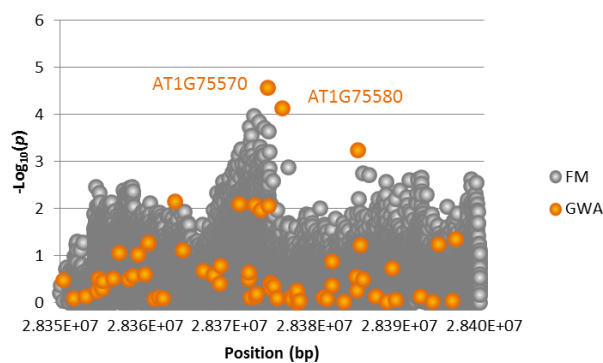
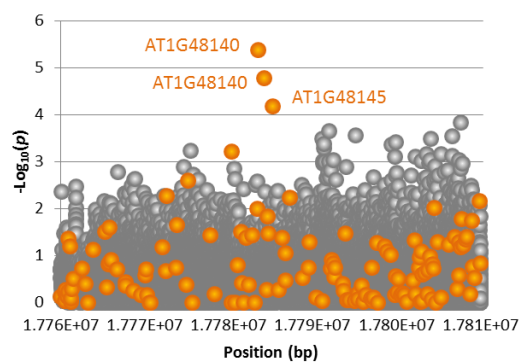
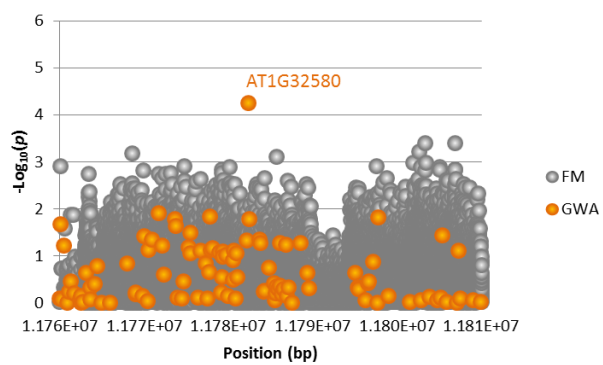
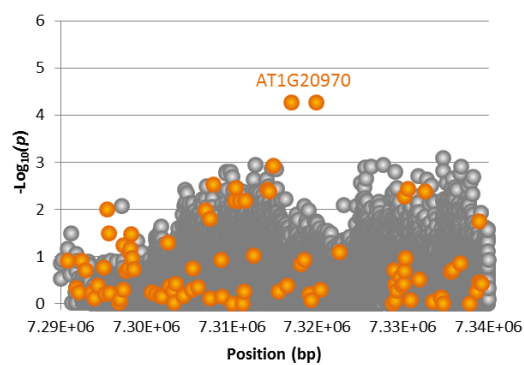
Pr+Bc residuals

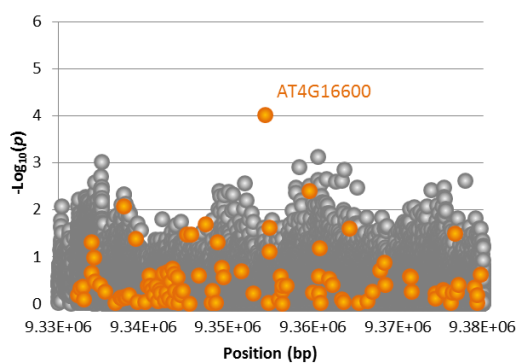
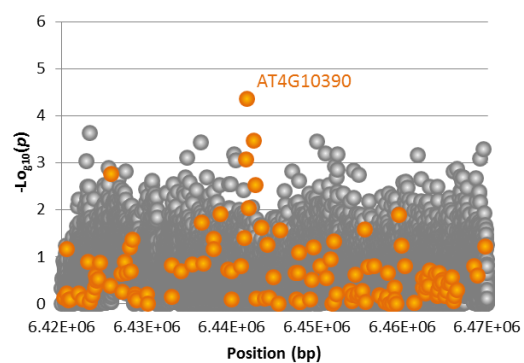
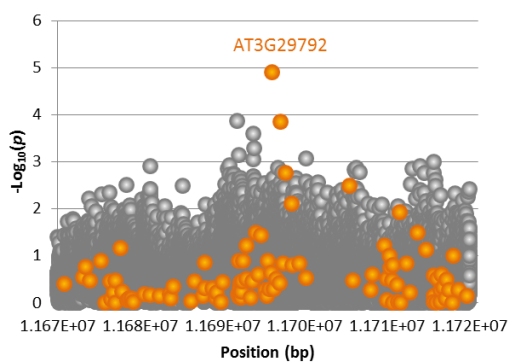
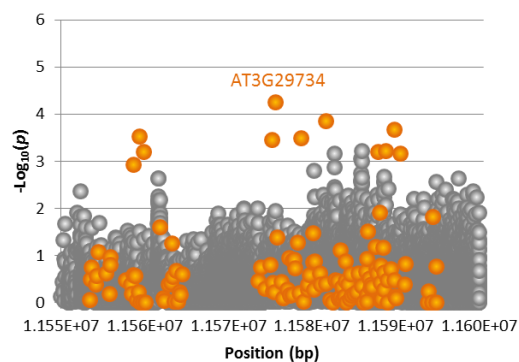
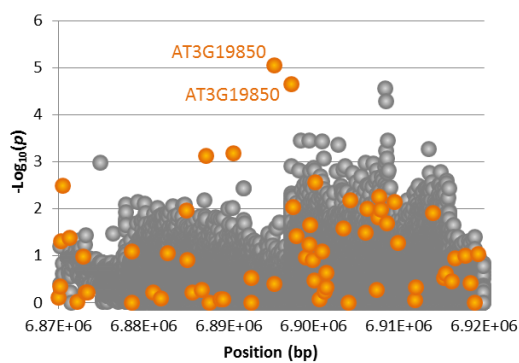
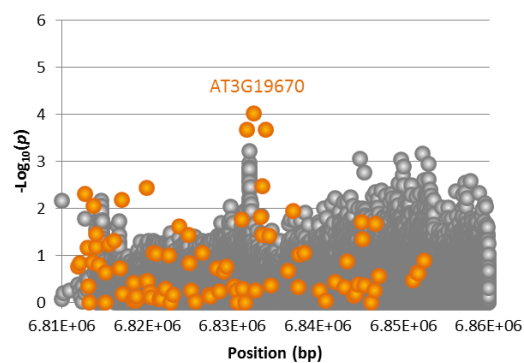
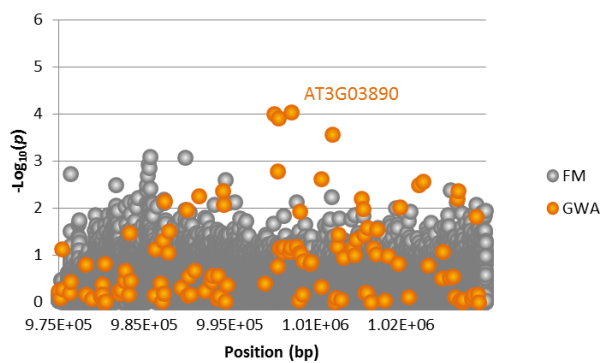
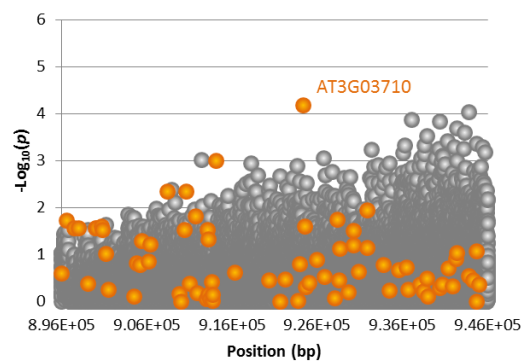


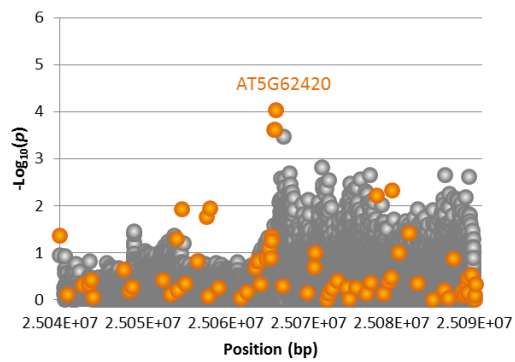
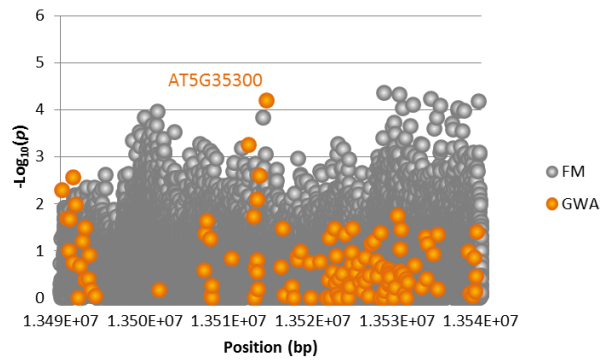
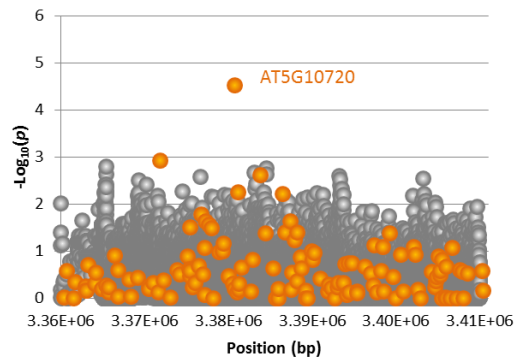


Dr+Bc

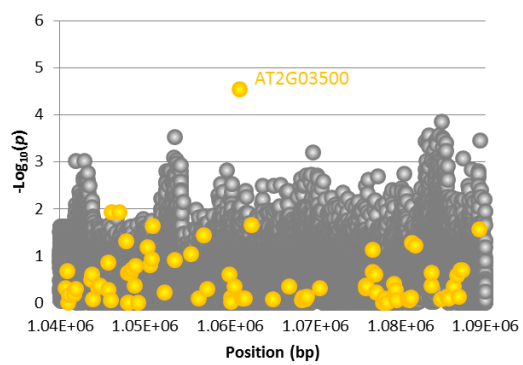
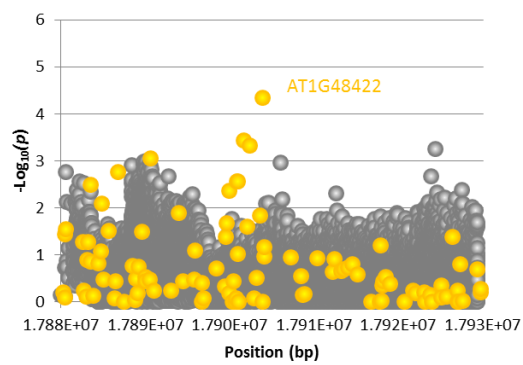
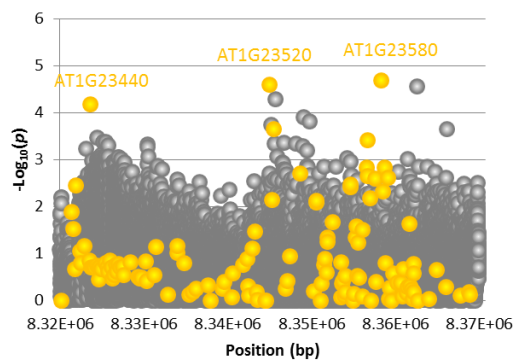
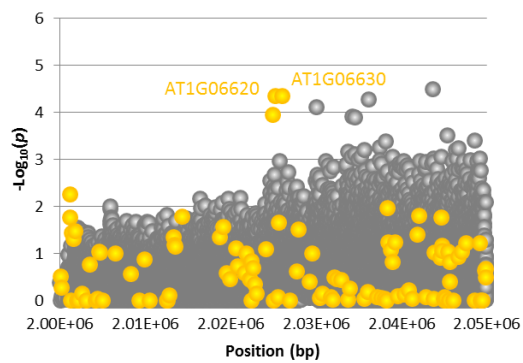


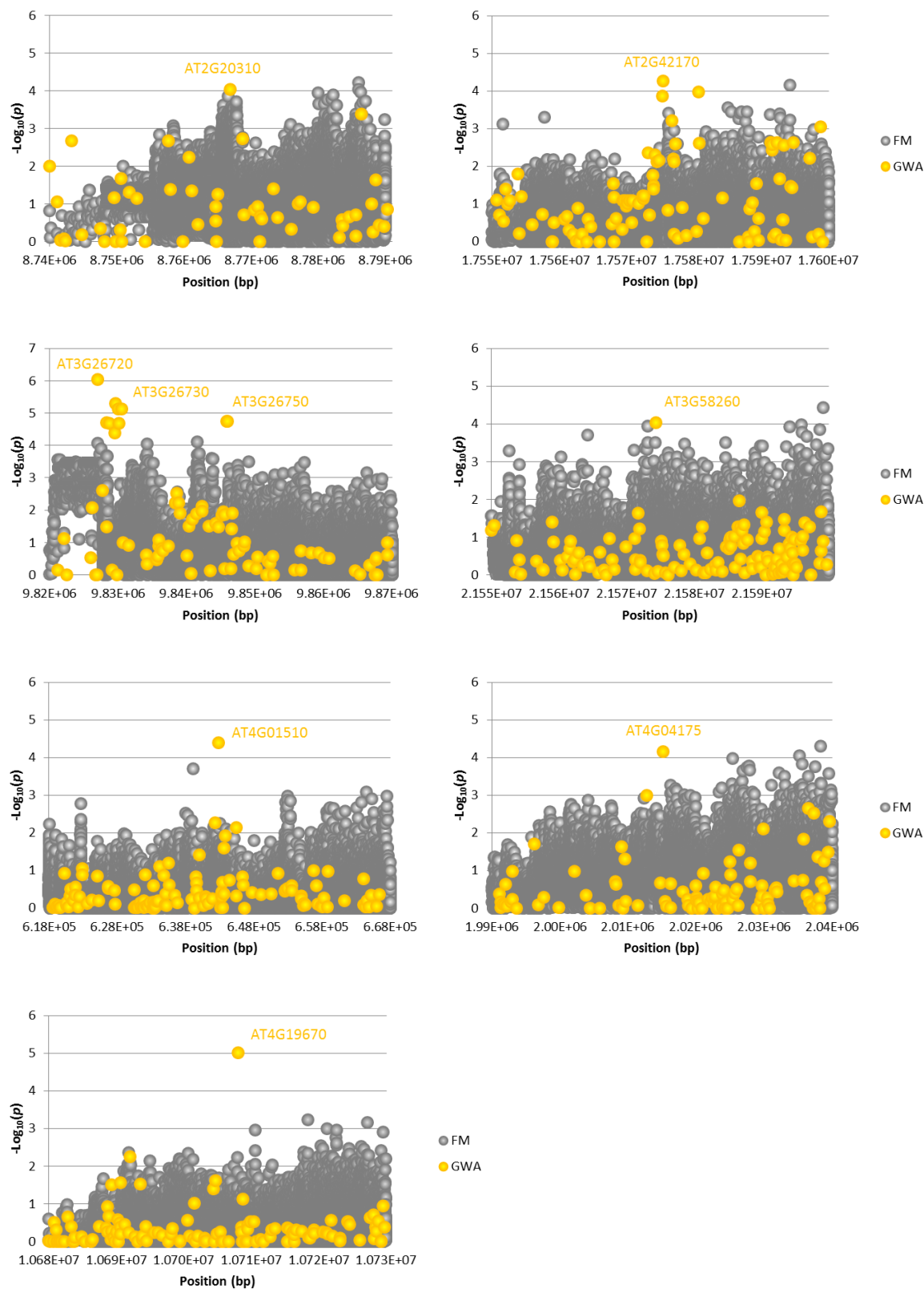




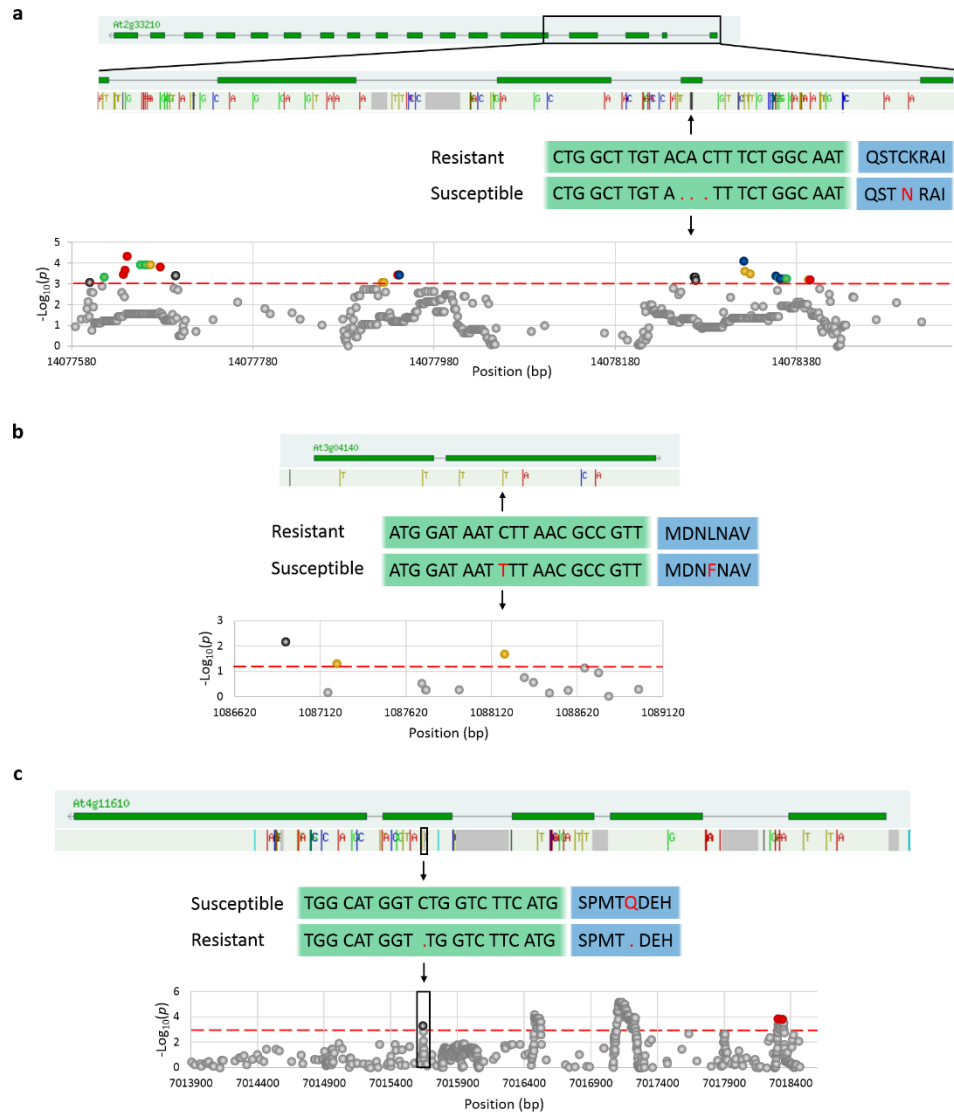


Dr+Bc residuals

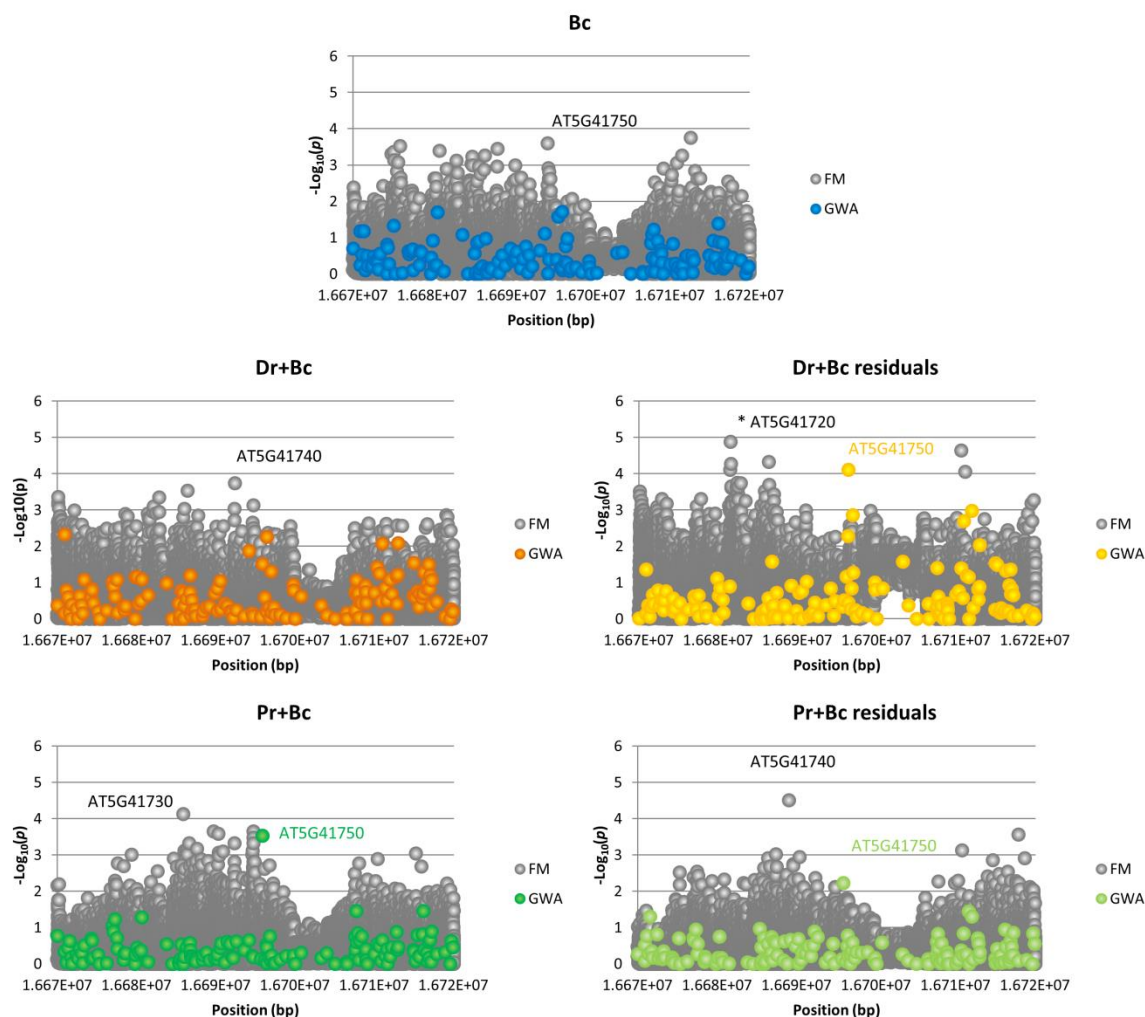




==== End Suppl. Fig. S7 ====



Suppl. Fig. 8 Schematic overview of amino acid changes associated with the phenotypic variation in *B. cinerea* disease severity during single and sequential double stress. Manhattan plots for the fine mapping results showing the $-\log_{10}(p)$ values of the SNP marker-trait (FDR-corrected) associations of three fine mapping-verified candidate genes: **(a)** AT2G33210, **(b)** AT3G04140, and **(c)** AT4G11610. On the x-axis the position in base pairs (bp) is shown. Genes are indicated by the green boxes (exons) and grey lines (introns). Gene direction is indicated with a grey arrow pointing to the transcribed direction. SNPs different from the Col-0 reference genome are marked with colors (A, red; C, blue; G, green; T, yellow), deletions are indicated with black lines and unsequenced regions with grey blocks (picture from <http://signal.salk.edu/atg1001/3.0/gebrowser.php>). Nucleotide sequences within exons (green boxes) are shown for both Col-0 (upper sequence) and the natural variant. Corresponding amino acid sequences are indicated by the blue boxes. Changes within the nucleotides and amino acids are indicated in red.



Suppl. Fig. S9 Fine mapping results of non-confirmed GWA SNP-trait associations at the TIR-NBS-LRR *R*-gene locus AT5G41750. Manhattan plots for the fine mapping results (grey dots) showing the $-\log_{10}(p)$ values of the SNP marker-trait associations of the GWA *R*-gene AT5G41750 locus for the Bc single stress (blue dots), Pr+Bc sequential double stress (green dots), Pr+Bc residual (light green dots), Dr+Bc sequential double stress (orange dots) and Dr+Bc residuals (yellow dots). On the x-axis the position in base pairs (bp) is shown. Genes indicated in blue, green, light green, orange and yellow show SNP-trait associations obtained with GWA mapping and the genes indicated in black corresponds to the non-significant fine mapping associations. The significant (FDR-corrected) fine mapping SNP-trait association for the Dr+Bc residual data (Fig. 3) is marked with an asterisk