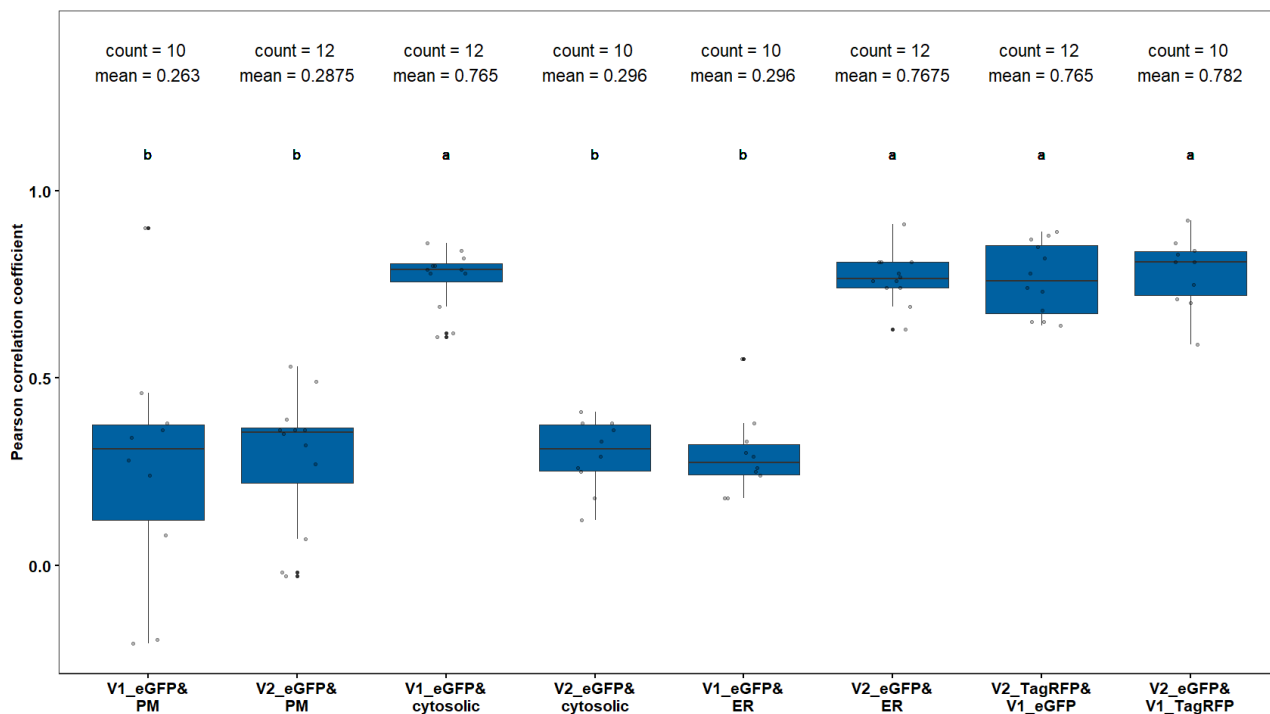
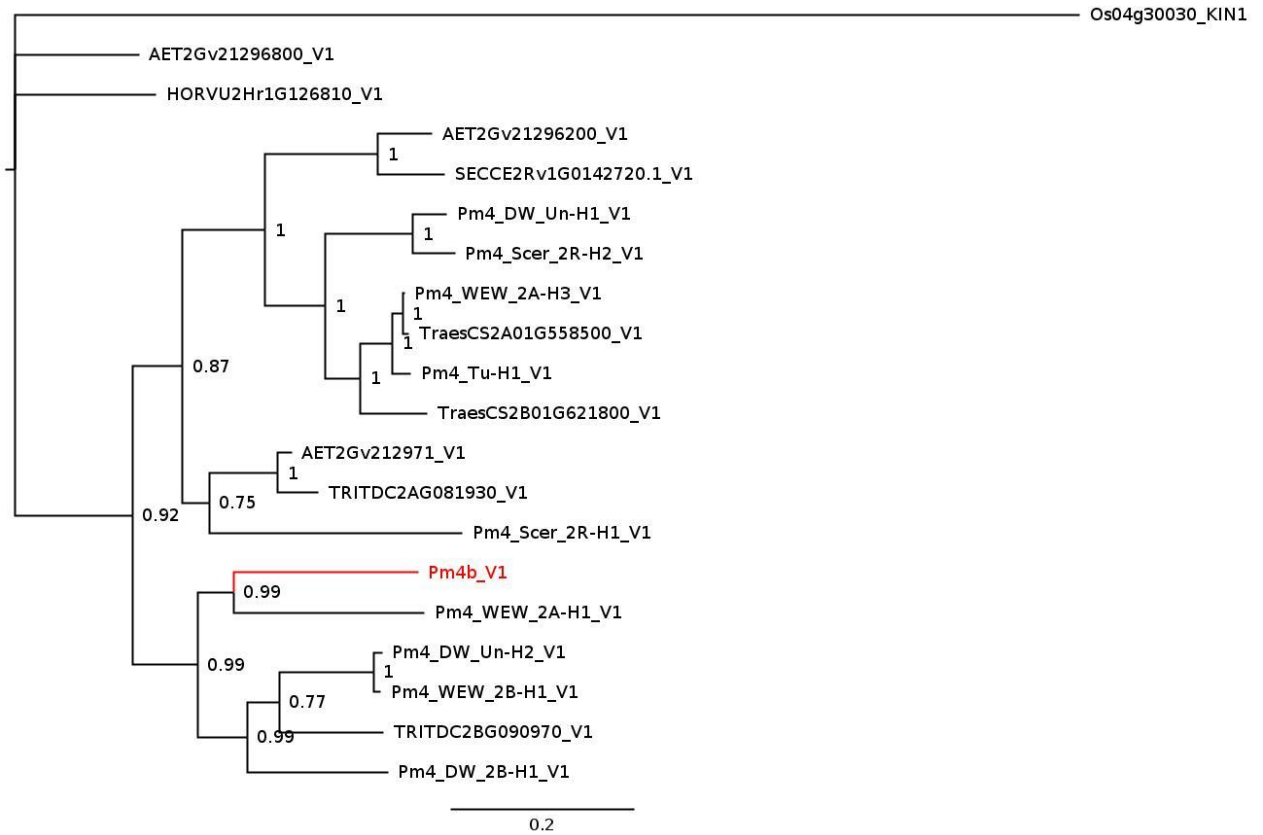
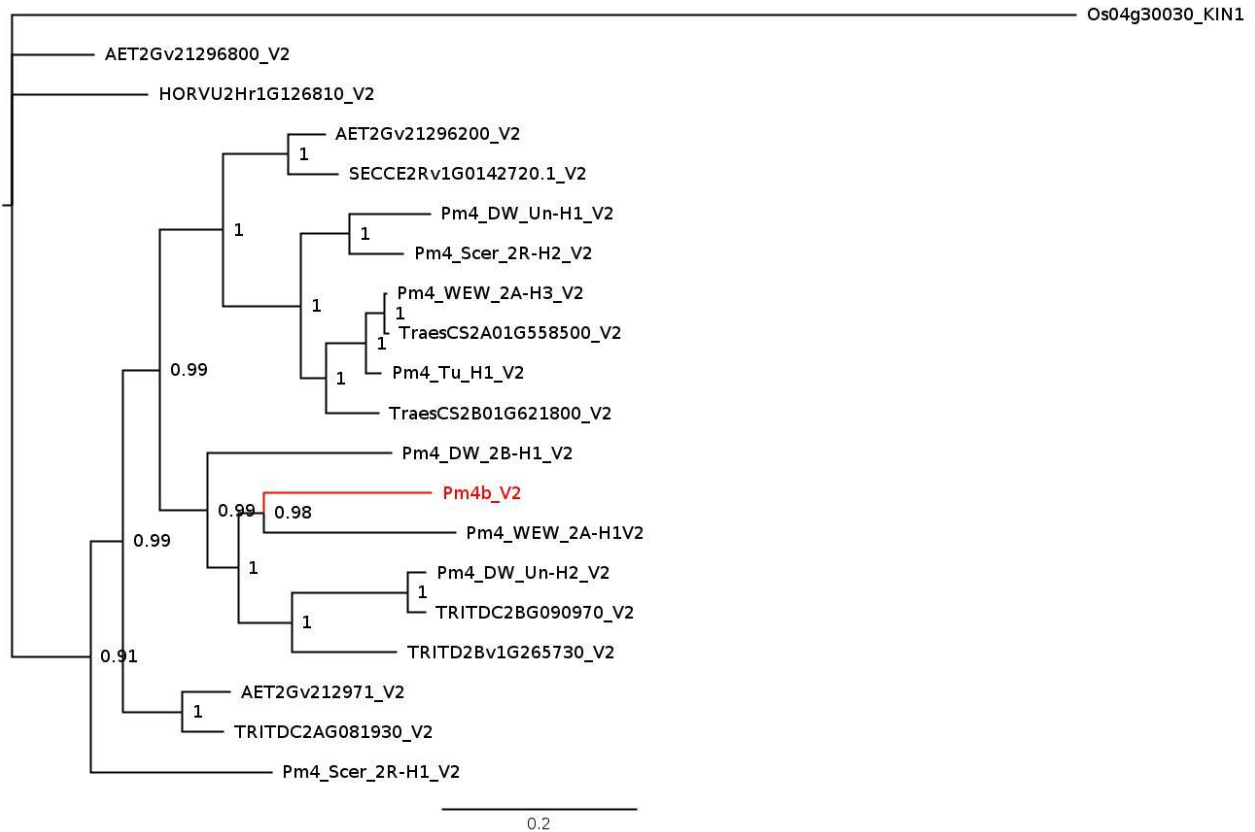


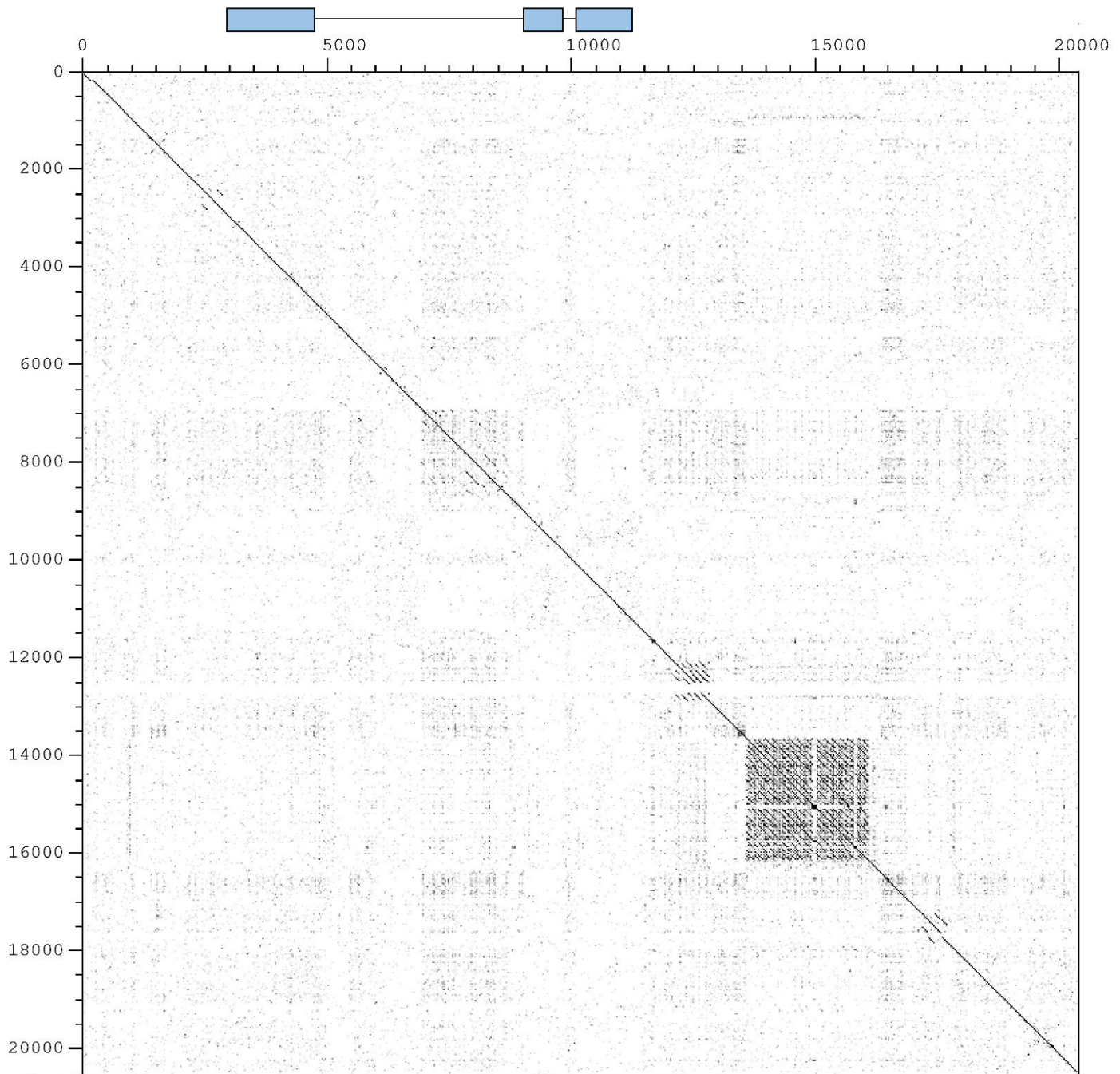
Supplementary Fig. 1 | Phylogenetic analysis of core kinase domains of described resistance proteins and Pm4b. The phylogenetic tree is based on the core kinase domains delimited based on Conserved Domain Database (CDD) from NCBI. The location of the domain is indicated by the sequence range numbers. In red, the core kinase domain of Pm4b. cAPK-alpha was used as outgroup.

a

Supplementary Fig. 3 | Co-localization analysis of Pm4b_V1 and Pm4b_V2 with characterized markers. Pm4b_V1 and Pm4b_V2 isoforms were co-infiltrated with the plasma membrane-marker (35S:REM 1.2 m_RFP40), the mRFP-fused cytosolic localization sequence (pGWB45538) and the ER-marker (ER-ck, CD3-95939) to examine their subcellular localization. Pm4b_V1 mainly co-localizes with the cytosolic marker while Pm4b_V2 with the ER marker. High Pearson correlation coefficients of Pm4b_V1 and Pm4b_V2 indicate their co-localization when co-expressed. On top of each boxplot, number of observations and means. Different letters indicate significant differences using ANOVA followed by Tukey honest significant difference (HSD) test ($P < 0.05$). At least $n = 10$ single-scanned cell images per experiment were collected and analyzed using the same conditions of laser intensity, pinhole size, and gain levels. In the boxplots, center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by the `geom_boxplot` function of the `ggplot2` R package; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, individual data points are represented by dots.

a**b**

Supplementary Fig. 4 | Phylogenetic analysis of Pm4 homologues. The tree on the top corresponds to full-length predicted proteins based on Pm4b_V1 isoform. Likewise, isoform Pm4_V2 is displayed in the bottom. In red, Pm4b_V1/V2. For both cases, the kinase domain of the rice Os04g30030 was used as outgroup.



Supplementary Fig. 5 | Sequence comparison of the contig_18057 in wheat cultivars Fed-*Pm4b* and SYMattis. Dotplot alignment of the Pm4 contig_18057 from Fed-*Pm4b* (horizontal) and SYMattis (vertical). On top of the dotplot, it is displayed a schematic drawing of the Pm4 CDS. The first blue box corresponds to exons one to five. The second and third blue boxes, to exons six and seven, respectively. SYMattis contained the *Pm4* contig_18057 sequence spanning physical positions 788'726'801-788'747'264, at the very distal end of chromosome arm 2AL. Around 27 bp downstream of the stop codon of Pm4b_V2 lies a novel TE of the Mutator superfamily (<https://www.botinst.uzh.ch/en/research/genetics/thomasWicker/trep-db.html>). Since this TE lies so close to the gene, it provides downstream regulatory sequences to *Pm4*. For example, two putative poly-adenylation signals are located inside this TE.