Supplementary Figures

Fig. S1. Alignment of *PR3b* genomic sequence and CDS (coding sequence) amplified from tobacco Burley 21.

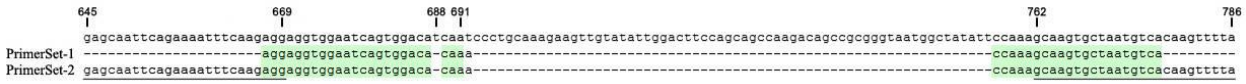

Fig. S2. Sequence analysis of *PR3b* splicing region amplified with different primer sets.

Top line shows the sequence of native *PR3b*. Numbers over native *PR3b* sequence indicates nucleotide positions in the full-length coding sequence. PrimerSet-1 indicates the sequence amplified with primers mentioned in the main text for detection of both native and spliced *PR3b*. The sequence labeled as PrimerSet-2 was amplified with primers according to the underlined sequences.

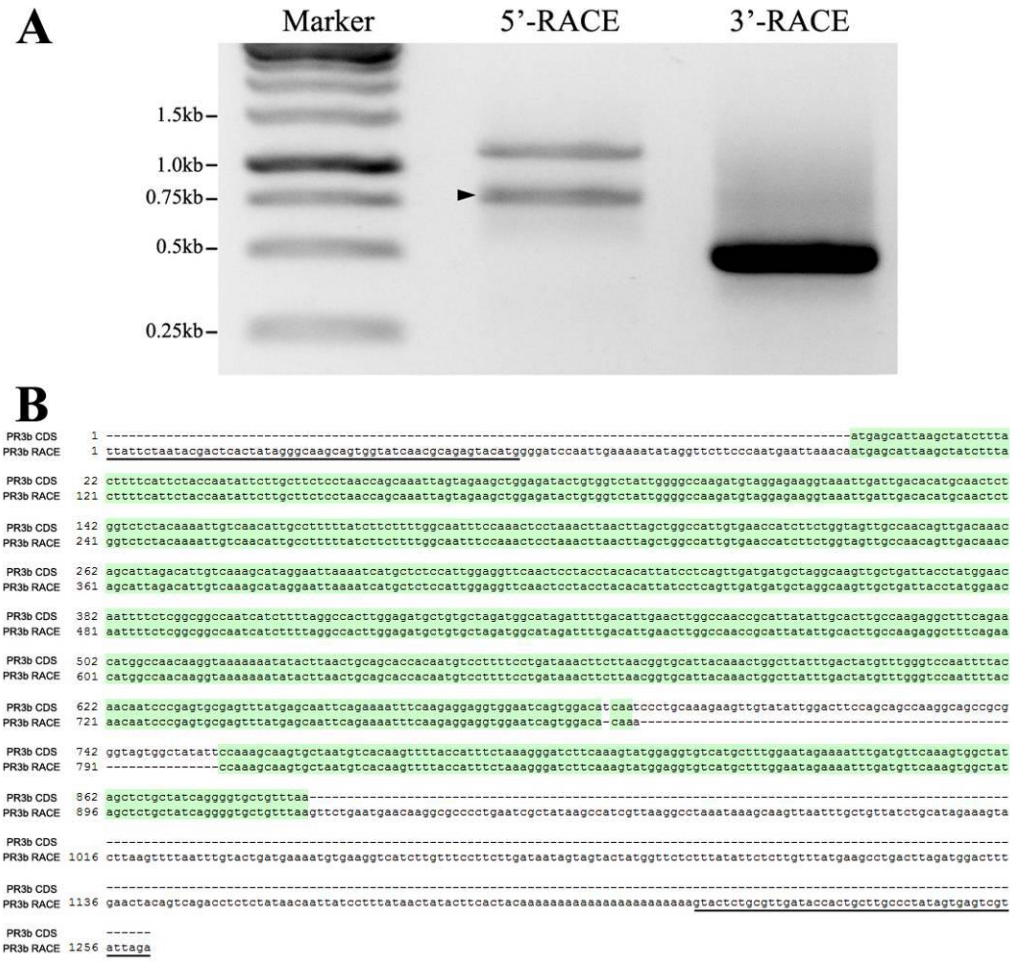

Fig. S3. Rapid amplification of cDNA ends (RACE) of alternatively spliced *PR3b*. (A) Electrophoresis of RACE PCR products. Triangle in the lane of 5'-RACE indicates the band containing target RACE PCR product. (B) Sequence alignment of spliced *PR3b* obtained by RACE PCR and the coding sequence of native *PR3b*. Underlined sequences indicate amplified adapters in the universal primers of SMARTer RACE kit.

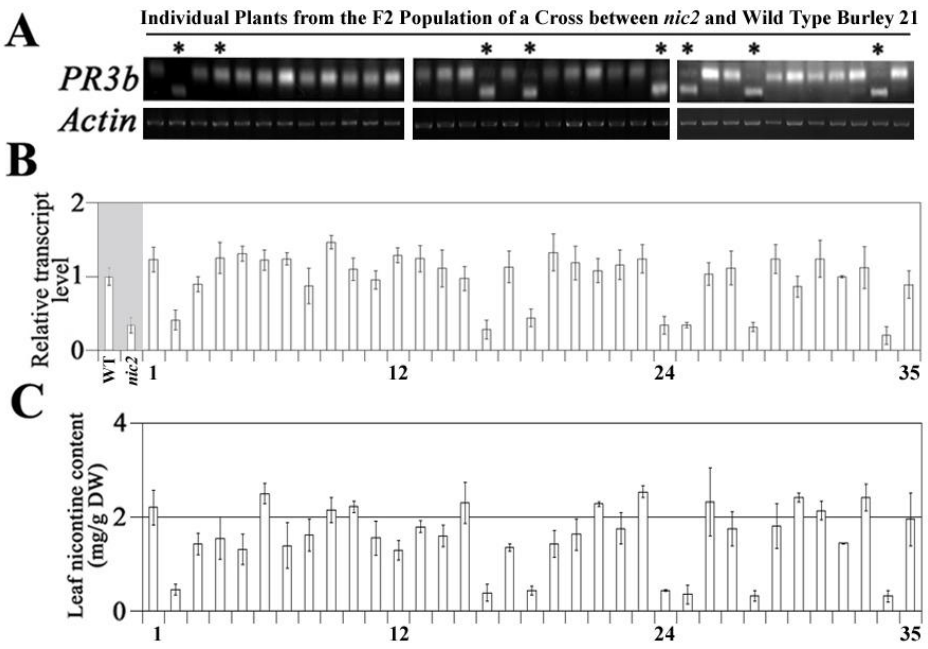

Fig. S4. Alternative splicing of *PR3b* in the F2 individuals of a cross between *nic2* and wild type Burley 21. (A) Alternative splicing of *PR3b* in individual F2 plants. (B) Transcript levels of *PMT1* in the roots of individual F2 plants. Transcript level of *PMT1* in the roots of wild type Burley 21 was set as "1". *Actin* was used as an internal control. (C) Leaf nicotine content of individual F2 plants. Shown values are means of three technical replicates. Error bar, mean ± SD.

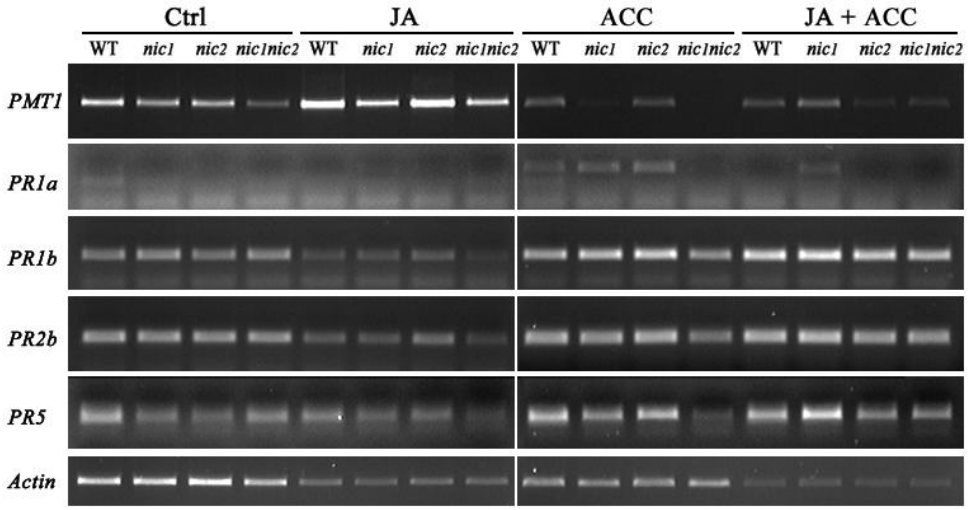

Fig. S5. Phytohormone-induced transcription patterns of PR protein genes. The transcription patterns of PR protein genes in wild type (WT) and low-nicotine mutants (*nic1*, *nic2*, and *nic1nic2*) were analyzed following treatment with JA, ACC, or the combination of JA and ACC (JA+ACC). Shown are representative results of three independent replicates. Ctrl indicates the untreated controls. *Actin* was used as an internal control.