

## Supplementary Figures

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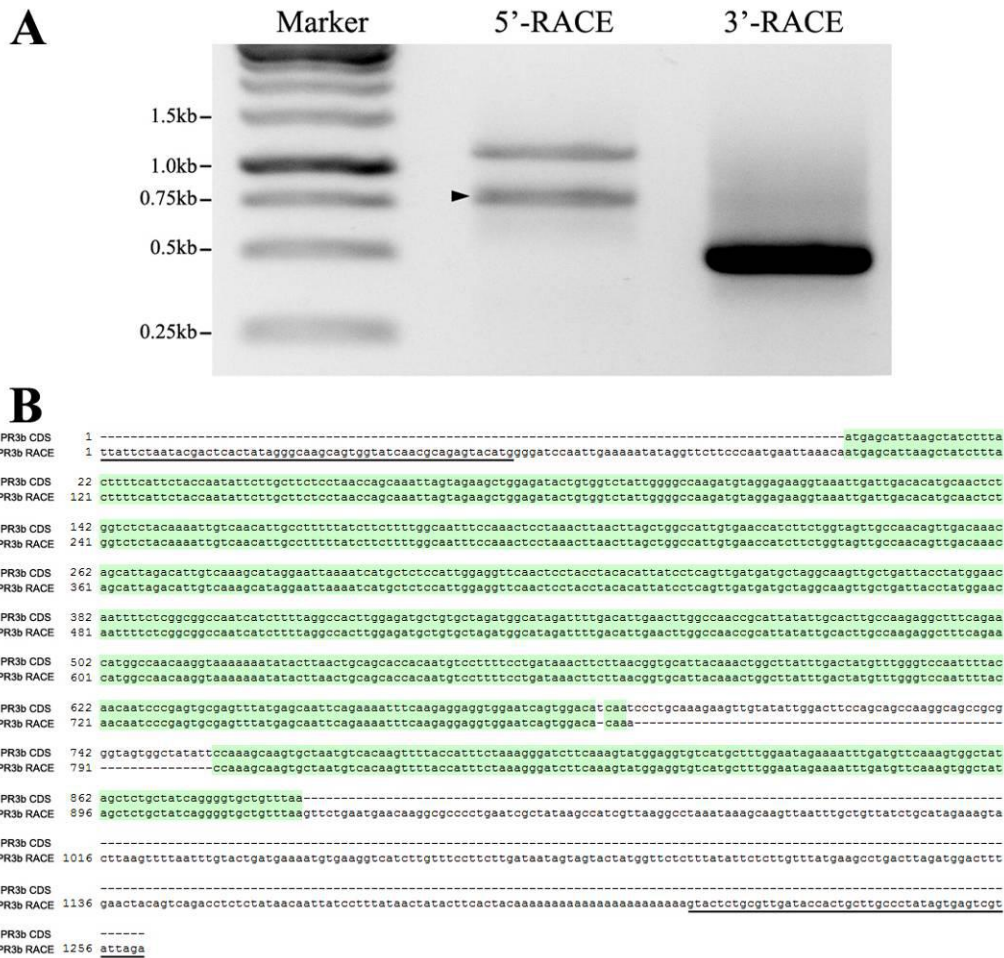
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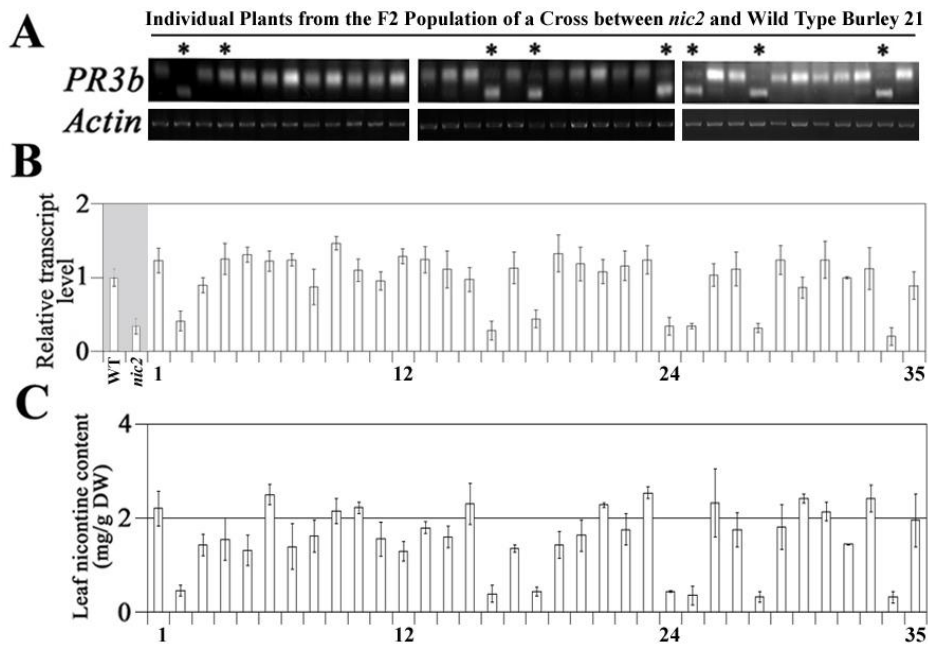
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**Fig. S1.** Alignment of *PR3b* genomic sequence and CDS (coding sequence) amplified from tobacco Burley 21.

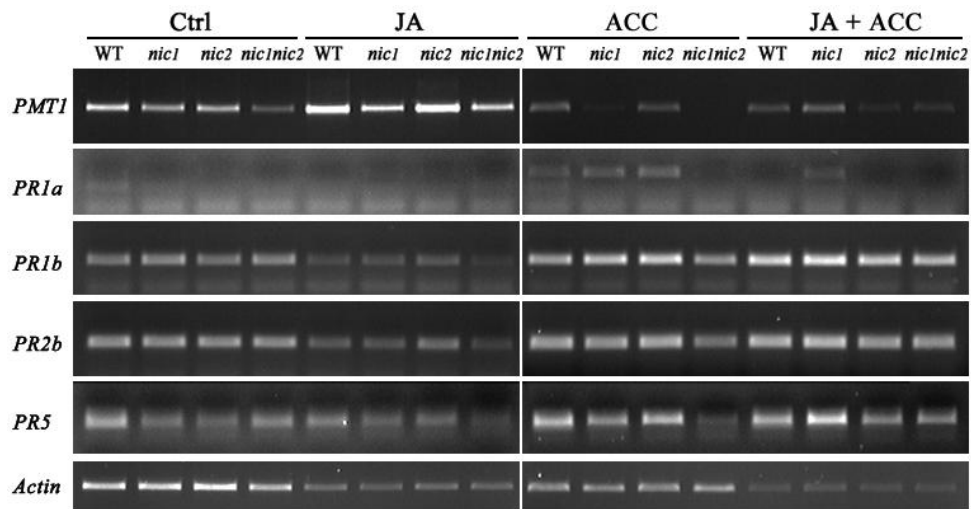




**Fig. S3.** Rapid amplification of cDNA ends (RACE) of alternatively spliced *PR3b*. (A) Electrophoresis of RACE PCR products. Triangle in the lane of 5'-RACE indicates the band containing target RACE PCR product. (B) Sequence alignment of spliced *PR3b* obtained by RACE PCR and the coding sequence 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  $\pm$  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.