


SHORT COMMUNICATION

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The Arabidopsis spliceosomal protein SmEb modulates ABA responses by maintaining proper alternative splicing of *HAB1*



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Abstract

Abscisic acid (ABA) signaling is critical for seed germination and abiotic stress responses in terrestrial plants. Pre-mRNA splicing is known to regulate ABA signaling. However, the involvement of canonical spliceosomal components in regulating ABA signaling is poorly understood. Here, we show that the spliceosome component Sm core protein SmEb plays an important role in ABA signaling. *SmEb* expression is up-regulated by ABA treatment, and analysis of Arabidopsis *smeb* mutant plants suggest that SmEb modulates the alternative splicing of the ABA signaling component *HAB1* by enhancing the *HAB1.1* splicing variant while repressing *HAB1.2*. Overexpression of *HAB1.1* but not *HAB1.2* rescues the ABA-hypersensitive phenotype of *smeb* mutants. Mutations in the transcription factor *ABI3*, 4, or 5 also reduce the ABA hypersensitivity of *smeb* mutants during seed germination. Our results show that the spliceosomal component SmEb plays an important role in ABA regulation of seed germination and early seedling development.

Keywords: ABA signaling, Alternative splicing, Cotyledon greening, *HAB1*, SmEb

Alternative splicing occurs in many genes involved in diverse processes during plant development and responses to abiotic stresses (Staiger and Brown 2013). mRNA splicing is mainly mediated by the spliceosome consisting of five conserved small nuclear ribonucleoproteins (snRNPs) in eukaryotes. The seven Sm core proteins (B/B', D1, D2, D3, E, F and G) are common components of the five snRNPs and are essential for their assembly (Reddy 2007; Wahl et al. 2009). Among the Sm core proteins, SmEb has been reported to positively regulate cold stress response by modulating pre-mRNA splicing (Capovilla et al. 2018). In this study, we found that the transcript level of *SmEb* was induced by ABA treatment (Fig. 1a), which suggests that SmEb might be involved in ABA response. To uncover the molecular mechanism

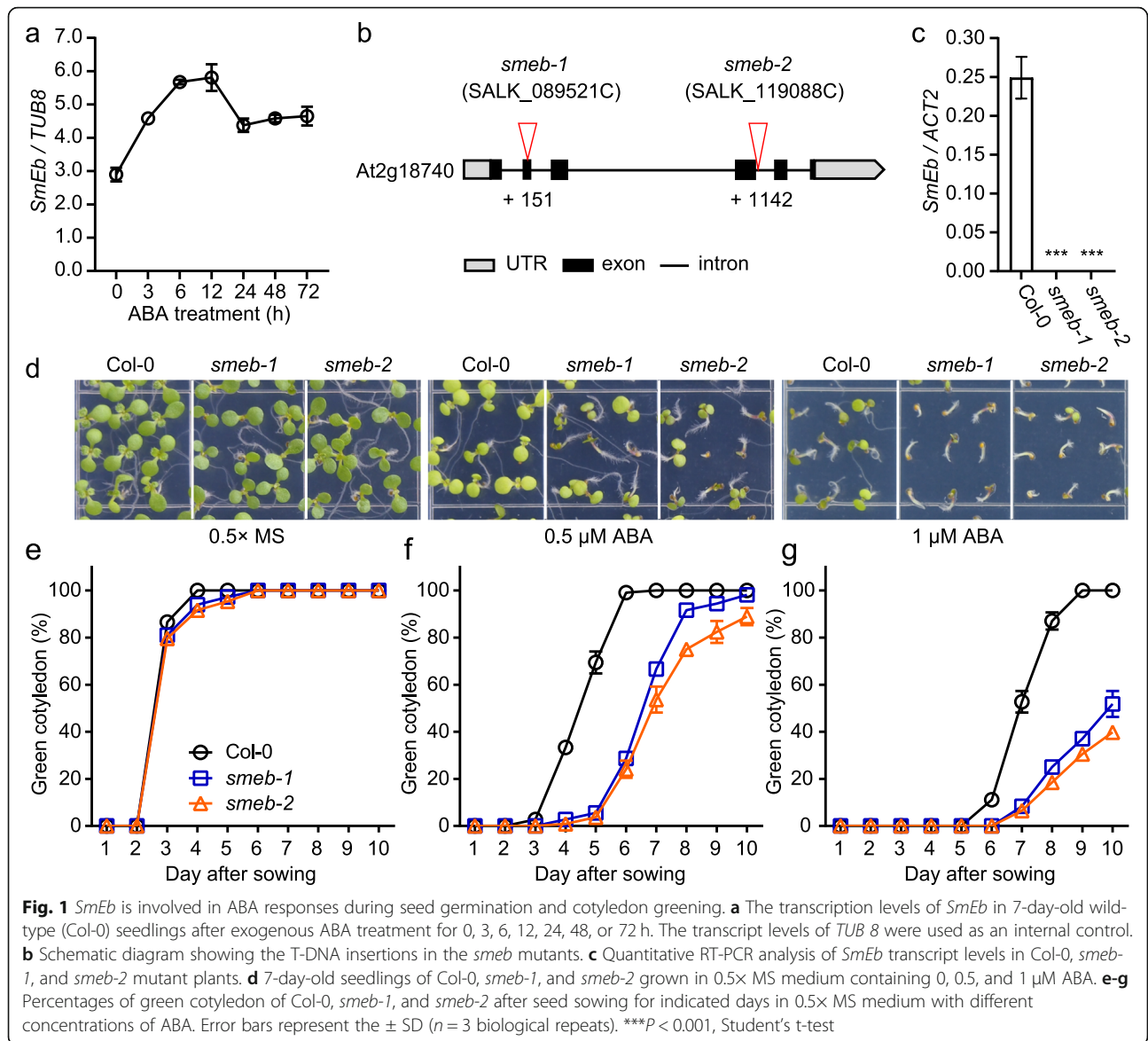
underlying SmEb-modulated ABA response, we first characterized two T-DNA insertion mutant alleles of *SmEb*, designated *smeb-1* and *smeb-2*. The *smeb-1* and *smeb-2* mutants have T-DNA insertions in the second exon and fourth intron of the At2g18740 gene, respectively (Fig. 1b). Quantitative reverse transcription-PCR (qRT-PCR) analysis determined that the transcript level of *SmEb* was nearly undetectable in *smeb-1* and *smeb-2* mutants (Fig. 1c). Phenotyping of *smeb-1* and *smeb-2* showed that these two mutants were much more sensitive to ABA treatment than the Col-0 wild type plants in cotyledon greening during seed germination (Fig. 1d). Under normal growth conditions, the rates of cotyledon greening were not different between *smeb* mutants and wild type plants (Fig. 1e). However, with 0.5 μM ABA supplemented in the medium, the emerged seedlings with green cotyledons in *smeb-1* and *smeb-2* mutants were only approximately 67% and 54%, in contrast to the near 100% green cotyledons in the wild type at the seventh day of the treatment (Fig. 1f). In addition,

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compared with the ~52% green cotyledon rate in the wild type, only approximately 8.3% of *smeb-1* and 6.5% of *smeb-2* mutant cotyledons turned green after 1 μM ABA treatment for 7 days (Fig. 1g). Together, these results indicate that *SmEb* is a positive regulator of ABA responses in Arabidopsis.

The core ABA signaling pathway is initiated by ABA binding to the PYR/PYL/RCAR receptors, which then interact with and inhibit the type 2C protein phosphatases (PP2Cs) co-receptors such as HYPERSENSITIVE TO ABA1 (*HAB1*) (Ren et al. 2017; Gong et al. 2020). *HAB1* serves as a negative regulator for ABA signaling in seed germination and cotyledon greening, and previous studies have shown that the transcriptional up-regulation of *HAB1* is critical for ABA signaling (Melcher et al. 2009; Wang et al. 2018a). As a result,

suppression of the accumulation of *HAB1* transcripts leads to the activation of ABA signal transduction in plants. Recent reports have shown that splicing factors play critical roles in plant response to ABA through regulating the splicing of *HAB1* transcripts (Wang et al. 2015; Zhan et al. 2015; Bi et al. 2021). However, it is unclear whether the canonical spliceosomal component *SmEb* may contribute to the regulation of *HAB1* pre-mRNA splicing and ABA responses. To determine the role of *SmEb* in the regulation of ABA signaling, we first examined the transcripts of *HAB1* by using RT-PCR and Sanger sequencing. As shown in Fig. 2a, alternatively spliced forms of *HAB1* transcripts, *HAB1.1* to *1.5*, were identified under normal and ABA treatment conditions. Only two types of *HAB1* polypeptides were encoded by these five spliced forms. The *HAB1.1*, *HAB1.3*, and

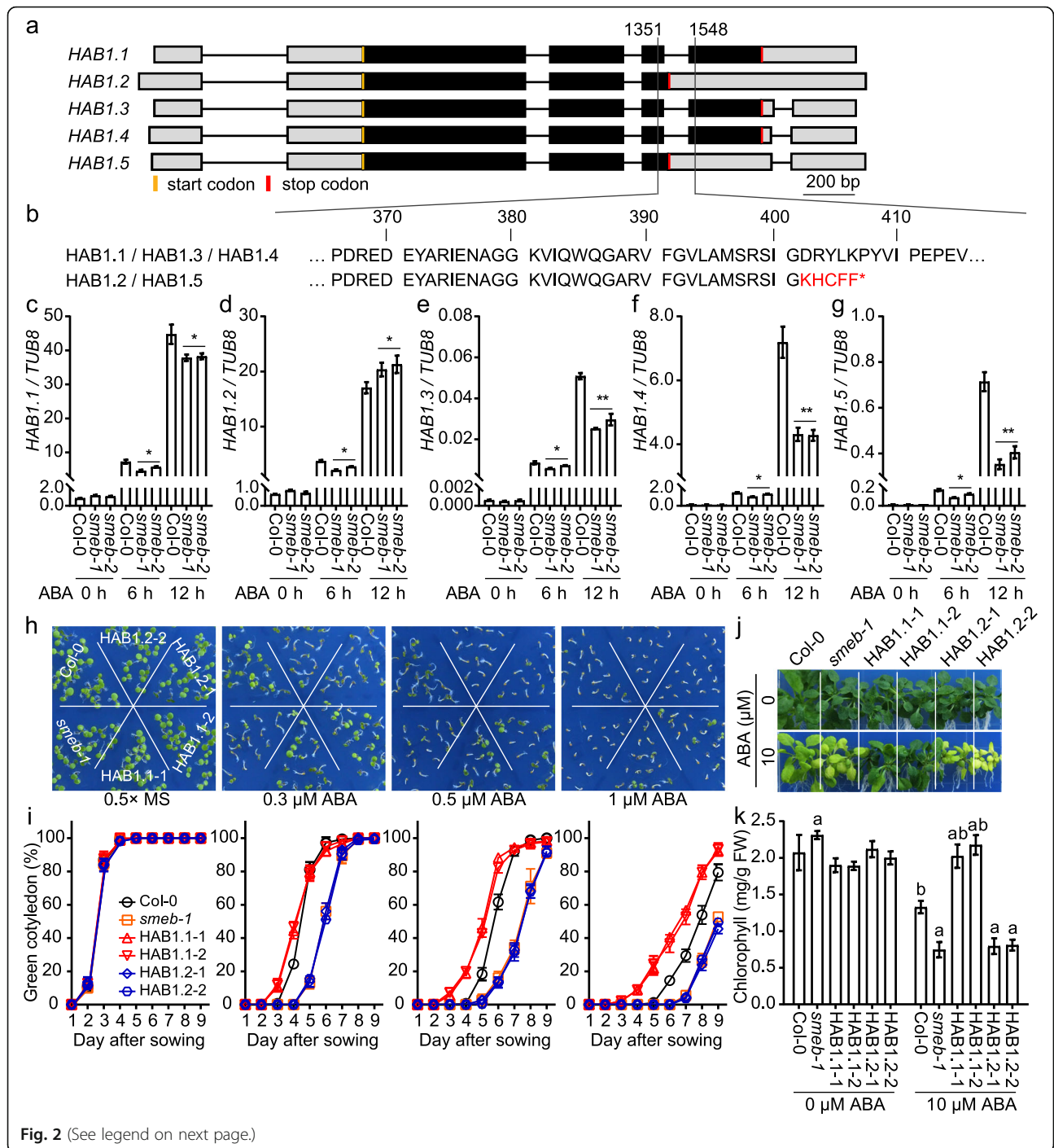


Fig. 2 (See legend on next page.)

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Fig. 2 SmEb modulates the alternative splicing of *HAB1* in response to ABA. **a** Different alternative splicing variants of *HAB1* as determined by sequence analysis of cloned PCR products. The lines between boxes indicate introns. The grey and black boxes represent the UTR and exon, respectively. **b** Amino acid sequence resulting from alternative splicing and the formation of a full-length and truncated *HAB1* proteins in the close-up view. *HAB1.1*, *HAB1.3* and *HAB1.5* encode the full-length *HAB1* protein, while the *HAB1.2* and *HAB1.4* encode the truncated *HAB1* protein. **c-g** Relative transcript levels of different alternative splicing variants of *HAB1* in Col-0, *smeb-1* and *smeb-2* plants subjected to 50 μ M ABA for 0, 6, or 12 h, as determined by qRT-PCR analysis. Values are mean \pm SD ($n = 3$), * $P < 0.05$, ** $P < 0.01$, Student's t-test. **h** Cotyledon greening assay of Col-0, *smeb-1*, *HAB1.1-1*, *HAB1.1-2*, *HAB1.2-1*, and *HAB1.2-2* after seed sowing for 6 days in 0.5 \times MS medium containing 0, 0.3, 0.5, or 1 μ M ABA. *HAB1.1-1* and *HAB1.1-2*, two independent lines of *smeb-1* expressing 35S:*HAB1.1-3* \times *FLAG* transgene. *HAB1.2-1* and *HAB1.2-2*, two independent lines of *smeb-1* expressing 35S:*HAB1.2-3* \times *FLAG* transgene. **i** Percentages of green cotyledon of Col-0, *smeb-1*, *HAB1.1-1*, *HAB1.1-2*, *HAB1.2-1*, and *HAB1.2-2* after seed sowing for indicated days in 0.5 \times MS medium supplementing 0, 0.3, 0.5, or 1 μ M ABA. Error bars represent \pm SD ($n = 6$). **j** Seven-day-old Col-0, *smeb-1*, *HAB1.1-1*, *HAB1.1-2*, *HAB1.2-1*, and *HAB1.2-2* seedlings grown in 0.5 \times MS medium were transferred to 0.5 \times MS medium with or without 10 μ M ABA and grown for an additional 3 weeks. **k** The chlorophyll content of rosette leaves of Col-0, *smeb-1*, *HAB1.1-1*, *HAB1.1-2*, *HAB1.2-1*, and *HAB1.2-2* plants shown in (j). Values are mean \pm SD ($n = 6$). The letters a and b above the columns indicate a significant difference relative to Col-0 and *smeb-1* mutant, respectively ($P < 0.05$, Student's t-test)

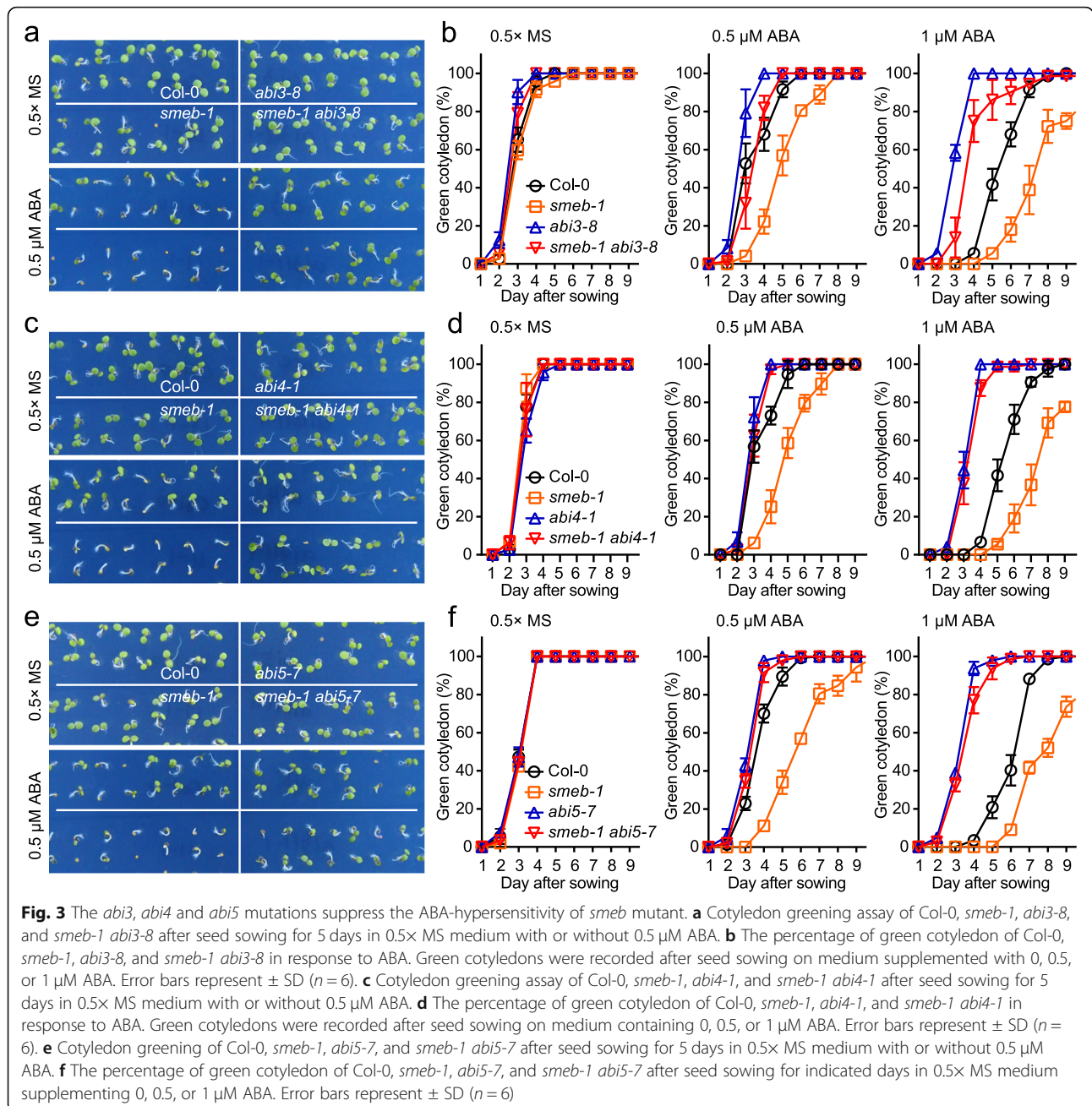
HAB1.4 transcripts are translated into full-length *HAB1* protein, named as *HAB1.1*, while the *HAB1.2* and *HAB1.5* transcripts produce a truncated *HAB1*, designated as *HAB1.2* (Fig. 2b). Under normal growth conditions, the transcript levels of the five *HAB1* spliced forms showed no difference between *smeb* mutants and Col-0 wild type plants (Fig. 2c - g). However, the transcript levels of all *HAB1* variants in *smeb* mutants were significantly reduced after ABA treatment for 6 h when compared with those of the Col-0 wild type. After 12 h of ABA treatment, the accumulation of *HAB1.1*, *1.3*, *1.4*, and *1.5* transcripts were clearly lower in *smeb* mutants than in Col-0 wild type, whereas the transcript level of *HAB1.2* was higher in *smeb* mutants than in Col-0 wild type plants (Fig. 2c - g). Since the expression level of *HAB1.3* is very low compared with the other four variants (Fig. 2e), and the *HAB1.1* and *HAB1.2* proteins function antagonistically (Wang et al. 2015), it is likely that the reduction of *HAB1.1* and *HAB1.4* transcripts and thus the decreased level of *HAB1.1* protein contributes to the ABA hypersensitive phenotype of *smeb* mutants.

To test the hypothesis that the reduction of *HAB1.1* protein is responsible for the ABA hypersensitive response phenotype of *smeb* mutants, we expressed the full-length cDNAs of *HAB1.1* and *HAB1.2* driven by the CaMV 35S promoter in *smeb-1* mutant plants. Two independent homozygous lines of 35S:*HAB1.1-3* \times *FLAG*/*smeb-1* (designated as *HAB1.1-1* and *HAB1.1-2*) and 35S:*HAB1.2-3* \times *FLAG*/*smeb-1* (named as *HAB1.2-1* and *HAB1.2-2*) were identified, and qRT-PCR analysis showed that the expression levels of *HAB1.1* and *HAB1.2* in the overexpression lines are approximately six- to nine-fold higher than the wild type controls. Cotyledon greening assays showed that overexpression of *HAB1.1* but not *HAB1.2* could fully rescue the hypersensitive phenotype of *smeb-1* in response to ABA treatment (Fig. 2h and i). These results further support that *HAB1* is a downstream target of SmEb and decreased

accumulation of *HAB1.1* protein is responsible for the *smeb* mutant phenotype. The transgenic seedlings were further tested on media supplemented with 10 μ M ABA. The results showed that the ectopic expression of *HAB1.1* but not *HAB1.2* also suppressed the chlorosis phenotype of *smeb-1* in response to ABA treatment (Fig. 2j and k). These results indicate that the *HAB1.1* but not the *HAB1.2* spliced form is the functional variant in SmEb-modulated ABA response, and it is very likely that the reduction of *HAB1.1* protein contributes to the ABA-hypersensitive phenotype of *smeb-1* mutant plants.

Inhibition of the full length *HAB1* protein results in the activation of SnRK2 protein kinases, leading to the activation of downstream transcription factors such as ABA INSENSITIVE (ABI) 3, ABI4, and ABI5 in the ABA signaling pathway (Feng et al. 2014; Zhu 2016). Although ABI3, ABI4, and ABI5 belong to different transcription factor families, they are all positive regulators in plant ABA responses (Skubacz et al. 2016). The genetic interaction between *SmEb* and *ABI3*, *ABI4*, and *ABI5* in modulating seed germination and cotyledon greening in response to ABA treatment was studied by generating *smeb-1 abi3-8*, *smeb-1 abi4-1*, and *smeb-1 abi5-7* double mutant lines. Cotyledon greening assays revealed that the *abi3*, *abi4*, or *abi5* mutation could rescue the ABA-hypersensitive phenotype of *smeb-1* mutant plants (Fig. 3). However, among the three *abi* mutations, *abi3* showed weaker suppression of the *smeb-1* mutant phenotype (Fig. 3), suggesting that the effect of *smeb* mutation on ABA response is partially independent of *ABI3*. Taken together, it is likely that the *smeb-1* mutation causes reduced production of *HAB1.1* and/or other PP2Cs protein, which leads to enhanced ABA signaling and activation of ABA responses that are dependent on the downstream transcription factors ABI3, 4, and 5, resulting in ABA-hypersensitivity of the *smeb-1* mutant.

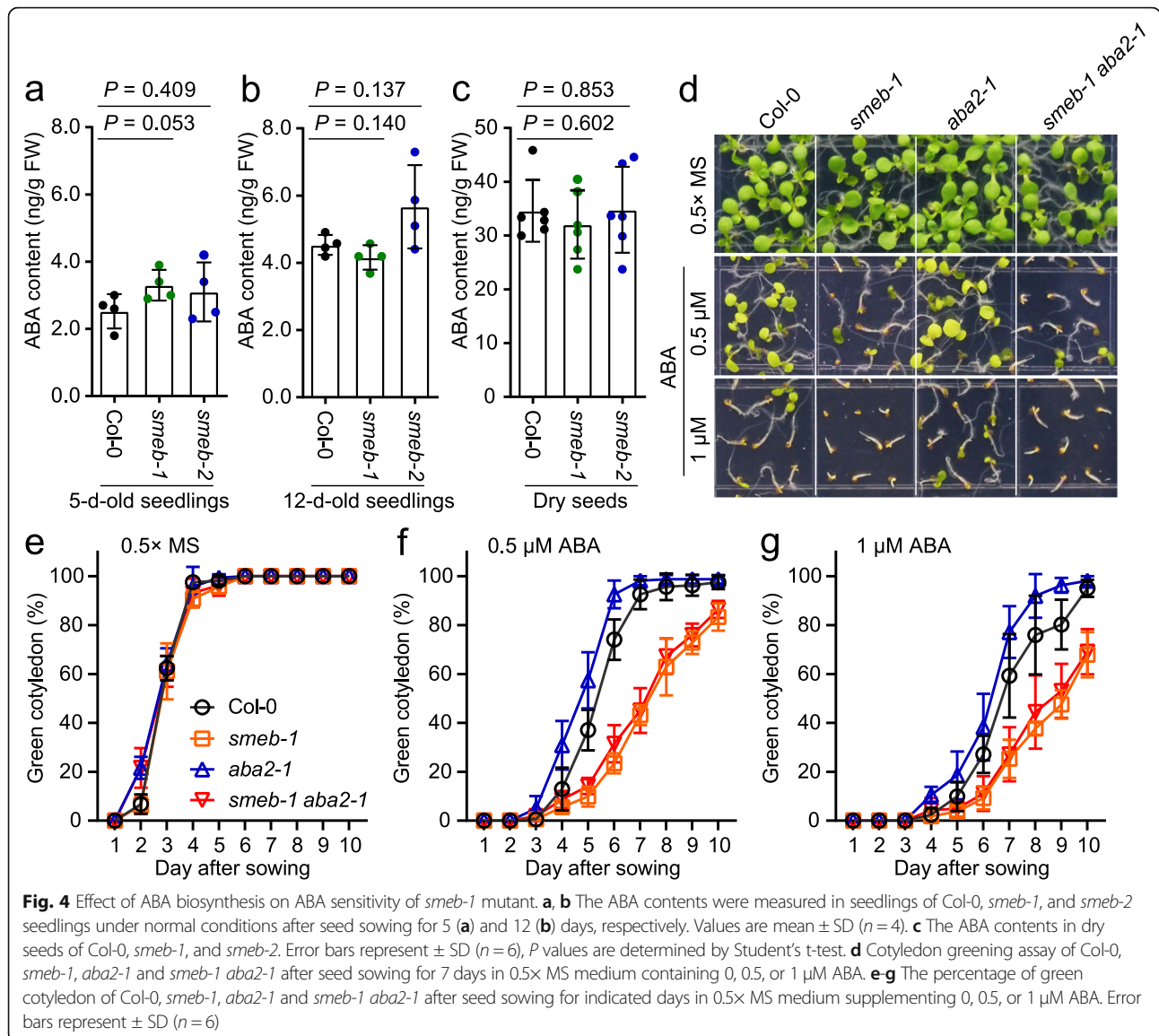
In addition to ABA signaling, ABA biosynthesis plays a role in controlling plant ABA responses (Nakashima



and Yamaguchi-Shinozaki 2013; Wang et al. 2018a). The de novo ABA biosynthesis pathway is well known in Arabidopsis, and the xanthoxin dehydrogenase encoded by *ABA DEFICIENT 2* (*ABA2*) is a critical enzyme for ABA biosynthesis (Seo and Koshiba 2002). To determine whether the ABA-hypersensitive phenotype of *smeb* mutants may be due to enhanced ABA accumulation, we measured ABA contents, and found that the two *smeb* mutants and Col-0 wild type had comparable cellular ABA levels in seedlings and dry seeds (Fig. 4a - c). This result indicates that the *smeb* mutations do not affect ABA biosynthesis. We further tested the genetic

interaction between *aba2* and *smeb-1* by analyzing the double mutant *smeb-1 aba2-1*. The *aba2* mutant is hyposensitive to ABA in seed germination (Lin et al. 2007). The results indicated that the *aba2* mutation could not rescue the ABA-hypersensitive phenotype of *smeb-1* mutant in cotyledon greening during seed germination (Fig. 4d - g). These results further support that SmEb modulates ABA responses by regulating ABA signaling rather than ABA accumulation.

In conclusion, we have identified that SmEb, a core spliceosome component involved in pre-mRNA splicing, modulates the alternative splicing of *HAB1* that encodes



an ABA co-receptor critical for ABA responses in Arabidopsis. Our results suggest that this regulatory mechanism helps to fine-tune ABA signaling during seed maturation, germination and cotyledon greening by maintaining proper levels of *HAB1* splicing variants in response to changes in endogenous ABA contents. When ABA concentration is low, SmEb is not critical for *HAB1* pre-mRNA splicing. When ABA concentration increases, *SmEb* expression is up-regulated, and SmEb becomes critical for maintaining a proper ratio of the two splicing variants that encode antagonistic HAB1 proteins, HAB1.1 and HAB1.2. Our findings highlight the crucial roles of mRNA splicing control of key genes in plant hormonal signaling and stress responses. Future work will determine how increased *SmEb* may affect the splicing of *HAB1* pre-mRNAs in response to ABA treatment.

Materials and methods

Plant materials and growth conditions

All Arabidopsis (*Arabidopsis thaliana*) plants used in this study are in the Columbia-0 background. The T-DNA insertion mutants, including *smeb-1* (SALK_089521C) and *smeb-2* (SALK_119088C), were obtained from the Arabidopsis Biological Resource Center (ABRC; The Ohio State University, Columbus, OH, USA). The *abi3-8*, *abi4-1*, *abi5-7*, and *aba2-1* mutants were collected from previous studies. The double mutants, including *smeb-1 abi3-8*, *smeb-1 abi4-1*, *smeb-1 abi5-7*, and *smeb-1 aba2-1*, were generated by genetic crossing and subsequent PCR-based genotyping in the F2 population. Seeds were surface-sterilized and stratified at 4 °C for 2 days, and then sown in 0.5x Murashige and Skoog (MS) medium plates and grown at 22 °C under 16 h light / 8 h dark photoperiod.

To construct the *HAB1.1* and *HAB1.2* transgenic plants, the coding sequence (CDS) of two *HAB1* splicing isoforms, *HAB1.1* and *HAB1.2*, were amplified and cloned into pCambia1305 binary vector (Hong et al. 2020) to obtain the *35S:HAB1.1-3 × FLAG* and *35S:HAB1.2-3 × FLAG* constructs, respectively. These two constructs were then respectively introduced into *smeb-1* mutant plants by using *Agrobacterium*-mediated floral dip method (Clough and Bent 1998). The seeds of T3 homozygous transgenic plants were identified for further analysis.

Physiological phenotype assays

For seed germination assay, Arabidopsis seeds were surface-sterilized and stratified at 4 °C for 2 days before sowing on 0.5× MS medium plates containing indicated concentrations of ABA. The plates were kept in a growth chamber at 22 °C under 16 h light / 8 h dark conditions. The percentages of green cotyledon were calculated with three to six replicates for indicated days after sowing. For analysis of ABA sensitivity at post-germination stage, seven-day-old seedlings of Col-0, *smeb-1* and transgenic plants grown in 0.5× MS plates were transferred to 0.5× MS medium with or without 10 μM ABA for additional 3 weeks, and the chlorophyll content were then determined in rosette leaves. The average content of total chlorophyll was calculated from six independent experiments ($n = 3$ plants).

Quantification of *HAB1* splice variants

The transcripts of *HAB1* gene were amplified by RT-PCR from the cDNA of 7-day-old Col-0 and *smeb* mutant seedlings after 50 μM ABA treatment for 12 h. The PCR products with three bands were cloned into the vector using pMD™18-T Vector Cloning Kit (TaKaRa), followed by Sanger sequencing. The *HAB1* alternative splicing variants were distinguished in qRT-PCR by using primer pairs that only amplify one of the five variants, which were designed according to the last three exons and 3'UTR of At1g72770. The primer pairs used to determine the transcript levels of the five splicing variants of *HAB1* by qRT-PCR are as follows. 5'-TCTAGG TCCATCGGTGACAGATATCT-3' as forward primer of *HAB1.1*, *1.3*, and *1.4*; 5'-GCACATCACACAAACG TGGCTTG-3' as forward primer of *HAB1.2* and *1.5*; 5'-TACGAAACTCGAACTTACCCCA-3' as reverse primer of *HAB1.1*; 5'-ACCTGTGCGAAATTAGATCCT TAACGATCT-3' as reverse primer of *HAB1.2*; 5'-TTTTCTTTATTTTTTTTACCCCCAGT-3' as reverse primer of *HAB1.3*; and 5'-TTCTTTATTTTTTCTCCC TTCCCCAGTA-3' as reverse primer of *HAB1.4* and *1.5*.

Determination of chlorophyll and ABA content

The chlorophyll and ABA contents were determined as described in previous study (Wang et al. 2018b). Briefly,

to quantify chlorophyll contents, the chlorophyll was extracted from 100 mg rosette leaves with 0.6 mL 80% (v/v) acetone buffer. The extract was then centrifuged for 5 min at 4 °C, and the supernatant was collected and the absorbance of chlorophyll at wavelengths 645 and 663 nm was measured using NanoDrop spectrophotometer 2000C (Thermo Scientific). To determine ABA contents, endogenous ABA was extracted from 50 mg fresh leaf tissue of each sample using 0.5 mL homogeneous buffer (70% methanol, 0.1% formic acid). 2 ng ABA-d6 (Olchemim, Olomouc, Czech) was added to the extracts as an internal standard. The mixture was diluted 2 times with deionized water, and the ABA content of 50 μL dilution of each sample was determined by the UPLC-Triple TOF 5600+ system (Sciex, Concord, Canada).

Quantification of gene expression

Total RNAs were extracted from rosette leaves of 7-day-old seedlings grown in 0.5× MS liquid medium with or without 50 μM ABA treatment using Quick RNA Isolation Kit (Huayueyang). Reverse transcription was carried out using iScript cDNA synthesis kit (*Bio-RAD*), followed by quantitative PCR on a CFX96™ Real-Time system (*BIO-RAD*) with AceQ qPCR SYBR Green Master Mix (Vazyme Biotech co., Ltd). The data of expression profiles were analyzed and illustrated by temporal changes using the Prism 7 (GraphPad Software). *TUB8* was used as an internal control.

Abbreviations

ABA: Abscisic acid; T-DNA: Transfer DNA; qRT-PCR: Quantitative reverse transcription-PCR; PP2C: Type 2C protein phosphatases; *HAB1*: HYPERSENSITIVE TO ABA1; *ABI3*: ABA INSENSITIVE 3; *ABI4*: ABA INSENSITIVE 4; *ABI5*: ABA INSENSITIVE 5; *ABA2*: ABA DEFICIENT 2; MS: Murashige and Skoog

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Accession numbers

Sequence data from this article could be found on the website of Arabidopsis Information Resource (www.arabidopsis.org) under the following accession numbers: *SmEb*, At2g18740; *HAB1*, At1g72770; *ABI3*, At3g24650; *ABI4*, At2g40220; *ABI5*, At2g36270; *TUB8*, At5g23860.

Authors' contributions

H.S. and Z.W. designed the experiments; Y.H., H.S., and Z.W. analyzed the data; Y.H., Y.C., J.Y., and Z.W. performed experiments; H.S. and Z.W. drafted the article; H.S., J.-K.Z., and Z.W. revised and finalized the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declaration

Competing interests

Author H.Z. and J.-K.Z. are members of the Editorial Board, and were not involved in the journal's review of, or decisions related to, this manuscript.

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References

- Bi Y, Deng ZP, Ni WM, Shrestha R, Savage D, Hartwig T, Patil S, Hong SH, Zhang ZZ, Osés-Prieto JA, Li KH, Quail PH, Burlingame AL, Xu SL, Wang ZY (2021) Arabidopsis ACINUS is O-glycosylated and regulates transcription and alternative splicing of regulators of reproductive transitions. *Nat Commun* 12(1):945. <https://doi.org/10.1038/s41467-021-20929-7>
- Capovilla G, Delhomme N, Collani S, Shutava I, Bezrukov I, Symeonidi E, Amorim MD, Laubinger S, Schmid M (2018) PORCUPINE regulates development in response to temperature through alternative splicing. *Nat Plants* 4(8):534–539. <https://doi.org/10.1038/s41477-018-0176-z>
- Clough SJ, Bent AF (1998) Floral dip: a simplified method for agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16(6):735–743. <https://doi.org/10.1046/j.1365-3113x.1998.00343.x>
- Feng CZ, Chen Y, Wang C, Kong YH, Wu WH, Chen YF (2014) Arabidopsis RAV1 transcription factor, phosphorylated by SnRK2 kinases, regulates the expression of ABI3, ABI4, and ABI5 during seed germination and early seedling development. *Plant J* 80(4):654–668. <https://doi.org/10.1111/tpj.12670>
- Gong ZZ, Xiong LM, Shi HZ, Yang SH, Herrera-Estrella LR, Xu GH, Chao DY, Li JR, Wang PY, Qin F, Li JJ, Ding YL, Shi YT, Wang Y, Yang YQ, Guo Y, Zhu JK (2020) Plant abiotic stress response and nutrient use efficiency. *Sci China-Life Sci* 63(5):635–674. <https://doi.org/10.1007/s11427-020-1683-x>
- Hong YC, Wang Z, Shi HZ, Yao JJ, Liu X, Wang FX, Zeng L, Xie Z, Zhu JK (2020) Reciprocal regulation between nicotinamide adenine dinucleotide metabolism and abscisic acid and stress response pathways in Arabidopsis. *PLoS Genet* 16(6):e1008892. <https://doi.org/10.1371/journal.pgen.1008892>
- Lin PC, Hwang SG, Endo A, Okamoto M, Koshiba T, Cheng WH (2007) Ectopic expression of ABSCISIC ACID 2/GLUCOSE INSENSITIVE 1 in Arabidopsis promotes seed dormancy and stress tolerance. *Plant Physiol* 143(2):745–758. <https://doi.org/10.1104/pp.106.084103>
- Melcher K, Ng LM, Zhou XE, Soon FF, Xu Y, Suino-Powell KM, Park SY, Weiner JJ, Fujii H, Chinnusamy V, Kovach A, Li J, Wang YH, Li JY, Peterson FC, Jensen DR, Yong EL, Volkman BF, Cutler SR, Zhu JK, Xu HE (2009) A gate-latch-lock mechanism for hormone signalling by abscisic acid receptors. *Nature* 462(7273):602–U672. <https://doi.org/10.1038/nature08613>
- Nakashima K, Yamaguchi-Shinozaki K (2013) ABA signaling in stress-response and seed development. *Plant Cell Rep* 32(7):959–970. <https://doi.org/10.1007/s00299-013-1418-1>
- Reddy ASN (2007) Alternative splicing of pre-messenger RNAs in plants in the genomic era. *Annu Rev Plant Biol* 58(1):267–294. <https://doi.org/10.1146/annurev.arplant.58.032806.103754>
- Ren ZZ, Wang Z, Zhou XE, Shi HZ, Hong YC, Cao MJ, Chan ZL, Liu X, Xu HE, Zhu JK (2017) Structure determination and activity manipulation of the turfgrass ABA receptor FePYR1. *Sci Rep* 7(1):14022. <https://doi.org/10.1038/s41598-017-14101-9>
- Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. *Trends Plant Sci* 7(1):41–48. [https://doi.org/10.1016/S1360-1385\(01\)02187-2](https://doi.org/10.1016/S1360-1385(01)02187-2)
- Skubacz A, Daszkowska-Golec A, Szarejko I (2016) The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Front Plant Sci* 7:1884
- Staiger D, Brown JWS (2013) Alternative splicing at the intersection of biological timing, development, and stress responses. *Plant Cell* 25(10):3640–3656. <https://doi.org/10.1105/tpc.113803>
- Wahl MC, Will CL, Luhrmann R (2009) The spliceosome: design principles of a dynamic RNP machine. *Cell* 136(4):701–718. <https://doi.org/10.1016/j.cell.2009.02.009>
- Wang K, He JN, Zhao Y, Wu T, Zhou XF, Ding YL, Kong LY, Wang XJ, Wang Y, Li JG, Song CP, Wang BS, Yang SH, Zhu JK, Gong ZZ (2018a) EAR1 negatively regulates ABA signaling by enhancing 2C protein phosphatase activity. *Plant Cell* 30(4):815–834. <https://doi.org/10.1105/tpc.17.00875>
- Wang Z, Wang FX, Hong YC, Yao JJ, Ren ZZ, Shi HZ, Zhu JK (2018b) The flowering repressor SVP confers drought resistance in Arabidopsis by regulating abscisic acid catabolism. *Mol Plant* 11(9):1184–1197. <https://doi.org/10.1016/j.molp.2018.06.009>
- Wang ZJ, Ji HT, Yuan BJ, Wang SF, Su C, Yao B, Zhao HT, Li X (2015) ABA signalling is fine-tuned by antagonistic HAB1 variants. *Nat Commun* 6(1):8138. <https://doi.org/10.1038/ncomms9138>
- Zhan XQ, Qian BL, Cao FQ, Wu WW, Yang L, Guan QM, Gu XB, Wang PC, Okusolubo TA, Dunn SL, Zhu JK, Zhu JH (2015) An Arabidopsis PWI and RRM motif-containing protein is critical for pre-mRNA splicing and ABA responses. *Nat Commun* 6(1):8139. <https://doi.org/10.1038/ncomms9139>
- Zhu JK (2016) Abiotic stress signaling and responses in plants. *Cell* 167(2):313–324. <https://doi.org/10.1016/j.cell.2016.08.029>

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