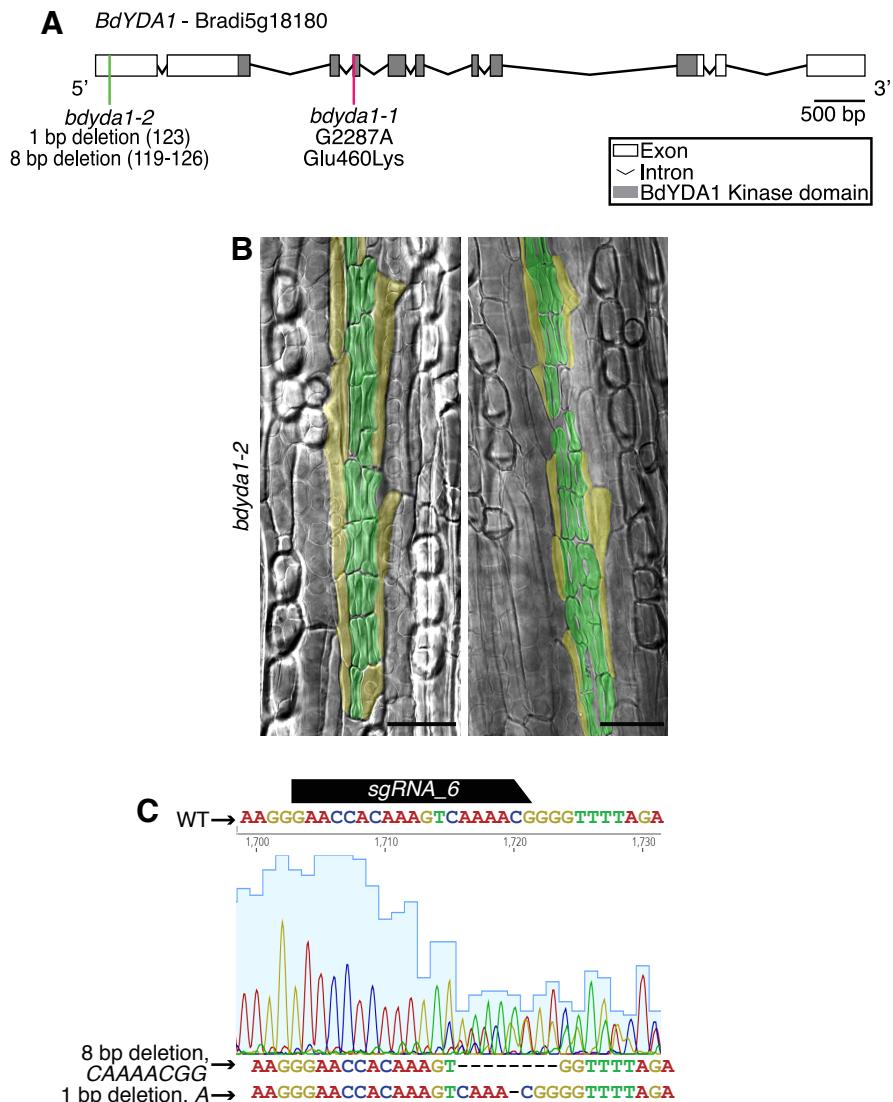


**Figure S1. Phylogenetic tree of YDA sequences from representative dicots, grasses, and basal plant lineages.**

Bootstrap value (%) of 1,000 replicates is shown. Protein sequences were obtained from the Phytozome v11 database (<https://phytozome.jgi.doe.gov/>). On MEGA7 (Kumar et al., 2016), the sequences were first aligned by MUSCLE, then the tree was calculated using the maximum likelihood method (1,000 bootstrap replicates).

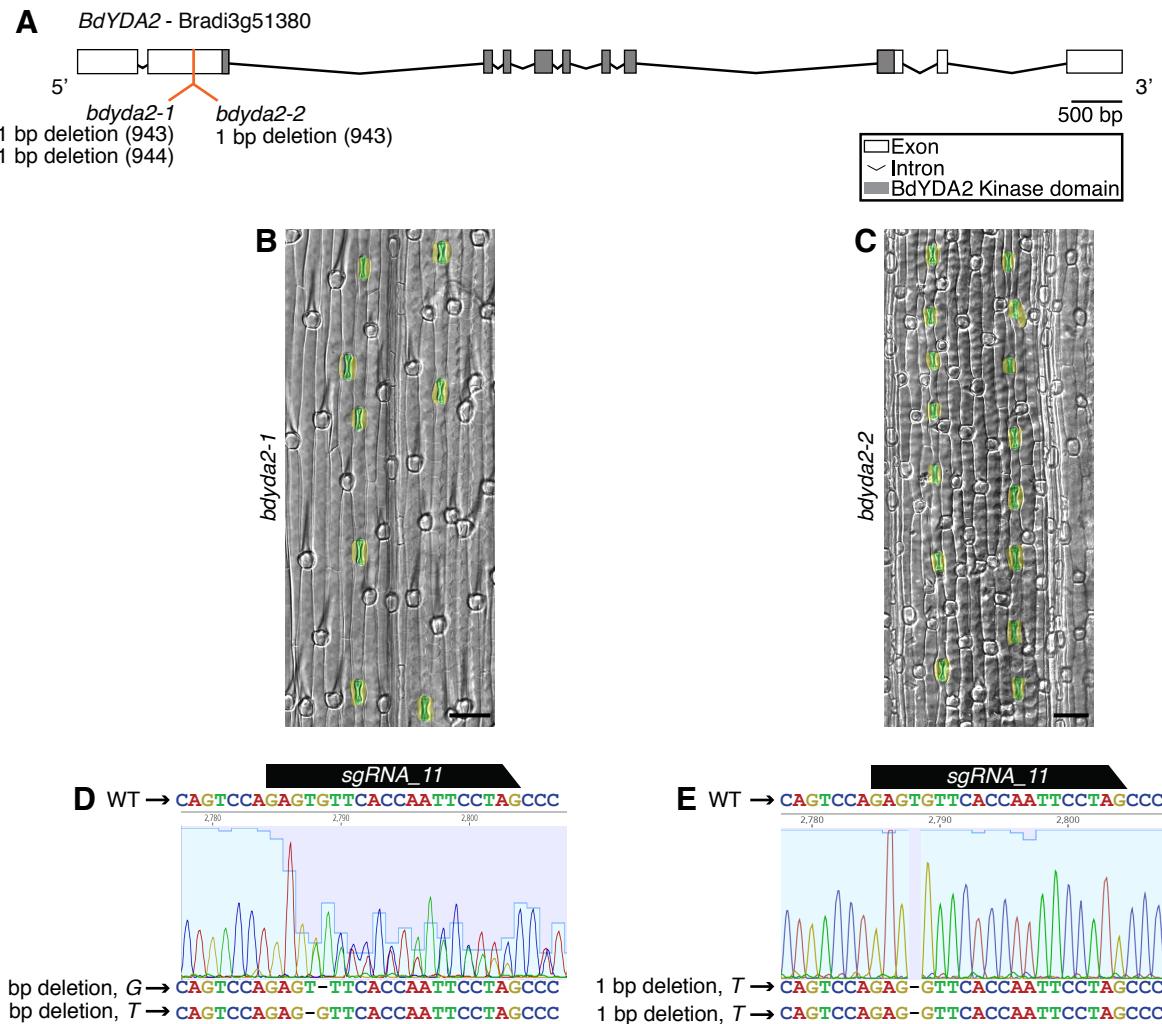


**Figure S2. Additional characterization of a CRISPR/Cas9-induced allele of *BdYDA1*.**

**(A)** Gene/Protein diagram of *BdYDA1*. The vertical magenta bar indicates the *b**dyda1-1* EMS mutation. A green bar indicates the *b**dyda1-2* CRISPR/Cas9 induced mutations. (two at same site). Models here and in Fig. S3A were generated in Gene Structure Display Server (Hu et al., 2015).

**(B)** Differential Interference Contrast (DIC) images of cleared *b**dyda1-2*. T0 regenerants. Guard cells and subsidiary cells are false-colored green and yellow, respectively. Scale bar = 40  $\mu$ m.

**(C)** Chromatogram of CRISPR-induced mutations in *b**dyda1-2*. The WT sequence is indicated above the chromatogram, and the Sanger sequence is shown below the chromatogram.

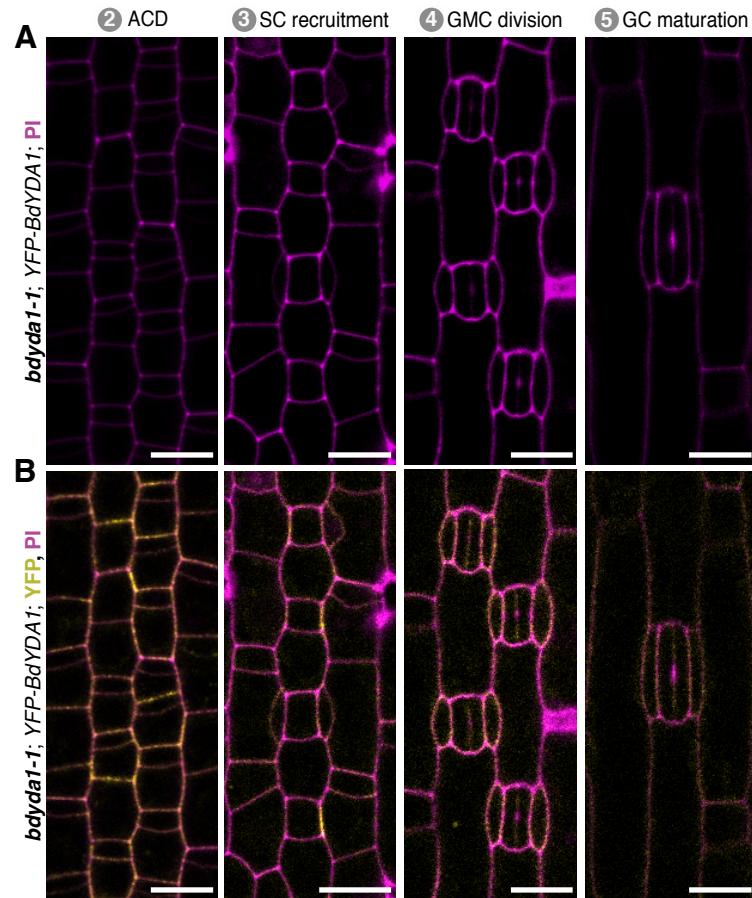


**Figure S3. Additional characterization of CRISPR/Cas9-induced alleles of *BdYDA2*.**

**(A)** Gene/Protein diagram of *BdYDA2*. The vertical orange bar indicates the location of the *bryda2-1* and *bryda2-2* CRISPR/Cas9 induced mutations.

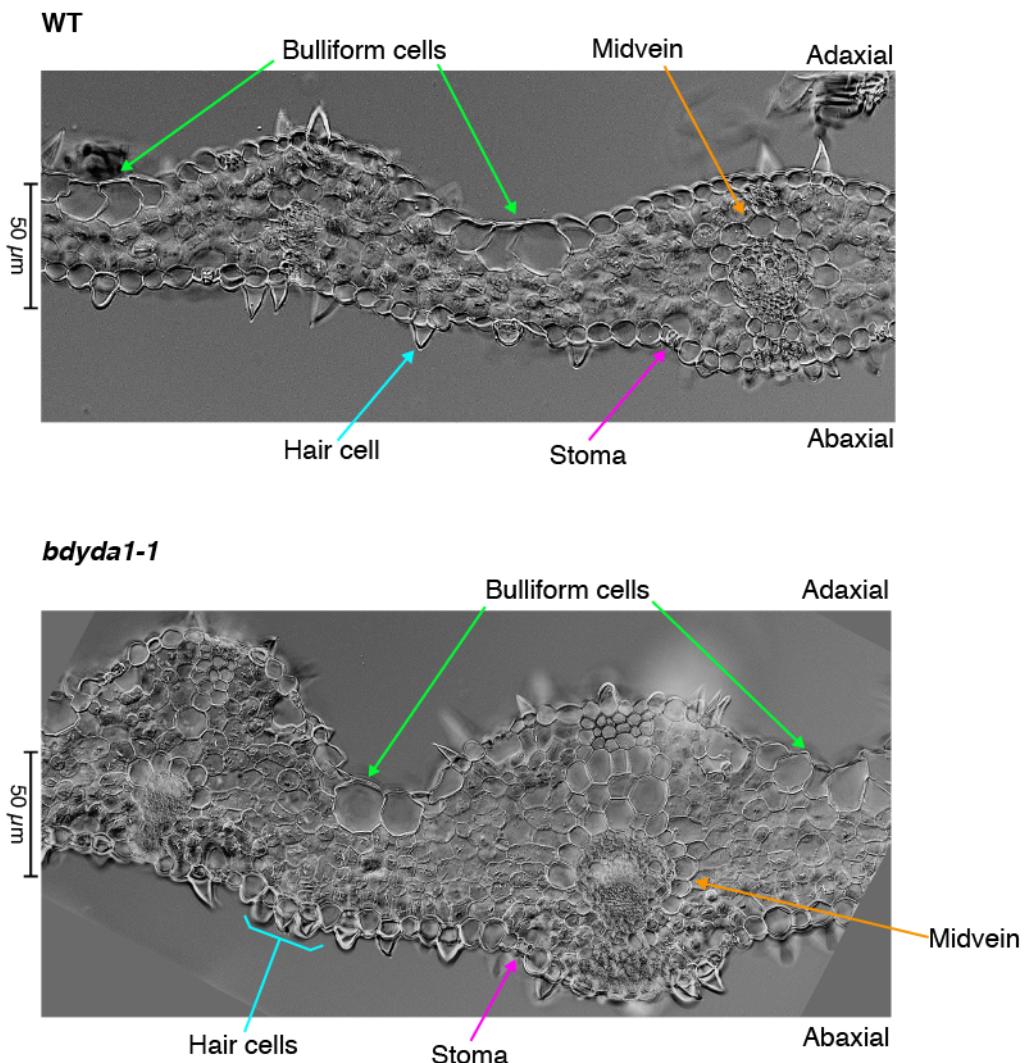
**(B-C)** Differential Interference Contrast (DIC) images of cleared *bryda2-1* (B) and *bryda2-2* (C). T0 regenerants. Guard cells and subsidiary cells are false-colored green and yellow, respectively. Scale bar = 40μm.

**(C-D)** Chromatogram of CRISPR-induced mutations *bryda2-1* (B) and *bryda2-2* (C). The WT sequence is indicated above the chromatogram, and the Sanger sequence is shown below the chromatogram.



**Figure S4. A rescuing *BdYDA1pro:BdYDA1-YFP:Yt* transgene co-localizes with PI.**

Confocal images of *BdYDA1pro:BdYDA1-YFP:Yt* in *bdyda1-1* (T1 plant; emerging 2<sup>nd</sup> leaf at 6 dpg) displaying (A) PI channel only and (B) merge of YFP and PI channel to show overlap of YFP signal with cell outlines in the leaf epidermis. YFP signal alone is shown in Fig. 3I-L. Scale bar = 10 µm. All images are oriented with the base of the leaf blade (younger cells) towards the bottom and the tip of the leaf (older cells) towards the top.



**Figure S5. Cross sections of mature leaf anatomy in WT and *bdyda1-1*.**

DIC images of cleared hand-sectioned WT (Bd21-3) and *bdyda1-1* leaves to show that inner tissues are also affected in the mutant. Compared to WT, *bdyda1-1* leaves have a thicker mesophyll due to the presence of additional cells and display higher numbers of ground tissue cells around the vasculature.



**Figure S6. Protein sequence alignment of AtYDA, BdYDA1, and BdYDA2.**

Detailed protein alignment of the YDA orthologues in *Arabidopsis* and *Brachypodium*. Kinase domains are shown in blue; site of EMS-generated mutation in *bdyda1-1* is bolded. Alignments were done using Clustal Omega at EMBL-EBI (Sievers et al., 2011).

**Table S1.** Primers used in construction of transgenes and genotyping of alleles (all shown 5' → 3').

Primer Name	Primer Sequence	Purpose
<b>Ascl_FP_noATG- 1F</b>	GGCGCGCCGTGAGCAAGGGCGAGGAG	<i>Citrine</i> YFP cloning
<b>Ascl_FP_stop-1R</b>	GGCGCGCCTTACTTGTACAGCTCGTCATGC	<i>Citrine</i> YFP cloning, <i>BdYDA1</i> cloning
<b>BdYDA1term-1F</b>	GATTAATTAAACTAAATTAAAGGCCAAGTGG	<i>BdYDA1</i> terminator cloning
<b>BdYDA1term-1R</b>	CTGAGCTCGACATATTCCCTCCGTTCC	<i>BdYDA1</i> terminator cloning
<b>BdYDA1pro5.1kb -1F</b>	CACCGGGCGCGCCGCTCGTTGGTTGTCGTATCC	<i>BdYDA1</i> promoter cloning
<b>BdYDA1pro_AscI -1R</b>	GGCGCGCCATCGAACATGTAGAAGAATTGTGGTGTG	<i>BdYDA1</i> promoter cloning
<b>BdYDA1proPacI- 1F</b>	GATTAATTAAACAATGCATGGGATTTTCCCAGC	<i>BdYDA1</i> genomic cloning
<b>BdYDA1cDNAns cAscl-1R</b>	CTGGCGCGCCCGTTATCATGCCTGAGTCCAAGG	<i>BdYDA1</i> genomic cloning
<b>BdYDA1-1F</b>	CACCATTGTGGTGAGTATTAGACGG	<i>BdYDA1-YFP</i> cloning
<b>Ala_linker-F</b>	CGCGCAGCAGCAGCGGCTGCCGCTGCCAGCAGGG	Poly-alanine linker
<b>Ala_linker-R</b>	CGCGCCCTGCTGCCGAGCGGCAGCCGCTGCTG	Poly-alanine linker
<b>BdMUTEpro- FWD</b>	TTACTAGTCAGGCTAGCAGCACTATT	<i>BdMUTE</i> promoter cloning
<b>BdMUTEpro- REV</b>	TAAGCTTGATCGTGTGTTCTTC	<i>BdMUTE</i> promoter cloning
<b>BdMUTE-CDS- FWD</b>	CACCATGTCGCACATCGC	<i>BdMUTE</i> genomic cloning
<b>BdMUTE-CDS- REV</b>	ATTGATCATGATGTCGCC	<i>BdMUTE</i> genomic cloning
<b>priMxa38</b>	GGCAGGAACCACAAAGTCAAAACG	<i>bdyda1-2</i> CRISPR (sgRNA_6) generation, F
<b>priMxa39</b>	AAACCGTTTGACTTGTGGTCC	<i>bdyda1-2</i> CRISPR (sgRNA_6) generation, R
<b>priMxa30</b>	GGCACTAGGAATTGGTGAACACTC	<i>bdyda2-1</i> , <i>bdyda2-2</i> CRISPR (sgRNA_11) generation, F

<b>priMxa31</b>	AAACGAGTGTTCACCAATTCTAG	<i>bdyda2-1</i> , <i>bdyda2-2</i> CRISPR (sgRNA_11) generation, R
<b>priMxa50</b>	TGGCTGACATTGTGGTGAGT	<i>bdyda1-2</i> genotyping, F
<b>priMxa52</b>	GGACTTCTAGATGGGCTTGACA	<i>bdyda1-2</i> genotyping, R
<b>priMxa48</b>	AAGCCCTCTCAAAGCCAA	<i>bdyda2-1</i> and <i>bdyda2-2</i> genotyping, R
<b>priMxa49</b>	AGGCAGGGACTCTATTCCAAA	<i>bdyda2-1</i> and <i>bdyda2-2</i> genotyping, R
<b>priMxa25</b>	CAAAGCAGTTGGGCAGG	<i>bdyda1-1</i> genotyping, F
<b>priMxa26</b>	AGCATAGATAATGGCAAGCGGCTAAGAGTGATAGTTC	<i>bdyda1-1</i> genotyping, WT R
<b>priMxa27</b>	TTCAGAACGGCTCAAGAGTGATGTTT	<i>bdyda1-1</i> genotyping, mut R