JIPB Journal of Integrative Plant Biology

Brief Communications
https://doi.org/10.1111/jipb.70298

Engineering herbicide-resistant sorghum with CRISPR/Cas9-mediated adenine base editing[FA]

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Sorghum (*Sorghum bicolor* (L.) Moench), the fifth most important cereal crop globally, is used for food, fodder, and biofuel production. Maximizing yield is challenging due to significant losses caused by weed competition, particularly from wild grasses (Pandian et al., 2022). Conventional breeding strategies, including utilization of natural variation and ethyl methanesulfonate (EMS) mutagenesis, have improved herbicide resistance in sorghum (Tang et al., 2025). However, these approaches are often labor-intensive and time-consuming, and their effectiveness is limited by the genetic diversity of cultivated varieties.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9)-based gene editing technologies, particularly base editors, offer efficient and precise genome modification. Adenine base editors (ABEs) and cytosine base editors (CBEs) catalyze A·T-to-G·C and C·G-to-T·A transitions, respectively. Glycosylase base editors (GBEs), C-to-G base editors (CGBEs), and A-to-Y base editors (AYBEs) expand the scope of base editing to include transversions (Li et al., 2024). These technologies have generated herbicide resistance in model species such as Arabidopsis (*Arabidopsis thaliana*) and crops including rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*), and soybean (*Glycine max*) (Zhang et al., 2019; Zhang et al., 2021; Niu et al., 2024; Fu et al., 2025; Nakazato et al., 2025). However, their applicability in sorghum remains unexplored.

Acetolactate synthase (ALS) is a key enzyme in branched-chain amino acid biosynthesis. Mutations at the conserved tryptophan 574 (Trp-574, W574) of Arabidopsis ALS or the equivalent position in ALSs from other species confer broad-spectrum resistance to all five classes of ALS-inhibiting herbicides in various species (Figure 1A), including rice, maize, wheat, and Arabidopsis (Pan et al., 2025). To introduce analogous herbicide tolerance in sorghum, we targeted the W545 residue in *SbALS*. We designed a single guide RNA (sgRNA) for single base editing on the non-coding strand of *SbALS* to introduce an A-to-G transition, resulting in a Trp-to-Arg substitution (W545R) (Figure 1B).

We developed a sorghum-specific ABE8e vector by cloning a codon-optimized *TadA-8e* in-frame and upstream of the sequence encoding the nickase Cas9 (nCas9; Cas9^{D10A}) variant. To enhance base-editing efficiency, we used the native *SbU6* promoter in combination with a composite cauliflower mosaic virus (CaMV) 35S promoter and the Cestrum yellow leaf curling virus (CmYLCV) promoter to drive *tRNA-sgRNA-HDV* expression (Jiang et al., 2020; Figure 1C). We tested the editing efficiency of this construct by transfecting it into sorghum protoplasts; deep sequencing of PCR products encompassing the targeted site using the Hi-TOM platform showed evidence of the expected A-to-G change, with an average editing efficiency of 11.6% based on three independent experiments (10.3%, 11.9%, 12.7%) (Figure S1).

We introduced this ABE construct into the "P184" cultivar using the Agrobacterium (*Agrobacterium tumefaciens*)-mediated immature embryo method, generating 17 regenerated plants from 217 immature embryos. Sanger sequence confirmed that 12 regenerated plants carried transgenic elements, among which four were heterozygous for the edited *SbALS*^{W545R} allele, corresponding to an editing efficiency of 33.3% at the desired target (Figures 1D, E, S2). No off-target editing was detected at the five predicted sites (Figure S3; Table S1). Future genome-wide analyses will be needed to fully evaluate potential off-target effects in sorghum.

To obtain stable, transgene-free homozygous mutant plants, we examined the progeny of the four *SbALS*^{W545R}/*SbALS* plants for the presence of transgene elements and the mutation. Segregation of the transgene elements from the T0 to the T1 generation followed Mendelian inheritance, indicating that the parental plants carried a single copy of the transgene (Table S2). However, none of the T1 progeny were homozygous for the *SbALS*^{W545R} allele, regardless of their transgene status. Further genotyping of the progeny from transgene-free T1 plants #5-5, #5-15, and #5-27 heterozygous for *SbALS*^{W545R} revealed 2:1 segregation between heterozygotes and plants with the wild-type genotype, with no plants homozygous for the *SbALS*^{W545R} allele recovered (Table S2). These findings suggest that the ABE-mediated *SbALS*^{W545R} edits can be stably transmitted to the next generation. However, the failure to recover homozygous *SbALS*^{W545} indicates that the W545R substitution may impair essential function when present as the sole ALS form and therefore be more suitable for deployment in the heterozygous state.

Before assessing the herbicide resistance of *SbALS*^{W545R}/*SbALS* plants, we first determined the lethal concentration of imazethapyr for P184 plants by spraying 4–5-leaf-stage plants with a range of imazethapyr concentrations. A concentration of 50 mg/L imazethapyr caused complete lethality and was therefore defined as 1× (Figure S4). We then sprayed WT and transgene-free *SbALS*^{W545R}/*SbALS* plants with 200 mg/L (4×) or 400 mg/L (8×) imazethapyr. Eight days after herbicide treatment, WT plants were chlorotic, and all WT plants had died by day 24. By contrast, the *SbALS*^{W545R}/*SbALS* plants had minimal growth inhibition at 200 mg/L and only mild growth suppression at 400 mg/L (Figure 1F). Thus, *SbALS*^{W545R}/*SbALS* plants exhibit greater tolerance to imazethapyr than WT plants.

We further evaluated the herbicide resistance of these *SbALS*^{W545R}/*SbALS* plants under field conditions. One month after sowing, we sprayed all plants with 200 mg/L imazethapyr (4×) or water only. At 16 d post-treatment, all imazethapyr-treated WT plants had died, whereas *SbALS*^{W545R}/*SbALS* plants sprayed with the herbicide displayed vigor similar to untreated WT plants (Figure 1G). At maturity, there were no significant differences in agronomic traits, such as plant height, stem diameter, tiller number, panicle length, panicle weight, and thousand-grain weight, between herbicide-treated *SbALS*^{W545R}/*SbALS* and untreated WT plants (Figure 1H). Consistent with the observed herbicide tolerance, the mutant ALS protein retained higher enzymatic activity than the wild-type protein in the presence of herbicides from five ALS-inhibitor classes (Figure S5).

In conclusion, this work demonstrates that an ABE gene editing system can precisely replace an intended single nucleotide in the grain crop sorghum. By targeted modification of *SbALS*, we created sorghum germplasm more resistant to imazethapyr, providing a resource for

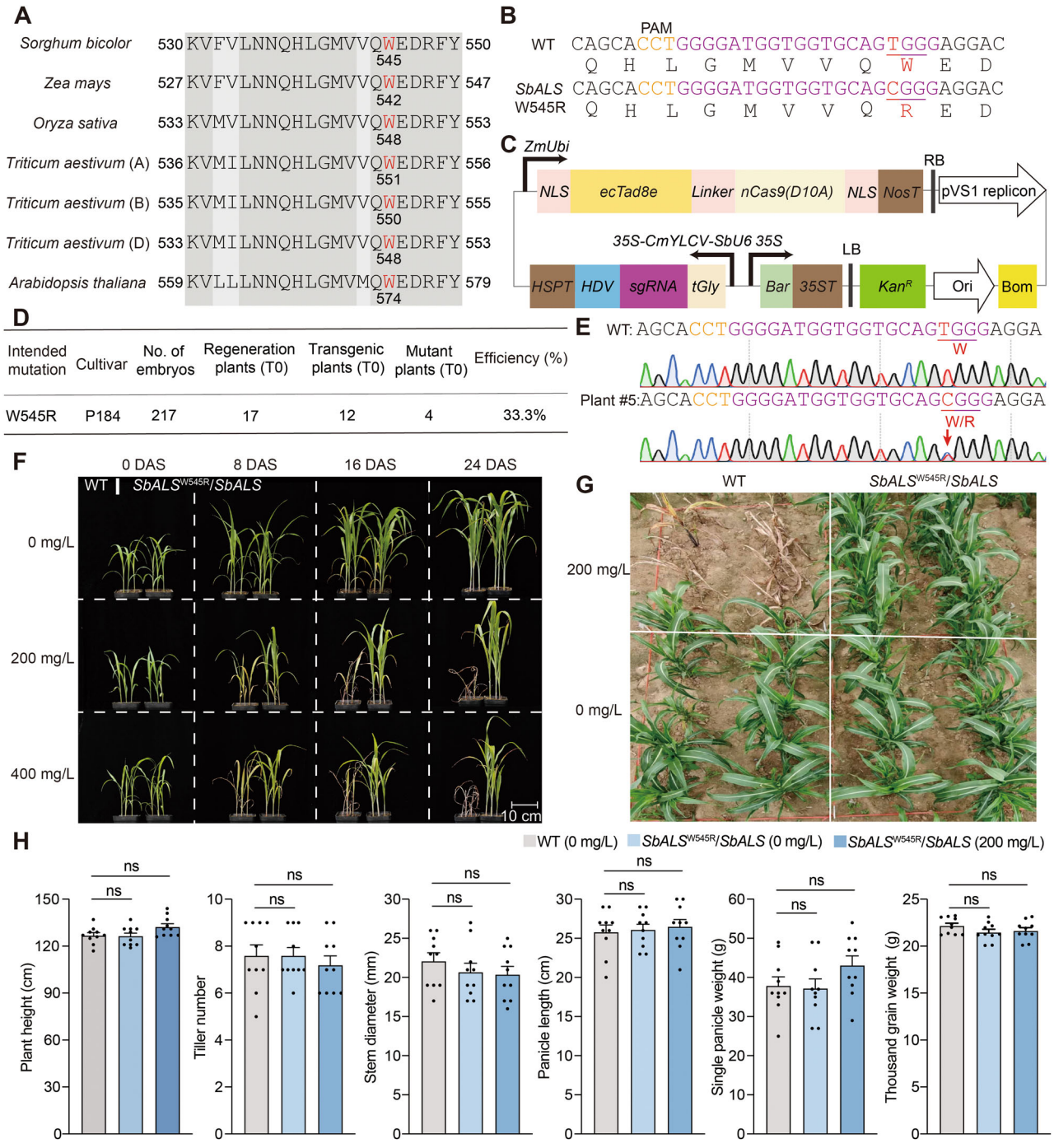

Figure 1. Creation of herbicide-resistant sorghum using a CRISPR-mediated adenine base editing system

(A) Partial amino-acid alignment of ALS proteins showing sequence conservation among ALSs from *Sorghum bicolor* (GenBank: XP_021315526.1), *Zea mays* (GenBank: NP_001151761.2), *Oryza sativa* (GenBank: NP_001403551.1), *Triticum aestivum* (GenBank: XP_044404156.1 for the A subgenome, GenBank: XP_044413937.1 for the B subgenome, and GenBank: XP_044404156.1 for the D subgenome), and *Arabidopsis thaliana* (GenBank: NP_190425.1). Identical and different amino-acid residues are denoted by dark gray and light gray, respectively. **(B)** Sequence of the single guide RNA (sgRNA) designed to introduce the W545R mutation in *SbALS*. The palindrome-adjacent motif (PAM) is in yellow; the target sequence recognized by the sgRNA is in light purple; the targeted codon is underlined, with the target base in red. WT, wild-type. **(C)** ABE8e vector. NLS, nuclear localization signal; LB, T-DNA left border; RB, T-DNA right border; 35S-CmYLCV-SbU6, composite promoter consisting of 35S, CmYLCV, and native *SbU6* promoters. **(D)** Table summarizing the *Agrobacterium tumefaciens*-mediated genetic transformation of P184, a conventional grain sorghum variety. **(E)** Sanger chromatograms showing the target site in WT (left) and regenerant T0 plant #5 (right). The PAM is in yellow; the target is in light purple; the targeted base is in red. **(F)** Representative photographs of WT and *SbALS*^{W545R}/*SbALS* plants at 8, 16, or 24 d after spraying with 200 or 400 mg/L imazethapyr (or water only as control [0 mg/L]) at the 4–5 leaf stage in the greenhouse. DAS, the day after spraying. **(G)** Representative photographs of WT and *SbALS*^{W545R}/*SbALS* plants at 16 d after spraying with 200 mg/L imazethapyr or water only (0 mg/L) in the field. **(H)** Plant height, tiller number, stem diameter, panicle length, single panicle weight, and thousand-grain weight of WT and *SbALS*^{W545R}/*SbALS* plants. Data are means ± standard error ($n = 10$). *P*-values were calculated by Student's *t*-test. ns, not significant.

breeding herbicide-resistant sorghum. As base-editing and prime-editing technologies continue to evolve, they will accelerate sorghum trait improvements, including herbicide resistance, yield, and quality.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (32241041) and the Agricultural Science, Technology Innovation Program of the Chinese Academy of Agricultural Sciences (01-ICS-22), and the Scientific Research Team of Zhongyuan Research Center, Chinese Academy of Agricultural Sciences (CAAS-ZRC-ZYZX20230203).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

B.D., Q.Q., and J.Z. designed the experiments. J.Z., R.L., Z.W., X.S., L.S., X.F., F.L., J.Z., and G.L. performed the experiments. J.Z. wrote the manuscript. B.D. and J.Z revised the manuscript. All authors read and approved the final manuscript.

Edited by: Qi Xie, Institute of Genetics and Developmental Biology, CAS, China

Received Dec. 1, 2025; **Accepted** May 4, 2026

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REFERENCES

Fu, X., Wang, N., Li, L., Qiao, D., Qi, X., Liu, C., Gao, Z., Xie, C., and Zhu, J. (2025). Development of cytosine and adenine base editors for maize precision breeding. J. Integr. Plant Biol. **67:** 2731–2743.

Jiang, Y.Y., Chai, Y.P., Lu, M.H., Han, X.L., Lin, Q., Zhang, Y., Zhang, Q., Zhou, Y., Wang, X.C., Gao, C., et al. (2020). Prime editing efficiently generates W542L and S621I double mutations in two ALS genes in maize. Genome Biol. **21:** 257.

Li, B., Sun, C., Li, J., and Gao, C. (2024). Targeted genome-modification tools and their advanced applications in crop breeding. Nat. Rev. Genet. **25:** 603–622.

Nakazato, I., Yamori, W., Matsumura, H., Qu, Y., Okuno, M., Tsutsumi, N., and Arimura, S.I. (2025). Resistance to the herbicide metribuzin conferred to *Arabidopsis thaliana* by targeted base editing of the chloroplast genome. Plant Biotechnol. J. **23:** 204–215.

Niu, Q., Xie, H., Cao, X., Song, M., Wang, X., Li, S., Pang, K., Zhang, Y., Zhu, J.K., and Zhu, J. (2024) Engineering soybean with high levels of herbicide resistance with a Cas12-SF01-based cytosine base editor. Plant Biotechnol. J. **22:** 2435–2437

Pan, W., Zhu, Y., Li, P., Li, Z., Xu, C., Jin, M., and Tang, X. (2025). Natural and artificial evolution of acetolactate synthase for crop breeding. Crop J. **14:** 95–106.

Pandian, B.A., Sexton-Bowser, S., Prasad, P.V., and Jugulam, M. (2022). Current status and prospects of herbicide-resistant grain sorghum (*Sorghum bicolor*). Pest. Manag. Sci. **78:** 409–415.

Tang, S., Shi, J., Li, X., Yang, M., Li, C., Zhang, D., Yang, S., Mei, C., Luo, Z., Zhang, L., et al. (2025). Development and breeding of herbicide-resistant sorghum for effective cereal-legume intercropping. Adv. Sci. **12:** 2503083.

Zhang, R., Chen, S., Meng, X., Chai, Z., Wang, D., Yuan, Y., Chen, K., Jiang, L., Li, J., and Gao, C. (2021). Generating broad-spectrum tolerance to ALS-inhibiting herbicides in rice by base editing. Sci. China Life Sci. **64:** 1624–1633.

Zhang, R., Liu, J.X., Chai, Z.Z., Chen, S., Bai, Y., Zong, Y., Chen, K.L., Li, J.Y., Jiang, L.J., and Gao, C.X. (2019). Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. Nat. Plants **5:** 480–485.

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