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Unraveling the Molecular Mechanism of Bispyribac-Sodium Resistance in *Echinochloa crus-galli* (Barnyardgrass): A Huge Threat to Effective Weed Control in Inbred and Hybrid Rice Productions

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The evolution of herbicide resistance in weed populations threatens the production of inbred and hybrid rice. There have been reports of herbicide failures to control *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass) specifically to the application of the recommended use rate (32 g a.i. ha⁻¹) of acetolactate synthase (ALS) inhibitor, bispyribac-sodium. This study sought to comprehensively investigate the underlying molecular mechanism behind the herbicide resistance phenomenon. Unraveling the molecular mechanism involved in the resistance will lead to the development of more efficient and sustainable strategies for herbicide control of resistant barnyardgrass biotypes in both inbred and hybrid rice cultivations. This study employed a herbicide dose-response assay to confirm and quantify the level of bispyribac-sodium resistance in the two putatively resistant *E. crus-galli* biotypes (R4 and R12). Seedlings (3-4 leaf stage) of R4 and R12 biotypes were treated with 0, 32, 64, 96, 128, 192, 256, and 288 g a.i. ha⁻¹ of the herbicide. The results demonstrated that the IN50 (herbicide rate resulting in 50% control) of R4 and R12 were 2.3 and 3.5 times greater than that of the susceptible (S) plants, corresponding to bispyribac-sodium rates of 57.84 and 86.95 g a.i. ha⁻¹, respectively. Moreover, BR50 (herbicide rate resulting in 50% biomass reduction) values of R4 and R12 showed a 6- and 9-fold resistance to bispyribac-sodium (109.21 and 157.38 g a.i. ha⁻¹) compared to the S plants (17.76 g a.i. ha⁻¹), respectively. Next, the underlying resistance mechanism in the resistant biotypes was investigated. Target-site resistance analysis detected a W-574-L mutation in the ALS2 gene of R4, while there was no mutation detected in any of the three ALS conserved regions of R12. This study confirmed that bispyribac-sodium resistance in R4 is due to a single-base target-site mutation, whereas resistance in R12 may be explained by a non-target site resistance mechanism. Further research is required to elucidate the mechanism of non-target site resistance in R12, which may involve herbicide-detoxifying enzymes such as cytochrome P450s and glutathione S-transferases.