Mechanisms of resistance to penoxsulam and quinclorac in *Echinochloa crus-galli*

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Summary Barnyard grass (*Echinochloa crus-galli*) is one of the major economically important weeds in the world rice production. ALS- and quinclorac-resistant barnyard grass populations are widespread in rice fields in China. To unravel the multiple-herbicide resistance mechanisms in *E. crus-galli*, the population with resistance (R) to penoxsulam and quinclorac was confirmed and characterised. Dose-response experiments showed that the R population has evolved a high level resistance to penoxsulam (RI>95) and quinclorac (RI = 158), relative to the susceptible (S) population. ALS gene sequencing revealed the known Trp574Leu mutation in the ALS2 gene in the R population is responsible for penoxsulam resistance. The full coding sequences of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene involved in ethylene biosynthesis were cloned from the R and S plants. Gene point mutations resulting in five amino acid substitutions (F98→Y, G101→D, Q206→R, G270→V, A272→G) were identified in the ACO-R versus ACO-S sequences, and three substitutions (F98→Y, G101→D, Q206→R) were located in the putatively conserved domains. The *E. crus-galli* ACO-R and ACO-S genes were expressed in *E. coli*, the ACO purified, and in vitro ethylene production measured. Results showed that ethylene production from ACO-R was 2-fold lower than that from ACO-S. The ACO gene expression of the R and S populations showed no significant difference. Therefore, reduced ethylene production from the R biotype, likely associated with ACO gene mutations, might be involved in quinclorac resistance in barnyard grass. In conclusion, the ALS Trp574Leu mutation and ACO gene mutations might endow multiple resistance in *E. crus-galli*.

Keywords *Echinochloa crus-galli*, penoxsulam, quinclorac, ethylene biosynthesis, 1-aminocyclopropane-1-carboxylate oxidase (ACO).