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Characterization of target-site resistance to ALS-inhibiting herbicides in *Ammannia multiflora* populations

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Research Article

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Abstract

Ammannia multiflora Roxb. is a dominant broadleaf weed that is a serious problem in southern China rice fields, and acetolactate synthase (ALS)-inhibiting herbicides have been used for its control for more than 20 years. Excessive reliance on ALS-inhibiting herbicides has led to herbicide resistance in *A. multiflora*. In this study, 10 *A. multiflora* populations from the Jiangsu Province of China were collected, and the resistance levels and target site–resistance mechanisms to ALS-inhibiting herbicides bensulfuron-methyl and penoxsulam were investigated. The dose–response assays showed that eight populations evolved resistance to bensulfuron-methyl (9.1- to 90.9-fold) and penoxsulam (5.0- to 103.1-fold). Amplification of *ALS* genes indicated that there were three *ALS* genes (*AmALS1*, *AmALS2*, and *AmALS3*) in *A. multiflora*. Sequence analysis revealed amino acid mutations at Pro-197 in either *AmALS1* (Pro-197-Ala, Pro-197-Ser, and Pro-197-His) or *AmALS2* (Pro-197-Ser and Pro-197-Arg) in resistant populations, and no mutations were found in *AmALS3*. Moreover, two independent mutations (Pro-197-Ala in *AmALS1* and Pro-197-Ser in *AmALS2* or Pro-197-Ala in *AmALS1* and Pro-197-Arg in *AmALS2*) coexisted in two resistant populations, respectively. In addition, the auxin mimic herbicides MCPA and florpyrauxifen-benzyl, the photosystem II inhibitor bentazon, and the protoporphyrinogen oxidase inhibitor carfentrazone-ethyl can effectively control the resistant *A. multiflora* populations. Our study demonstrates the wide prevalence of ALS inhibitor–resistant *A. multiflora* populations in Jiangsu Province and the diversity of Pro-197 mutations in *ALS* genes and provides alternative herbicide options for controlling resistant *A. multiflora* populations.

Introduction

As a primary food crop, rice (*Oryza sativa* L.) feeds nearly 50% of the world's population (Singh et al. 2011). Weed infestations have the potential to result in 40% to 60% rice yield losses, and up to 94% to 96% losses if weeds are not controlled (Dass et al. 2017). At present, weeds are the prime source of biotic stress in rice production and pose a great threat to global food security. *Ammannia multiflora* Roxb. is an annual dicot weed in the *Lythraceae* family, and it is often found in wetlands or rice fields, mainly in southern China. In recent years, *A. multiflora* has become a serious problem in some rice fields in Jiangsu and Zhejiang provinces in China, and it has become a dominant weed in some localized areas, adversely affecting rice production.

Currently, there are at least 169 weed species with documented resistance to acetolactate synthase (ALS) inhibitors globally (Heap 2022). Molecular resistance mechanisms causing ALS resistance have been well researched, and they can be grouped into target site–based resistance (TSR) and/or non–target site based resistance (NTSR) (Tranel and Wright 2002; Yu and Powles 2014). TSR includes *ALS* gene mutations or the overexpression of their enzymes. *ALS* mutations cause conformational changes at the binding site of herbicides, resulting in a herbicide-insensitive target-site protein (Yu and Powles 2014). So far, a total of 30 *ALS* mutation types have been established for *ALS* genes at the following codon positions Ala-122 (5), Pro-197 (11), Ala-205 (2), Asp-376 (1), Arg-377 (1), Trp-574 (4), Ser-653 (3), and Gly-654 (3) (numbers in brackets represent the numbers of distinct amino acid mutation substitutions) in weeds with field-evolved resistance (Cao et al. 2021; Tranel et al. 2021). In contrast, a few studies have shown that *ALS* overexpression is related to ALS resistance (Sen et al. 2021; Yu et al. 2020; Zhao et al. 2018). In the case of NTSR to ALS inhibitors, increased herbicide metabolism is most often demonstrated in resistant biotypes (Bai et al. 2018; Mei et al. 2017; Sen et al. 2021 Yang et al. 2018; Zhao et al. 2017). Moreover, both TSR and NTSR mechanisms can occur in resistant plants, not only within a population but also within individual plants, and the coexistence of multiple resistance mechanisms have been reported in ALS inhibitor–resistant weeds, including

Table 1. Primers for amplification of *Ammannia multiflora ALS* genes.

Primer	Sequence (5′→3′)	Amplification size	Annealing temperature	Containing the mutation sites
		——bp——	———C———	
AmALS-F1	TCCATGGAGATCCACCA	848	54	Ala-122, Pro-197, Ala-205
AmALS-R1	TTGTTCTTTCCAATTTCAGC			Asp-376, Arg-377
AmALS-F2	TGCGATTGTTGTTGACATTGACGGT	420	65	Trp-574, Ser-653
AmALS-R2	TAAGATGTCCGCCCGTCACCCTCAA			Gly-654

flixweed [*Descurainia sophia* (L.) Webb ex Prantl] (Yang et al. 2018), water starwort [*Myosoton aquaticum* (L.) Moench] (Bai et al. 2018), barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (Fang et al. 2019), shepherd's-purse [*Capsella bursa-pastoris* (L.) Medik.] (Zhang et al. 2021), *Glebionis coronaria* (L.) Cass. ex Spach (Hada et al. 2021), green foxtail [*Setaria viridis* (L.) P. Beauv.] (Huang et al. 2021), and redroot pigweed (*Amaranthus retroflexus* L.) (Cao et al. 2021).

ALS-inhibiting herbicides, represented by bensulfuron-methyl and penoxsulam in this study, are mainly used to control broadleaf weeds, including *A. multiflora*, in rice fields. Other ALS-inhibiting herbicides, pyrazosulfuron-ethyl and bispyribac-sodium, have also been widely used in rice fields. However, bensulfuron-methyl and penoxsulam have shown poor control of *A. multiflora* in some regions of China, which may be associated with the evolution of resistance in *A. multiflora*. Thus, we aimed to (1) determine the resistance levels of 10 *A. multiflora* populations to bensulfuron-methyl and penoxsulam in selected populations, (2) elucidate the potential TSR mechanisms of resistant populations, and (3) assess control of both the susceptible and resistant *A. multiflora* populations with alternative herbicides.

Materials and Methods

Plant Materials

Seeds of 10 *A. multiflora* populations were collected from rice fields in Jiangsu Province in 2018. The nine putatively resistant populations (JS2 to JS10) were harvested from rice fields where ALS inhibitors failed to control *A. multiflora*. The identified susceptible population (JS1) was harvested from roadside areas having no history of herbicide use and geographically close to the regions from which the resistant populations were taken. Seeds for each population were randomly collected from about 5 to 10 plants.

Dose Response to Bensulfuron-Methyl and Penoxsulam

Ammannia multiflora seeds were sown into 7-cm-diameter plastic pots. About 40 seeds were transplanted to pots with a brush, and the pots were kept in an artificial climate chamber (30/25, 14/10 h day/night) until treatment. Seedlings of *A. multiflora* were thinned to 15 to 20 plants of approximately equal size per pot. About a month later, when the *A. multiflora* plants grew to two to three pairs of true leaves, a series of different concentrations of bensulfuron-methyl or penoxsulam were applied using a sprayer delivering 400 L ha^{-1} at 0.20 MPa. The bensulfuron-methyl rates were 0, 1/256×, 1/64×, 1/16×, 1/4×, 1×, and 4× the recommended label rate (45 g ai ha^{-1}), and the penoxsulam rates were 0, 1/256×, 1/64×, 1/16×, 1/4×, 1×, and 4× the recommended label rate (30 g ai ha^{-1}). The pots were returned to the growth chamber until fresh weight of aboveground plants was sampled and weighed at 14 d after herbicide treatment (DAT). The experiment was conducted twice, and each herbicide treatment contained three replications.

ALS Gene Cloning and Sequencing

Surviving *A. multiflora* plants from dose–response assays were collected for subsequent ALS sequencing. Total RNA was isolated from each plant using Protocol-II of a Plant RNA Extraction Kit (TaKaRa, Beijing, China), and cDNA was obtained using the FastKing RT Kit with gDNase (Tiangen, Shanghai, China). The primer set (*AmALS*-F1/*AmALS*-R1 and *AmALS*-F2/*AmALS*-R2) was designed based on the *ALS* gene sequence from *Ammannia arenaria* Kunth to amplify the partial *A. multiflora ALS* genes spanning the known eight resistance-related mutation sites (Table 1). The PCR component and program were carried out using 2× Taq PCR MasterMix (Tiangen). The amplified products were then purified using the TIANgel Maxi Purification Kit (Tiangen) and directly sequenced with the forward and reverse primers by Sangon Biotech (Shanghai, China). Because multiple *ALS* genes were detected in *A. multiflora*, the purified PCR products were cloned into a pLB vector (Tiangen), and the identified insertions were sequenced with the sequencing primers of the pLB vector. At least 12 clones of each sample were sequenced to distinguish the different *ALS* gene copies.

Response to Alternative Herbicides

The single-dose assays were conducted to evaluate the control efficacy of other herbicides against the susceptible (JS1) and resistant (JS2, JS3, JS5, and JS10) *A. multiflora* populations. The cultivation of plants was conducted as described earlier. Four herbicides, MCPA (1,000 g ai ha^{-1}), florpyrauxifen-benzyl (27 g ai ha^{-1}), bentazon (1,200 g ai ha^{-1}), and carfentrazone-ethyl (22.5 g ai ha^{-1}), at their field recommended doses were sprayed on plants with two pairs of leaves (Table 2). The experiment was conducted twice, and each herbicide treatment contained three replications. The plant survival rates and aboveground fresh weight were recorded 21 DAT. The data were subjected to ANOVA and compared by Duncan test ($P < 0.05$).

Statistical Analysis

The fresh weight per herbicide treatment from the whole-plant dose–response assays was converted into a percentage of control treatment. The herbicide rates causing a 50% plant growth inhibition (GR_{50}) were calculated by the following three- or four-parameter equation using SigmaPlot v. 12.0 (Systat Software, San Jose, CA, USA) (Seefeldt et al. 1995):

$$y = C + (D - C)/\left[1 + (x/GR_{50})^{b}\right] \quad [1]$$

where y is the fresh weight response (percentage of the control) at rate x of herbicide, C is the lower limit, D is the upper limit, and b is the slope at GR_{50}. The resistance index (RI) was expressed as the ratio of GR_{50} of the resistant *A. multiflora* population to that of the susceptible population (JS1).

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Table 2. Information on herbicides used in this research.

Herbicide	Mode of action (HRAC group)[a]	Formulation[b]	Recommended field dose	Company
			—g ai ha^{-1}—	
Bensulfuron-methyl	ALS inhibitor (2)	10% WP	45	Shandong Shengbang LVYE Chemical Co., Ltd, Shandong, China
Penoxsulam	ALS inhibitor (2)	25 g L^{-1} OD	30	Jiangsu Changqing Biotechnology Co., Ltd, Jiangsu, China
MCPA	Auxin mimic (4)	56% WP	1000	Shandong Lvbang Crop Science Co., Ltd, Shandong, China
Florpyrauxifen-benzyl	Auxin mimic (4)	3% EC	27	Corteva Agriscience Co., Ltd, Shanghai, China
Bentazon	PSII inhibitor (5)	480 g L^{-1} AS	1200	Anhui Zhongshan Chemical Co., Ltd, Anhui, China
Carfentrazone-ethyl	PPO inhibitor (14)	10% WP	22.5	Anhui Shanghe Woda Biotechnology Co., Ltd, Anhui, China

[a]ALS, acetolactate synthase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.
[b]AS, aqueous solution; EC, emulsifiable concentrate; OD, oil dispersion; WP, wettable powder.

Table 3. GR_{50} and resistance index (RI) values to bensulfuron-methyl and penoxsulam in *Ammannia multiflora* populations and target-site mutations in *ALS* genes of *A. multiflora* populations.[a]

	Bensulfuron-methyl					Penoxsulam							
Population	GR_{50}	SE	*b*	R^2	RI	GR_{50}	SE	*b*	R^2	RI	Amino acid substitution	*AmALS1*	*AmALS2*
	—g ha^{-1}—					—g ha^{-1}—							
JS1	1.05	0.84	0.36	0.98	1.0	0.65	0.19	0.49	0.99	1.0	—	—	—
JS4	2.14	1.12	0.42	0.98	2.0	1.75	0.59	0.46	0.96	2.7	—	—	—
JS8	9.57	2.89	0.45	0.95	9.1	3.25	0.93	0.38	0.98	5.0	CCG to TCG	—	Pro-197-Ser
JS6	9.82	1.89	0.40	0.98	9.4	5.04	1.94	0.37	0.99	7.8	CCT to CAT	Pro-197-His	—
JS5	16.78	4.29	0.50	0.98	16.0	17.27	5.20	0.54	0.98	26.6	CCT to TCT	Pro-197-Ser	—
JS10	25.11	5.23	0.48	0.97	23.9	33.72	9.28	0.64	0.99	51.9	CCT to TCT or CAT	Pro-197-Ser/His	—
JS7	36.53	9.15	0.44	0.95	34.8	7.93	2.14	0.40	0.98	12.2	CCT to CAT	Pro-197-His	—
JS9	44.15	18.75	0.31	0.91	42.0	21.95	6.36	0.50	0.97	33.8	CCT to CAT	Pro-197-His	—
JS3	61.36	7.63	10.3	0.95	58.4	67.01	9.76	2.42	0.99	103.1	CCT to GCT and CCG to CGG	Pro-197-Ala	Pro-197-Arg
JS2	95.43	12.85	1.62	0.95	90.9	35.46	19.10	0.41	0.97	54.6	CCT to GCT and CCG to TCG	Pro-197-Ala	Pro-197-Ser

[a]GR_{50} is the herbicide dose required to reduce fresh weight by 50%, *b* is the slope in GR_{50}, and R is the coefficient of the curve.

Results and Discussion

Dose Response to Bensulfuron-Methyl and Penoxsulam

In this study, we investigated and characterized sensitivity of 10 *A. multiflora* populations in Jiangsu Province to bensulfuron-methyl and penoxsulam. The results showed that eight *A. multiflora* populations (JS2, JS3, JS5, JS6, JS7, JS8, JS9, and JS10) displayed different resistance levels to bensulfuron-methyl (Table 3; Figure 1A). The JS2 and JS3 populations were highly resistant to bensulfuron-methyl, and the RIs were 90. 9 and 58.4, respectively, compared with the JS1 population. In addition, four populations (JS5, JS7, JS9, and JS10) showed high resistance levels (16.0- to 42.0-fold), and two population (JS6 and JS8) showed moderate resistance levels (9.1- to 9.4-fold) to bensulfuron-methyl.

The results of penoxsulam sensitivity tests indicated that six *A. multiflora* populations (JS2, JS3, JS5, JS7, JS9, and JS10) exhibited high levels of resistance to penoxsulam, with 54.6-, 103.1-, 26.6-, 12.2-, 33.8-, and 51.9-fold resistance, respectively, and two populations (JS6 and JS8) displayed a low to moderate levels of resistance, with RIs ranging from 5.0 to 7.8 (Table 3; Figure 1B).

The results revealed that all but one of the nine tested *A. multiflora* populations were resistant to both bensulfuron-methyl and penoxsulam, suggesting the prevalence of ALS resistance in this weed species. According to the prediction of the *E. crus-galli* resistance evolution model developed by Bagavathiannan et al. (2014), when ALS inhibitors were used to control *E. crus-galli* three times a year, it was expected that there would be resistant biotypes within 4 yr and that 80% of *E. crus-galli* would be at risk of developing resistance after 30 yr of continuous use. The widespread occurrence of ALS herbicide resistance in *A. multiflora* is not surprising, because bensulfuron-methyl has been used for more than 30 yr since its registration in China in 1986, and penoxsulam for more than 10 yr after registration in 2008 in China. In addition, various paddy weeds in China, including *A. arenaria* (Wang et al. 2013; Zhang et al. 2020), smallflower umbrella sedge (*Cyperus difformis* L.) (Li et al. 2020), and *Ludwigia prostrata* Roxb. (Deng et al. 2021), have been demonstrated to evolve resistance to ALS inhibitors, especially to bensulfuron-methyl. This phenomenon was strongly associated with intensive and persistent use of herbicides with the same mechanism of action. Herbicides with a different site of action or an integrated weed management approach should be considered as alternative management options.

ALS Gene Sequencing

Partial *ALS* genes of *A. multiflora* were amplified from cDNA, and the direct sequencing results displayed a chromatogram of double or triple peaks in some sequence regions, indicating that there are multiple gene copies encoding ALS in *A. multiflora* (Figure 2). As expected, isolation of the PCR products by cloning revealed three copies of *ALS* gene sequences (named *AmALS1*, *AmALS2*, and *AmALS3*), and all were actively expressed in *A. multiflora*.

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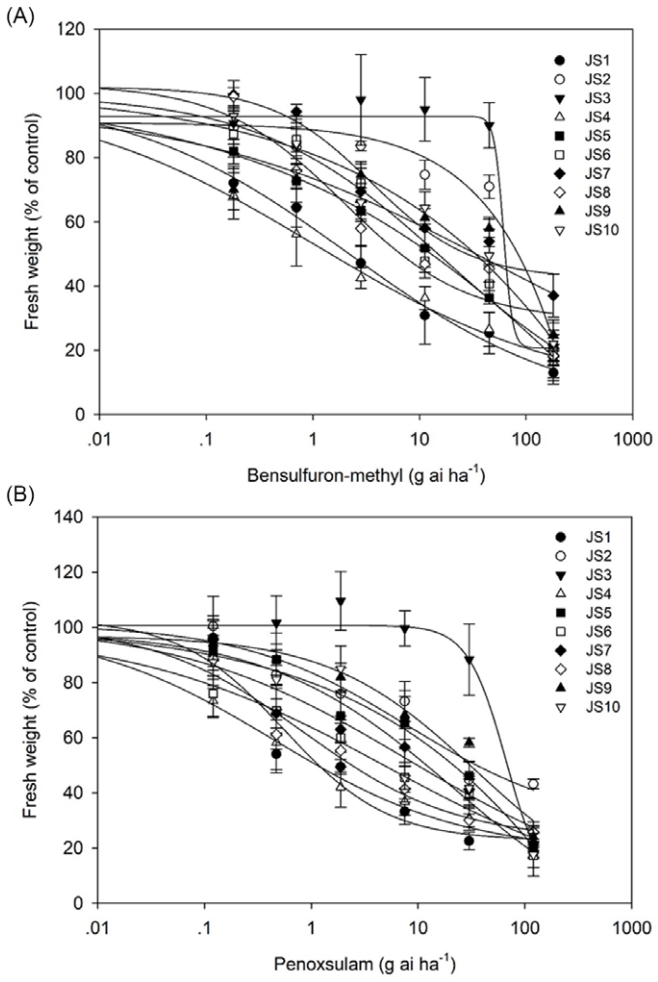

Figure 1. Dose–response curves of 10 *Ammannia multiflora* populations to bensulfuron-methyl (A) and penoxsulam (B).

The alignment of each *ALS* gene sequence from the susceptible and resistant *A. multiflora* populations disclosed nucleotide mutations at the Pro-197 codon in one or two *ALS* genes. Four different mutation types were found at the Pro-197 site, namely, CCT to GCT (JS2 and JS3), CCT to TCT (JS5 and JS10) or CCG to TCG (JS2 and JS8), CCT to CAT (JS6, JS7, and JS9), and CCG to CGG (JS3), resulting in alanine (Ala), serine (Ser), histidine (His), and arginine (Arg) amino acid substitutions, respectively (Table 3). Interestingly, the identified Pro-197 mutations were exclusively observed in *AmALS1* and *AmALS2*, and not in *AmALS3*. In addition, two mutated *ALS* genes in a single plant were also found in two populations that exhibited the strongest resistance. JS2 populations contained the Pro-197-Ala in *AmALS1* and Pro-197-Ser in *AmALS2* mutations, and JS3 populations contained the Pro-197-Ala in *AmALS1* and Pro-197-Arg in *AmALS2* mutations.

Multiple *ALS* gene copies have been frequently observed in weed species, such as rock bulrush [*Schoenoplectus juncoides* (Roxb.) Lye] (Uchino et al. 2007), *D. sophia* (Deng et al. 2017; Xu et al. 2015), *C. bursa-pastoris* (Wang et al. 2019), and *Monochoria vaginalis* (Burm. f.) C. Presl ex Kunth (Iwakami et al. 2020). In our study, three partial *ALS* genes were cloned from all tested *A. multiflora* populations. This phenomenon was generally identical with results in previous studies (Uchino et al. 2007; Xu et al. 2015). The reduced ALS enzyme sensitivity to herbicides resulted from amino acid changes at eight sites of the *ALS* gene, which endowed resistance to ALS herbicides. In the current study, four different Pro-197 mutations (Pro-197-Ala, Pro-197-Ser, Pro-197-His, and Pro-197-Arg) were found in eight resistant populations, indicating these mutations are mainly responsible for bensulfuron-methyl and penoxsulam resistance in the tested *A. multiflora*, although any potential additional NTSR mechanisms were not determined in these populations. No mutations at other sites of the *ALS* genes were detected in resistant *A. multiflora* populations. This is likely due to the major use of sulfonylurea herbicides, which is known to select for mutations at Pro-197 (Yu and Powles 2014). As far as we know, our studies report for the first time the target-site mutations in *A. multiflora*, although these mutations have been found in other resistant weeds.

In our case, two mutated *ALS* genes were identified in two resistant populations, one each on separate copies, which conferred higher levels of resistance than in weeds carrying a single *ALS* gene mutation (Table 3). In recent years, high-level resistance endowed by double *ALS* mutations has been frequently documented in many resistant weeds. For instance, Wang et al. (2019) reported a resistant a *C. bursa-pastoris* population with the Pro-197-Ser mutation in *ALS1* and the Pro-197-His mutation in *ALS2*. Xu et al. (2021) found that a combination of two *ALS* mutations (Asp-376-Glu in *ALS1* and Pro-197-Ala in *ALS2*) in *D. sophia* showed more than 10,000-fold resistance to tribenuron-methyl. Tanigaki et al. (2021) reported a relatively rare resistant case in which the Pro-197-Ser and Asp-376-Glu mutations had occurred in a single *ALS* gene. In addition, double or triple mutations (Thr-102-Ile, Ala-103-Val, and Pro-106-Ser) have also been reported in glyphosate-resistant weeds (Perotti et al. 2018; Yu et al. 2015). Furthermore, the combinations of double ALS mutations have been incorporated in crop-breeding programs to improve tolerance to ALS inhibitors (Jiang et al. 2020; Walter et al. 2014). The accumulation of two mutations may be the consequence of herbicide-resistance evolution imposed by higher selection pressure. In our study, no *ALS* mutations were found in the *AmALS3* copy. Tanigaki et al. (2021) reported that amino acid substitutions were only observed in *MvALS1* or *MvALS3* in 60 resistant *M. vaginalis* populations and that high expression of two *ALS* genes is the driving force of resistance evolution in *M. vaginalis*. It is speculated that the three *ALS* genes may have different expression levels in *A. multiflora*, which requires further study.

Response to Alternative Herbicides

The susceptible and resistant populations of *A. multiflora* were completely controlled by field-recommended doses of MCPA, florpyrauxifen-benzyl, bentazon, and carfentrazone-ethyl (Table 4). The results indicated that control of ALS inhibitor–resistant *A. multiflora* is possible by rotating alternative herbicides with different sites of action. The four alternative herbicides are commonly used in rice fields to control broadleaf weeds. The resistant *A. multiflora* population has not yet evolved resistance to these herbicides of multiple modes of action. However, we should also pay attention to the type of herbicide when selecting herbicides to rotate to avoid the evolution of cross-resistance to the greatest extent possible. Hulme (2022) reported that herbicides could be clustered into three groups, and the same groups tend to be more closely linked in terms of cross-resistance in weed species. One of three herbicide clusters includes ALS inhibitors, synthetic

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Table 4. Control efficacy of MCPA, florpyrauxifen-benzyl, bentazon, and carfentrazone-ethyl at their field-recommended doses in the susceptible (JS1) and four resistant (JS2, JS3, JS5, and JS10) *Ammannia multiflora* populations.[a]

	Survivors					Aboveground fresh weight				
Herbicides	JS1	JS2	JS3	JS5	JS10	JS1	JS2	JS3	JS5	JS10
	% of tested					% of control				
MCPA	0 a	0 a	0 a	0 a	0 a	9.3 a	7.8 a	5.5 a	7.5 a	8.6 a
Florpyauxifen-benzyl	0 a	0 a	0 a	0 a	0 a	10.2 a	11.5 a	8.4 a	12.6 a	10.4 a
Bentazon	0 a	0 a	0 a	0 a	0 a	4.9 a	5.3 a	3.7 a	4.5 a	4.2 a
Carfentrazone-ethyl	0 a	0 a	0 a	0 a	0 a	8.5 a	11.6 a	9.6 a	9.0 a	8.4 a

[a]Survivors or aboveground fresh weight followed by the same letters in rows indicates no significant difference ($P < 0.05$).

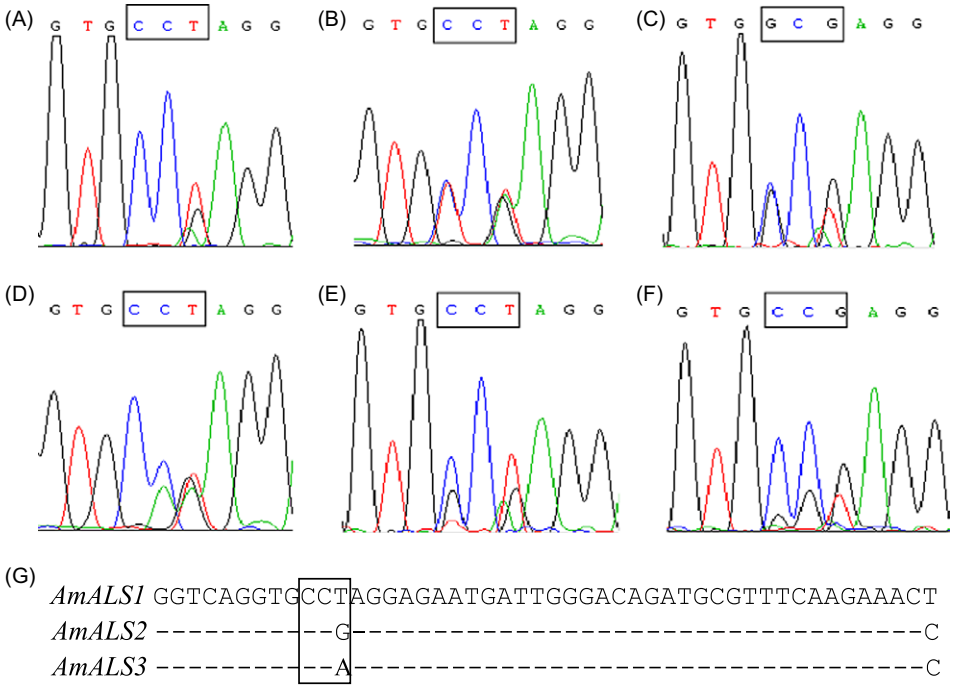

Figure 2. Sequence diagrams of *ALS* genes in *Ammannia multiflora*. All populations contain three *ALS* gene copies. (A) 197CCT(G/A) for Pro; (B) 197CCG(A) for Pro and TCT for Ser; (C) 197CCG(A) for Pro and GCT for Ala; (D) 197CCG(A) for Pro and CAT for His; (E) 197CCA for Pro, GCT for Ala, and TCG for Ser; (F) 197CCA for Pro, GCT for Ala, and CGG for Arg; (G) partial fragments of three *ALS* genes, and the boxed region represents the codon position 197 of *ALS*.

auxins, photosystem II (PSII) inhibitors, and 5-enolpyruvylshikimate-3-phosphate synthase inhibitors. Thus, from the point of view of developing cross-resistance, the protoporphyrinogen oxidase (PPO) inhibitor carfentrazone-ethyl may be a better option for management of resistant *A. multiflora*, rather than the synthetic auxins MCPA and florpyrauxifen-benzyl and the PSII inhibitor bentazon. In addition, utilizing herbicide mixtures or preemergence herbicides and integrated weed management programs should be encouraged to reduce the risk of resistance development in *A. multiflora*.

In conclusion, eight *A. multiflora* populations originating from rice fields where failure of ALS-inhibiting herbicides occurred have been shown to evolve resistance to bensulfuron-methyl and penoxsulam. Three *ALS* gene copies were observed in the collected *A. multiflora* populations, and *AmALS1* (Pro-197Ser, Pro-197Ala, and Pro-197His) and *AmALS2* (Pro-197Ser and Pro-197Arg) mutations or pairs of these mutations were found and appear to be the cause of resistance. Moreover, the alternative herbicides MCPA, florpyrauxifen-benzyl, bentazon, and carfentrazone-ethyl can be used to control both susceptible and resistant *A. multiflora* populations, but care should be taken to avoid evolution of further resistance.

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