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Resistance test of *Echinochloa crus-galli* from West Java toward metsulfuron-methyl and penoxsulam

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ABSTRACT

Barnyard grass is acknowledged as the most troublesome weed in rice fields in West Java province of Indonesia due to development of resistance to certain chemical control measures. Therefore, this study was conducted during 2020 at the greenhouse of Faculty of Agriculture, Universitas Padjadjaran, Indonesia aimed to confirm and classify the presence of herbicide-resistant of *Echinochloa crus-galli* toward metsulfuron-methyl and penoxsulam. The resistance level test of *E. crus-galli* was performed using the Whole Plant Pot Test method laid out under Split Plot Design with 3 replications. The main plot comprised of metsulfuron-methyl herbicide: 0, 1, 2, 4, 8, 16 and 32 g a.i./ha, whereas for the dose of penoxsulam herbicide was 0, 2.5, 5, 10, 20, 40 and 80 g a.i./ha. The subplot was the origin of weed: exposed and not exposed to herbicides. The results showed that *E. crus-galli* from East Karawang was included in to low level of resistance to metsulfuron-methyl with a resistance ratio of 5.88. This study has affirmed resistance to metsulfuron-methyl in *E. crus-galli* for the first time. In contrast, no resistance was observed in *E. crus-galli* samples to penoxsulam.

Key Words : *Echinochloa crus-galli*, metsulfuron-methyl, penoxsulam, weed resistance

INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food resource in Indonesia (Setyowati *et al.*, 2018). The second-largest rice producer in Indonesia is West Java Province with productivity of 57.54 q/ha (Badan Pusat Statistik, 2019). However, some efforts to maintain and increase rice productivity usually encounter several problems, one among that of which is a competition between rice plants and weeds. Weeds can reduce the rice yield by 21 - 56% in Pakistan (Rabbani *et al.*, 2011) and 11 - 66% in India (Singh and Maiti, 2016; Gharde *et al.*, 2018; Selvaraj and Hussainy, 2020).

Banyard grass [*Echinochloa crus-galli* (L.) P. Beauv.] is one of the most serious grass weeds in rice production in the world. Antralina (2012) specified that *E. crus-galli* can diminish the rice yield by 57% in Indonesia. The chemical use of herbicides is one way to control weeds in rice cultivation. Beltran *et al.* (2013) have shown that the utilization of herbicides is 80% more profitable than conventional weeding. However, the extensive use of herbicides will cause weed resistance. Weed resistance occurs frequently over a long period due to the utilize of the same herbicide or herbicide with the same mode of action (Battel, 2018).

Metsulfuron-methyl is a selective, systemic pre and post-emergence herbicides often used in lowland rice cultivation areas to control the growth of several types of weeds (Rao, 2000). Moreover, penoxsulam is also a widely used herbicide for lowland rice around the world because it is selective, effective, and has a broad spectrum (Johnson *et al.*, 2009). Metsulfuron-methyl and penoxsulam have the same mode of action, *i.e.*, inhibiting the acetolactate synthase (ALS) enzyme with the aim that the chains of amino acids valine, leucine and isoleucine are not formed (Su, 2008; Ross and Childs, 2010). Acetolactate synthase inhibitor herbicide (ALS) is the most common herbicide used in lowland rice cultivation in the world (Tranel and Wright, 2002).

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Echinochloa crus-galli biotypes from 11 nations have been reported to be resistant to herbicides that inhibit acetolactate synthase (ALS) (Heap, 2020). In Jiangsu and Anhui Provinces of China, *E. crus galli* was reported as being resistant to penoxsulam herbicides from the triazolopyrimidine chemical compound. In addition, cross-resistance exists in the biotype of Anhui province against herbicides that inhibit ALS of all chemical compounds, namely: pyrimidinyl-thiobenzoate, imidazolinone, triazolopyrimidine, sulfonyl-carbonyl-triazolinone, and sulfonylurea (Fang *et al.*, 2019).

In Indonesia, *E. crus-galli* is still not officially registered as a resistant weed in paddy fields. A number of populations of *E. crus-galli* have been found to be resistant to ALS-inhibiting herbicides in West Java province, especially metsulfuron-methyl. However, this report has not been supported by an in-depth analysis, thus resistant weeds in West Java are still unclear. Therefore, this experiment was necessary to identify and classify the presence of herbicide-resistance in *E. crus-galli* for commonly used herbicides *viz.*, metsulfuron-methyl and penoxsulam.

MATERIALS AND METHODS

A field experiment was conducted during August to October of 2020 at the greenhouse of Faculty of Agriculture, Universitas Padjadjaran, Indonesia to confirm the presence of herbicide-resistant of *E. crus-galli* toward metsulfuron-methyl and penoxsulam and classify the resistance level of *E. crus-galli* toward metsulfuron-methyl and penoxsulam.

Plant Materials and Growth Conditions

Seeds of suspected resistant *E. crus-galli* populations were collected from rice fields in Rancaekek (6°57′37.6″S 107°45′53.4″E), East Karawang (6°18′22.0″S 107°19′35.4″E), and Majalaya (6°19′39.7″S 107°21′48.0″E) districts. *E. crus-galli* seeds were also obtained from herbicide-untreated paddy fields in Banyusari (6°16′58.0″S 107°34′06.8″E), subsequently referred to as susceptible biotype. Seeds were stored at 4°C for one month to break dormancy (Chen *et al.*, 2016). The Whole Plant Pot Test system (Burgos, 2015) is the method used in this study. The plants were cultivated in plastic pots (18 cm in diameter) that were filled with rice paddy field soil. All plants were kept in the greenhouse and watered every 3 days.

Dose Response Experiment

Seedlings have been thinned to 10 plants per pot before herbicide treatment. At the 2-3 leaf stage, herbicides were applied using a YOTO 5 litre pressure sprayer with Anvil flood jet green nozzle 1.2 L/min. Metsulfuron-methyl was applied at 0, 1, 2, 4, 8, 16 and 32 g a.i./ha and penoxsulam was applied at 0, 2.5, 5, 10, 20, 40 and 80 g a.i./ha. These rates are equivalent to 0, 0.25 0.5, 1, 2, 4 and 8 times of the recommended field rate of the products. Recommended rates of metsulfuron-methyl and penoxsulam levels are 4 and 10 g a.i./ha, respectively. Dose-response experiments for each herbicide were conducted based on a completely randomized design with three replicates. The plants were cut on the soil surface twenty-one days after treatment (DAT), oven-dried for 48 h at 80°C (Marambe and Amarasinghe, 2002; Valeriy *et al.,* 2020) and shoot dry weights were recorded.

Data Analysis

The whole-plant dose-response data were exposed to ANOVA utilizing SPSS v. 25.0. Non-linear regressions of equation were used to analyze data (Seefeldt *et al.,* 1995) :

$$y = C + \frac{D - C}{1 + (x/I_{50})^b}$$

Where, C = Lower limit, D = Upper limit, b = Slope, and I_{50} = A dosage that gives a 50% response.

An analysis of the dose-response curves was performed using Origin Pro 2016 (Originlab Corp, 2016). For each herbicide, Resistance-Susceptible (R/S) ratio was determined as follows: GR_{50} of the resistant (R) population/GR_{50} of the susceptible (S) population. According to Ahmad-Hamdani *et al.* (2012), the herbicide sensitivity is further divided into four classes: susceptible (R/S< 2), low resistance (R/S = 2–6), moderate resistance (R/S = 6–12) and high resistance (R/S>12).

RESULTS AND DISCUSSION

Sensitivity to ALS Herbicides

There was a significant difference between assay repetitions in the ANOVA results, hence, the outcomes for the repeat assays were summed. The GR50 values for Rancaekek, East Karawang, Majalaya, and susceptible biotypes were 1.36, 7.59, 2.37 and 1.29 g a.i./ha metsulfuron-methyl, respectively (Fig. 1). The GR50 value with metsulfuron-methyl was 1.90 times higher (7.59 g a.i./ha) than the recommended application dose (4 g a.i./ha) for East Karawang. Based on the interview results, it was found that there were differences in the frequency of herbicide application in one growing season and the length of land use at each location. The rice field from East Karawang was treated with herbicides twice per planting season with land use reaching 10 years; the frequency of herbicide applications at that location is higher than at other locations.

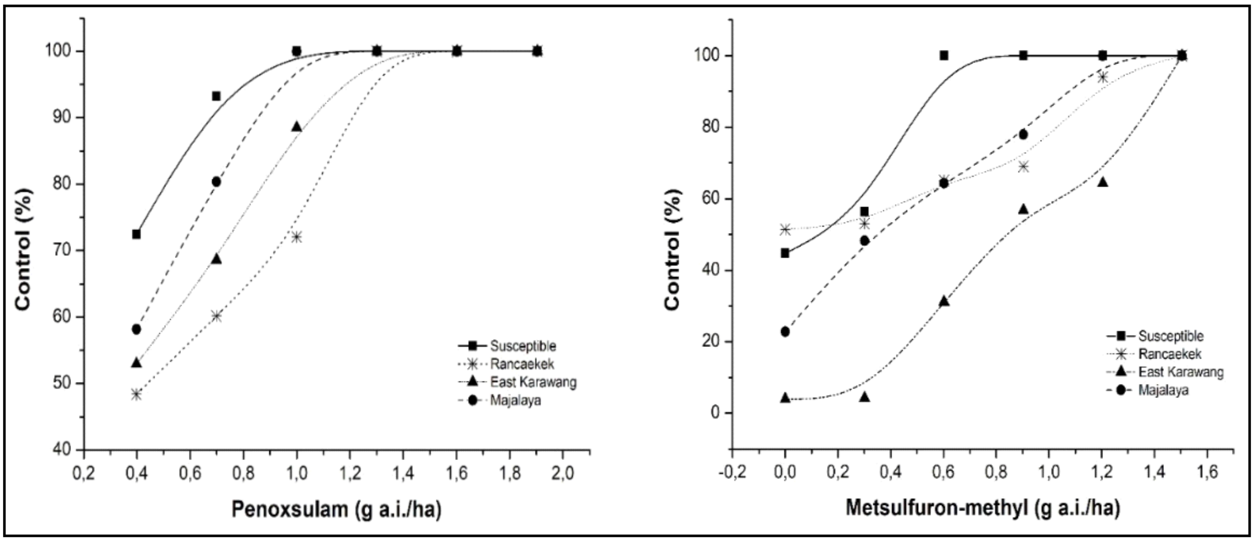

Fig. 1. Inhibition curves by metsulfuron-methyl and penoxsulam from four populations of *E. crus-galli.* Lines are the reaction bends foreseen by non-linear regression; symbols represent shares average survival, upheld the untreated controls.

The GR50 values with penoxsulam were 3.08, 2.46, 2.16 and 1.70 g a.i./ha for Rancaekek, East Karawang, Majalaya, and susceptible biotypes, respectively (Fig. 1). The GR50 values of the E. crus-galli population, which was thought to be resistant were less than the recommended dose of penoxsulam (10 g a.i./ha), which means they were susceptible to penoxsulam. This confirms the information during the interview that the rice fields were treated with metsulfuron-methyl and never with penoxsulam.

Unlike the results of this study, weeds have the potential to develop resistance in the same mode of action against many herbicide families, even though some herbicides have never been used to control weeds. For example, a population of *E. phyllopogon* developed bensulfuron and bispyribac-sodium cross-resistance from California. However, only bensulfuron was used in the rice fields and never with bispyribac-sodium (Fischer *et al.,* 2000).

Confirmation of Resistance

The resistance levels of the ALS-resistant accessions were calculated as the R/S ratios of the GR50 values (Table 1). *Echinochloa crus-galli* biotypes from Rancaekek and Majalaya were susceptible to metsulfuron-methyl, yet those from East Karawang showed low resistance to methyl metsulfuron (R/S 5.88), suggesting that it was 5.88 times more resistant to metsulfuron-methyl compared to the susceptible population. To date, 33.33% of the West Java population of *E. crus-galli* has a low resistance (R/S = 2-6) and 66.67% are susceptible to metsulfuron-methyl (R/S = <2). Nevertheless, all biotypes were susceptible to penoxsulam. Based on the GR50 value, penoxsulam R/S ratios for Rancaekek, East Karawang and Majalaya biotypes were 1.81, 1.45 and 1.27, respectively. The East Karawang population was resistant to metsulfuron-methyl but was susceptible (not cross-

Table 1. Metsulfuron-methyl and penoxsulam dose needed to kill 50% of plants (GR50) and R/S ratios obtained from the curves of population S and R *E. crus-galli*

Herbicide	Population	R^2	$GR_{50}{}^a$	R/S ratiosb
Metsulfuron-methyl	Susceptible	0.87	1.29	-
	Rancaekek	0.81	1.36	1.04
	East Karawang	0.94	7.59	5.88
	Majalaya	0.97	2.37	1.84
Penoxsulam	Susceptible	0.99	1.70	-
	Rancaekek	0.90	3.08	1.81
	East Karawang	0.97	2.46	1.45
	Majalaya	0.97	2.16	1.27

[a]GR_{50} alludes to the successful measurements of herbicide causing 50% restraint of dry weight and is demonstrated as grams of active ingredient per hectare (g a.i./ha)
[b]R/S ratio, resistance index calculated as the ratio of GR_{50} R over GR_{50} S

resistant) to penoxsulam, indicating that penoxsulam may viably be utilized to control this population of *E. crus-galli.*

The different levels of resistance to metsulfuron-methyl and penoxsulam from each location can be seen visually in Fig. 2. Other researchers have identified populations of *Echinochloa crus-galli* resistant to sulfonylurea. *Echinochloa crus-galli* biotype from China is considered to have resistance to flucetosulfuron, propyrisulfuron and rimsulfuron with ratios (R/S) of 16.37, 10.79 and 8.34, respectively (Fang *et al.*, 2019).

Similar to the results of this study, some of the biotypes of *E. crus-galli* (TVA8 and TEV9) from Uruguay were susceptible to penoxsulam with R/S ratios of 1.1 and 1.9 (Marchesi and Saldain, 2019). Furthermore, *E. glabrescens* from the Province of Anhui (China) could be effectively controlled by the oxidant in the field at the recommended dosage level, but not by oxaziclomefone and butachlor. In addition, it was confirmed that the other five species (*E. colonum*, *E. crus-galli* var.

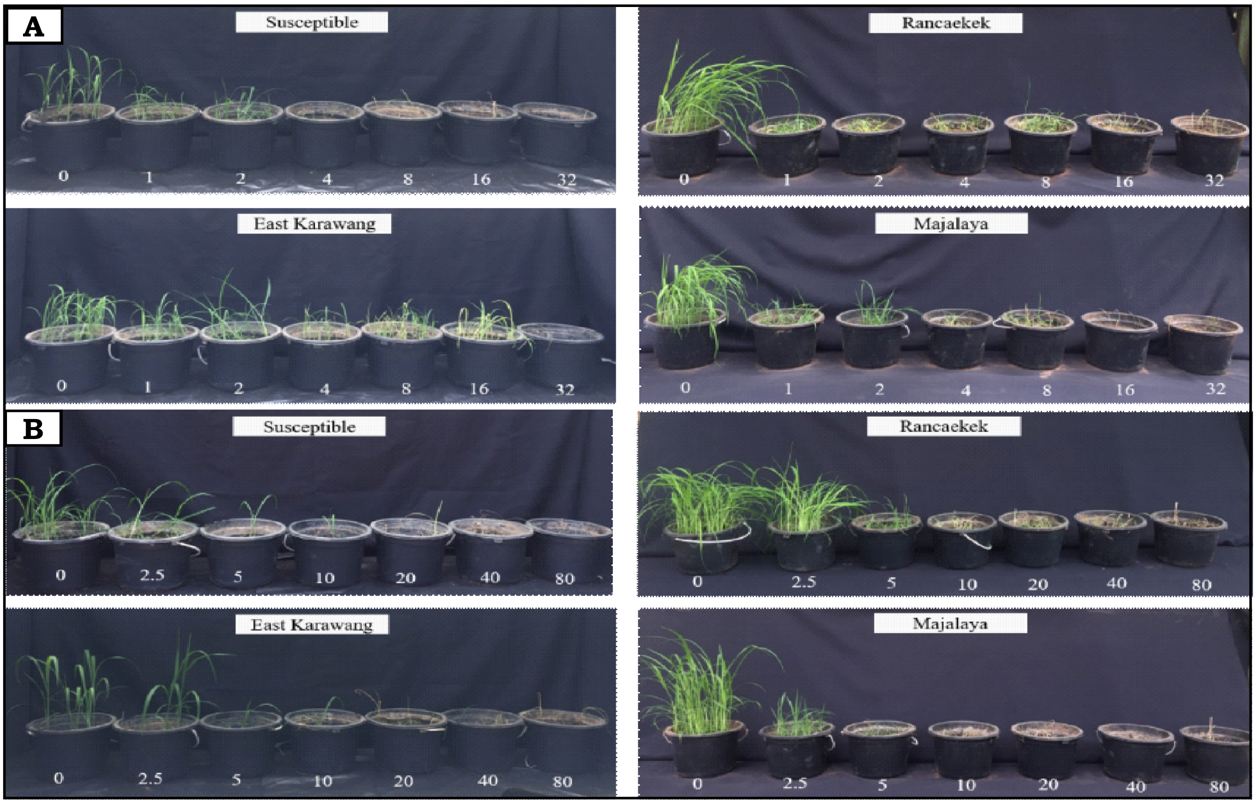

Fig. 2. The growth of resistance (R) and susceptible (S) *E. crus-galli* biotypes at various concentrations of the herbicides metsulfuron-methyl (A) and penoxsulam (B). The dose of each herbicide was in g a.i./ha.

zelayensis, *E. caudata*, *E. phyllopogon* and *E. oryzoides*) were susceptible to penoxsulam (Wang *et al.*, 2015).

In contrast to the results of this study, a low level of resistance to penoxulam was observed in *E. phyllopogon* (R/S 5) and a non-target location component was recognized (Yasuor *et al.*, 2009). The lower than anticipated R/S ratio (based on *in vitro*) bioassays, especially for penoxsulam might be due to the polyploidy of *E. crus-galli* and the existence of un-mutated alleles (Panozzo *et al.*, 2013). In addition, *E. crus-galli* biotypes from a few countries have experienced high resistance to penoxsulam with ratios (R/S) of 39 (Turkey) and 69 (China) (Chen *et al.*, 2016; Kacan *et al.*, 2020).

Herbicide resistance mechanisms can be divided into two categories: the target-site resistance mechanism (TSR) and the non-target site resistance mechanism (NTSR). The mechanism of NTSR includes decreased assimilation or translocation and expanded sequestration or metabolic debasement. On the other hand, TSR mechanisms are advanced processes, unique to a single site of action. The TSR may be attributed to the improved expression of the content of the target site, producing more protein than can be considerably restrained by ordinary application rates of herbicides. Increased expression of the gene can be due to regulatory changes and/or an expanded amount of genomic copy of the target-site gene, which also results in increased transcription (Gaines *et al.*, 2020). In expansion, the results of other studies suggest that mutations in Pro-197 lead to resistance to SU (sulfonylurea) (Tranel *et al.*, 2020). Up to the present, TSR mutation detection has progressively highlighted its significance compared to NTSR although the mechanisms of TSR and NTSR can coexist in the same population (Bai *et al.*, 2019; Chen *et al.*, 2020). For instance, two CYP450 genes, namely CYP94A1 and CYP71A4 are constitutively expressed by the short foxtail resistance mechanism to metsulfuron-methyl (Zhao *et al.*, 2019).

Management Implications

In general, the strategies include a mix of early preventive steps and one or more essential control measurements (chemical, cultivation innovation, biological, and physical). Early preventive steps include identification of resistance and risk evaluation (Liu *et al.*, 2020). In postponing resistance, herbicide rotation has been connected to combat weed resistance (Marochi *et al.*, 2018). For cultivation management, crop rotation is as a rule received by rotating of a grass crop (e.g. rice) with a broadleaf, or by rotating of a wetland crop (e.g. rice) with a dry land crop (Le *et al.*, 2018; Liu *et al.*, 2018). Additionally, logical information shown in bioorganic fertilizers can effectively regulate *E. crus-galli* and *M. vaginalis* in rice areas with a normal rate of more than 80% weed concealment (Li *et al.*, 2018). In expansion, the stale seedbed physical action is valuable for depleting the seed bank before planting crops (Feng *et al.*, 2015). The effectiveness of multiple resistant control on *E. crus-galli* weeds will surpass 85% by using an integrated method (Ma *et al.*, 2012; Zhang *et al.*, 2018).

CONCLUSION

The population of *Echinochloa crus-galli* originating from East Karawang was classified as low resistance to methyl metsulfuron (R/S 5.88) but susceptible to penoxsulam. This study confirms the first case of metsulfuron-methyl (sulfonylurea) resistance in *E. crus-galli*. Meanwhile, the population of *E. crus-galli* originating from Rancaekek and Majalaya was classified as being susceptible to both metsulfuron-methyl and penoxsulam (R/S<2).

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