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Management of *Eleusine indica* (L.) Gaertn Resistance to Glyphosate Herbicide in Indonesia

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Abstract: *Eleusine indica* (L.) Gaertn, commonly known as goosegrass or wiregrass, is a type of grass that is widespread in many parts of the world. The broad-spectrum herbicide glyphosate is most frequently used in Indonesian oil palm plantations to get rid of weeds and other undesirable plants. However, improper rotation of herbicide types by farmers has led to an increased risk of resistant weed emergence. This investigation tries to validate *E. indica*'s glyphosate resistance, investigate mutations in the EPSPS gene of the resistant biotype, and determine the type of herbicides that can control *E. indica* glyphosate-resistant biotypes. The whole plant pot test method was used to measure the resistance level, while DNA sequencing using the PCR method was conducted on all samples to identify mutations in the EPSPS gene of the resistant biotype. The study results showed that all biotypes of *E. indica* were identified as resistant to glyphosate but susceptible to propaquizafop, ametryn, and sulfentrazone herbicides. Several biotypes, such as the North Sumatra biotype, were identified as having multiple resistances to glyphosate, paraquat, and ammonium glufosinate. Thr102Iso and Pro106Ser amino acid substitutions were found in the EPSPS gene of *E. indica*-resistant biotypes. The findings of this study showed that *E. indica* was resistant to paraquat and ammonium glufosinate; further research is required to determine the mechanism.

Keywords: glyphosate; herbicide resistance; *Eleusine indica*; amino acid substitution

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1. Introduction

In a crop field, weeds are any uninvited plants that proliferate and compete with the intended crop for resources like water, nutrients, and light. They reduce crop yields and quality and may also harbor pests and diseases that damage crops [1]. Weed control is an integral aspect of crop cultivation, and various methods are used, including mechanical methods (hand-weeding and tillage), cultural methods (crop rotation and use of cover crops), and chemical methods (herbicides) [2]. The choice of weed control method depends on factors such as the crop type, the type of weeds, the severity of the weed infestation, and the resources available. Effective weed management is critical for maximizing crop yields and reducing losses due to weed competition [3].

Eleusine indica (L.) Gaertn, often called goosegrass or wiregrass, is a grass that grows widely throughout the world. It is a fast-growing annual or perennial weed that thrives in various environments, including cultivated fields, gardens, lawns, and roadsides [4]. Goosegrass possesses flat, narrow leaves and a fibrous root system. It produces small, greenish-white flowers (a self-pollinating monoecious species) and can reach a height of approximately 60 cm. The seeds can germinate at any time of the year and are carried in clusters. In agricultural regions, this weed is seen as a severe issue since it competes with crops for water, nutrients, and sunlight. It is also difficult to control because of

its ability to produce large quantities of seeds and its tolerance to many herbicides [4]. Besides being tolerant, *E. indica* has become a significant problem weed in agricultural fields, largely due to its ability to develop resistance to herbicides. Reports indicate that it has developed resistance to several classes of herbicides, including glyphosate, paraquat, ACCase inhibitors, atrazine, and acetolactate synthase (ALS) inhibitors [5].

Weed resistance occurs when a weed population evolves to become less susceptible or completely resistant to the effects of herbicides. The resistance mechanism is divided into two categories: non-target site resistance (NTSR) and target site resistance (TSR). The NTSR is a mechanism of weed resistance by reducing absorption, translocation, and/or metabolism of herbicides into non-toxic compounds, while the TSR mechanism is a mutation at the target site that is targeted by herbicides so that the target site is not obstructed by the application of herbicides [6]. The overuse or improper application of herbicides, the recurrent application of herbicides with the same mechanism of action, and genetic heterogeneity among weed populations are only a few causes of this [7]. The development of TSR involves NTSR mechanisms such as physiological adaptations (absorption, translocation), biochemical adaptations (metabolism), TSR mechanisms such as molecular adaptations (mutation, gene amplification), and even the accumulation of both types of mechanisms [8].

Herbicide resistance has a complicated genetic foundation that changes based on the particular herbicide and its mechanism of action. Resistance to different herbicides is often associated with different genetic mechanisms [8]. Cross-resistance (weed resistance to the same mode of action with different chemical groups) can be caused by a single mutation, while multiple resistance (weed resistance to several herbicides with different modes of action) is mostly caused by more than one mutation or a combination of TSR and NTSR mechanisms [9]. It is a significant problem in agriculture because it can reduce the effectiveness of herbicides, which are vital tools in weed management. When a weed population becomes resistant to herbicides, it can spread rapidly and become difficult to control, resulting in reduced crop yields and increased costs for farmers [10].

A broad-spectrum herbicide known as glyphosate is frequently used to eradicate weeds and other undesirable plants. It was first made available in the 1970s, and today, especially in agriculture, it is one of the most often used herbicides worldwide [11]. However, some plant species, including *E. indica*, have developed glyphosate resistance over time. This resistance is linked to a mutation in the EPSPS gene and physiological adaptations [12,13]. Glyphosate works by inhibiting an enzyme called EPSP synthase, which is involved in the production of certain amino acids that are essential for plant growth. When this enzyme is inhibited, the plant is unable to produce these amino acids and eventually dies [14]. The development of glyphosate-resistant weeds has been facilitated by the widespread use of glyphosate. Resistance to glyphosate is the second-largest case of resistance in the world after atrazine. Currently, there are 355 cases of resistance to glyphosate occurring in more than 50 weed species in 29 countries with Thr102, Ala103, and pro106 EPSPS gene mutations [15]. There have been reports of glyphosate-resistant weeds in a number of crops in Indonesia, including oil palm and rubber [16].

The use of herbicides in Indonesia is often characterized by improper application, such as exceeding recommended doses, spraying at inappropriate times, and neglecting proper safety protocols. Moreover, most farmers in Indonesian oil palm cultivation do not apply herbicide rotation [17]. This may result in weed populations becoming resistant to herbicides as well as other negative effects such as environmental contamination, health risks for farmers, and others. In Indonesian oil palm plantations, herbicides are commonly utilized for weed control, with glyphosate, paraquat, and ammonium glufosinate being the most frequently used [18]. In this study, we tested *E. indica*'s resistance to glyphosate. Other herbicides marketed in Indonesia, such as paraquat, ammonium glufosintae, propaquizafop, ametryn, and sulfentrazone, were also tested to determine whether these herbicides could be used to control glyphosate-resistant biotypes of *E. indica*. The nucleotide sequence of the EPSPS gene from the resistant and susceptible *E. indica* biotypes was also determined to determine the mechanism of resistance.

2. Material and Methods

2.1. Plant Materials

Susceptible and resistant biotypes of *E. indica* seeds were collected from oil palm cultivation. After harvesting the seeds, they were cleaned and sun-dried for a week to minimize moisture and improve maturation. The coordinates of the sampling locations for each weed biotype, including five glyphosate-resistant biotypes that had passed the resistance screening test and one biotype that proved susceptible to glyphosate, are shown in Table 1. The five resistant weed samples were obtained from oil palm plantations and identified by local farmers as weeds that could not be effectively controlled with glyphosate herbicide.

Table 1. *Eleusine indica* sampling locations.

Province	District	Coordinate	*Eleusine indica*
North Sumatra	Langkat	3°38′49.0″ N 98°19′44.3″ E	Susceptible
Lampung	Pesawaran	5°10′07.0″ S 105°06′12.0″ E	Resistant
Belitung	East Belitung	3°1′23″ S 107°52′23″ E	Resistant
West Kalimantan	Ketapang	1°28′28.1″ S 110°12′23.4″ E	Resistant
North Sumatra	Langkat	3°38′49.046″ N 98°19′34.458″ E	Resistant
Riau	Pelalawan	0°9′22.439″ S 102°12′51.997″ E	Resistant

Rotation of herbicides with alternatives to glyphosate is uncommon, with only paraquat herbicide being used when rotation is done. Farmers at the *E. indica* biotype sampling sites reported a history of using glyphosate herbicides, subsequently transitioning to paraquat when glyphosate was perceived as ineffective in weed control. Eventually, they switched to ammonium glufosinate when paraquat also lost its efficacy. Herbicide substitution in these locations was not accompanied by a rotation of the herbicide mode of action during implementation. Farmers simply changed the herbicide type without utilizing the previous herbicide, thereby increasing the likelihood of weeds developing multiple resistances.

2.2. Herbicide Dose–Response Experiments

Herbicide dose–response research was conducted using the whole plant pot test dose–response approach [19]. Pots (20 cm in diameter) were filled with soil (3 kg) after being sterilized in an autoclave at 120 °C and a pressure of 15 kPa for 2 h, allowing only *E. indica* seeds to sprout and thrive. Then, in the pots, 10–20 *E. indica* seeds were sown on the soil surface. During the studies, enough water was supplied to the pots. Four weeks after sowing herbicides were applied at seven dosage levels (0, 0.25, 0.5, 1, 2, 4, and 8 times of herbicide recommended dosage): Glyphosate (0, 187.5, 375, 750, 1500, 3000, 6000 g a.i. ha^{-1}; Roundup 486 SL, PT. Nufarm, Jakarta, Indonesia), Paraquat (0, 100, 200, 400, 800, 1600, 3200 g a.i. ha^{-1}; Gramoxone 276 SL, PT. Syngenta Indonesia, Jakarta, Indonesia), ammonium Glufosinate (0, 75, 150, 300, 600, 1200, 2400 g a.i. ha^{-1}; Basta 150 SL, PT. BASF Indonesia, Jakarta, Indonesia), Propaquizafop (0, 25, 50, 100, 200, 400, 800 g a.i. ha^{-1}; Agil 100 EC, PT. Royal Agro Indonesia, Jakarta, Indonesia), Ametryn (0, 206.25, 412.5, 825, 1650, 3300, 6600 g a.i. ha^{-1}; Kingdom 550 SC, PT. Sinar General Industries, Serang, Banten, Indonesia), and Sulfentrazone (0, 180, 360, 720, 1440, 2880, 5760 g a.i. ha^{-1}; Boral 480 SC, PT. Bina Guna Kimia, Semarang, Central Java, Indonesia). Herbicide application was carried out with a spray volume of 400 L ha^{-1} and a pressure of 138 kPa utilizing a semiautomatic backpack sprayer with a flat fan nozzle. *Eleusine indica* was harvested 4 weeks after application of the herbicides to determine dry weight.

2.3. Dose–Response Experiments Statistical Analyses

Data on the dry weight of the weeds were used to calculate the growth reduction (GR_{50}) using non-linear regression analysis and the log-logistic model [20]. Weed dry weight data were converted to growth reduction percentages, which were obtained by comparing the dry weights of the weeds to which herbicide was applied (T) and the dry weights of weeds

with no herbicide application (C). (Growth decrease (%) = [1 − (T/C)] × 100) [21] and the nonlinear log-logistic regression equations were used to calculate the sigmoidal growth curve. To get the GR_{50} value of each herbicide, the sigmoid growth curve is employed to match the distribution of growth data. Furthermore, the regression analysis was carried out using the Origin Pro version 2018 software (Originlab Corporation, Northampton, MA, USA, 2018). The formula for the non-linear log-logistic regression curve is as follows:

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

where b is the slope, I_{50} is the dose that results in a 50% response, and C and D are the lower and upper limits of the data range, respectively.

The level of resistance is obtained from a comparison between the GR_{50} resistant and susceptible biotypes (GR_{50} Resistant/GR_{50} Susceptible); the classification is as follows: high resistance: R/S > 12, moderate resistance: R/S = 6–12, low resistance: R/S = 2–6, Susceptible: R/S < 2 [22].

2.4. DNA Isolation and EPSPS Gene Sequence

The "Quick-DNA Plant/Seed Miniprep Kit" was used to isolate genomic DNA from the leaves of *E. indica* biotypes that were susceptible and resistant (Zymo Research, D6020, Irvine, CA, USA). Using the primers F-CTCTTCTTGGGGAATGCTGGA and R-TAACCTTGCCACCAGGTAGCCCTC, a 300-bp EPSPS fragment was amplified [13] for PCR amplification of the EPSPS genes of *E. indica*. The amplification procedure was carried out in a total volume of 35 µL, which contained 25 µL of "PCR buffer for KOD FX Neo" (KFX-201 Toyobo Co., Ltd., Osaka, Japan), 1.5 µL of each primer (0.3 µM), 6 µL of dd H_2O, and 1 µL of *E. indica* DNA (10 ng). The PCR conditions were as follows: 94 °C for 2 min for denaturation; 30 cycles of 98 °C for 15 s and 64 °C for 10 s; and 72 °C for final extension. Thermo Fisher Scientific Inc.'s VeritiPro Thermal Cycler, 96 well (Applied Biosystems, Waltham, MA, USA, A48141) were used to perform capillary electrophoresis (Sanger sequencing method) after the DNA amplification process.

3. Results

The study's findings showed that weeds from Lampung, Belitung, West Kalimantan, North Sumatra, and Riau had a low level of glyphosate herbicide resistance (Figure 1, Table 2). *Eleusine indica* originating from Lampung and Riau also showed resistance to paraquat, while *E. indica* from North Sumatra was resistant to both paraquat and ammonium glufosinate herbicides. The lowest doses of ACCase inhibitor herbicides (propaquizafop), photosystem II (PSII) inhibitors (ametryn), and protoporfirinogen oksidase (PPO) inhibitors (sulfentrazone) efficiently controlled the resistant biotypes of *E. indica*. This indicates a high susceptibility of the resistant biotypes of *E. indica* to these three herbicides (Table 2).

The highest resistance index in the response of *E. indica* to glyphosate was 5.6 (North Sumatra biotype), indicating that a dose of herbicide 5.6 times higher than the one required to control susceptible *E. indica* was needed to control the resistant biotype. Similarly, the highest resistance indices in the response of *E. indica* to paraquat and ammonium glufosinate were 19.0 and 2.4, respectively. Each biotype showed a distinct response to the application of glyphosate herbicide. However, it is evident from Figure 1 that all biotypes of *E. indica* resistant did not experience a 100% growth reduction (death) at the highest application dose; the pressure of glyphosate herbicide application at the highest dose allowed all biotypes of *E. indica* resistant to survive.

The Lampung, North Sumatra, and Riau biotypes of *E. indica* experienced a similar outcome after being treated with paraquat. These biotypes could not be controlled by paraquat at the highest doses. Growth reduction at the highest dose of herbicide application only reached 71% in the Lampung biotype, 58.5% in the Riau biotype, and 47% in the North Sumatra biotype. *Eleusine indica* from North Sumatra showed resistance to ammonium

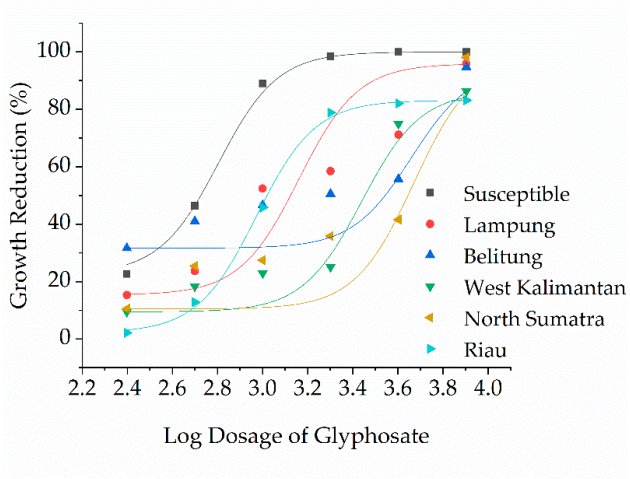
glufosinate; *E. indica* did not experience a complete growth reduction with a resistance index value of 2.4 and complete growth reduction values at four times the advised dose (1200 g a.i. ha^{-1}). Furthermore, 86.70% and 99.10% reductions were observed at one and two times the recommended dose (300 and 600 g a.i. ha^{-1}), respectively (Figure 1).

The nucleotide sequence of PCR-amplified DNA and the *E. indica* EPSPS genes were completely homologous [13]. The sequencing of the EPSPS gene revealed two nucleotide substitutions between the susceptible and resistant biotypes, resulting in amino acid substitutions at positions 102 (Thr102Iso, ACT; susceptible, ATT; resistant) and 106 (Pro106Ser, CCA; susceptible, TCA; resistant) (Table 3).

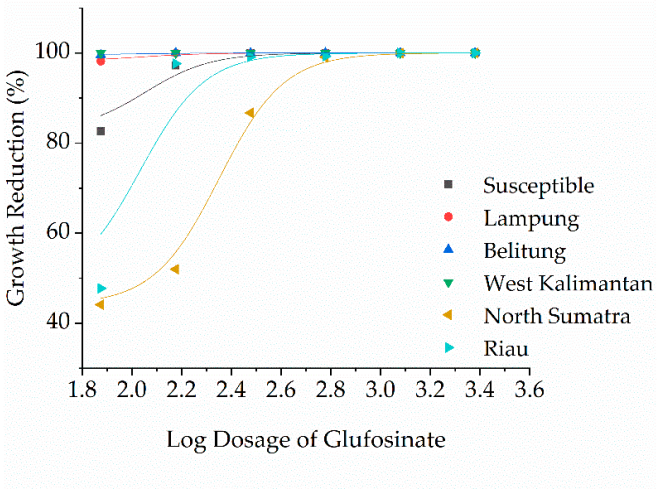
Table 2. Herbicide doses required for 50% reduction (GR_{50}) and resistance indexes.

Herbicide	Biotype of *Eleusine indica*	b^2	r^2	GR_{50} (g a.i. ha^{-1})	Resistance Index	Level of Resistance [22]
Glyphosate	Susceptible	2.34	0.98	483.4	-	S
	Lampung	1.07	0.95	1240.0	2.6	L
	Belitung	0.62	0.76	1163.4	2.4	L
	West Kalimantan	1.48	0.90	2573.3	5.3	L
	North Sumatra	1.06	0.78	2711.0	5.6	L
	Riau	1.75	0.94	1178.1	2.4	L
Paraquat	Susceptible	1.86	0.95	141.0	-	S
	Lampung	0.86	0.77	469.6	3.3	L
	Belitung	2.60	0.97	222.7	1.6	S
	West Kalimantan	1.47	0.95	247.7	1.8	S
	North Sumatra	0.59	0.80	2679.2	19.0	H
	Riau	0.65	0.95	1357.7	9.6	M
Glufosinate	Susceptible	2.94	0.99	44.1	-	S
	Lampung	-	-	-	-	S
	Belitung	-	-	-	-	S
	West Kalimantan	-	-	-	-	S
	North Sumatra	1.50	0.93	105.4	2.4	L
	Riau	5.48	0.99	76.3	1.7	S
Propaquizafop	Susceptible	-	-	-	-	S
	Lampung	-	-	-	-	S
	Belitung	-	-	-	-	S
	West Kalimantan	-	-	-	-	S
	North Sumatra	-	-	-	-	S
	Riau	-	-	-	-	S
Ametryn	Susceptible	-	-	-	-	S
	Lampung	-	-	-	-	S
	Belitung	-	-	-	-	S
	West Kalimantan	-	-	-	-	S
	North Sumatra	-	-	-	-	S
	Riau	-	-	-	-	S
Sulfentrazone	Susceptible	-	-	-	-	S
	Lampung	-	-	-	-	S
	Belitung	-	-	-	-	S
	West Kalimantan	-	-	-	-	S
	North Sumatra	-	-	-	-	S
	Riau	-	-	-	-	S

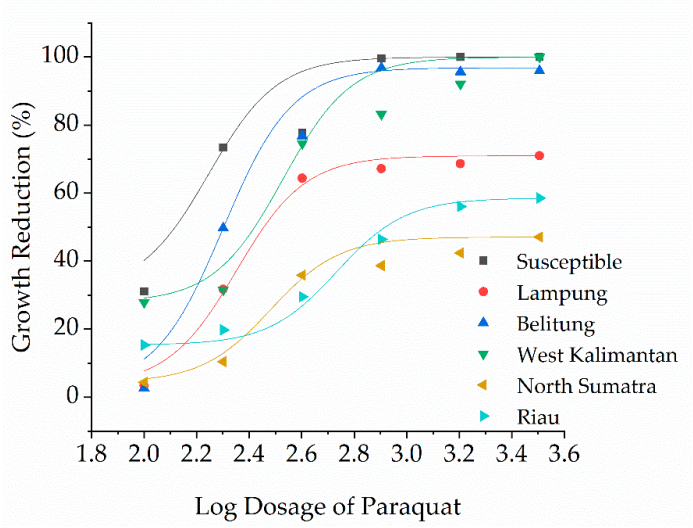
S: Susceptible, L: Low resistant, M: Moderate Resistant, H: High resistant.



Glyphosate



Glufosinate



Paraquat

Figure 1. Application of the herbicides glyphosate, paraquat, and ammonium glufosinate causes a decrease in growth for both susceptible and resistant biotypes of *Eleusine indica*.

Table 3. Amino acid substitution in *Eleusine indica* resistant biotypes compared to susceptible biotypes. There are two amino acid substitutions in the EPSPS gene of *Eleusine indica*-resistant biotypes at positions 102 (threonine to isoleucine) and 106 (proline to serine).

Susceptible Biotype Reference [13], Similar to GenBank Accession AY157642	**Position: Sequence: Amino Acid:**	**101 GGA Glycine**	**102 ACT Threonine**	**103 GCA Alanine**	**104 ATG Methionine**	**105 CGA Arginine**	**106 CCA Proline**
Susceptible	Position: Sequence: Amino Acid:	101 GGA Glycine	102 ACT Threonine	103 GCA Alanine	104 ATG Methionine	105 CGA Arginine	106 CCA Proline
Lampung	Position: Sequence: Amino Acid:	101 GGA Glycine	102 **ATT Isoleucine**	103 GCA Alanine	104 ATG Methionine	105 CGA Arginine	106 **TCA Serine**
Belitung	Position: Sequence: Amino Acid:	101 GGA Glycine	102 **ATT Isoleucine**	103 GCA Alanine	104 ATG Methionine	105 CGA Arginine	106 **TCA Serine**

Table 3. *Cont.*

Susceptible Biotype Reference [13], Similar to GenBank Accession AY157642	**Position: Sequence: Amino Acid:**	**101 GGA Glycine**	**102 ACT Threonine**	**103 GCA Alanine**	**104 ATG Methionine**	**105 CGA Arginine**	**106 CCA Proline**
West Kalimantan	Position:	101	102	103	104	105	106
	Sequence:	GGA	**ATT**	GCA	ATG	CGA	**TCA**
	Amino Acid:	Glycine	**Isoleucine**	Alanine	Methionine	Arginine	**Serine**
North Sumatra	Position:	101	102	103	104	105	106
	Sequence:	GGA	**ATT**	GCA	ATG	CGA	**TCA**
	Amino Acid:	Glycine	**Isoleucine**	Alanine	Methionine	Arginine	**Serine**
Riau	Position:	101	102	103	104	105	106
	Sequence:	GGA	**ATT**	GCA	ATG	CGA	**TCA**
	Amino Acid:	Glycine	**Isoleucine**	Alanine	Methionine	Arginine	**Serine**

Bold format: nucleotide and amino acid substitutions compared with susceptible biotypes.

4. Discussion

Eleusine indica from five Indonesian provinces was found to be resistant to glyphosate. According to farmers in the sampling area, it can be concluded that this resistance is a result of glyphosate being used continuously for a long period of time without herbicide rotation. Even though the application of herbicides has been carried out correctly, the repeated use of herbicides with the same mechanism of action and genetic heterogeneity in weed populations can lead to the appearance of resistance to herbicides [8]. The study's findings revealed that the North Sumatra and West Kalimantan biotypes of *E. indica* had the highest resistance index value of >5, making glyphosate ineffective in controlling them despite being in the low resistance category. Because of this, the biotypes' control by glyphosate had to be found in other ways. This is similar to the results of research in Italy, which reported that *E. indica* populations have developed glyphosate resistance, with resistance index values of 7.3 and 5.8 [23].

The point mutations ACT to ATT at position 102 and CCA to TCA at position 106 of the EPSPS gene induced an amino acid change from threonine to isoleucine and proline to serine in all *E. indica* glyphosate-resistant biotypes. The five resistant biotypes have two similar mutational sites, which makes this situation unusual. However, substitution of the amino acid threonine to isoleucine at position 102 and proline to serine at position 106 were the most common mutations in glyphosate-resistant weed species [15]; therefore, the similarity in the types of mutations that occurred in all *E. indica* biotypes is likely to occur. Double mutations of Thr102Iso and Pro106Ser have been identified previously as the TIPS mutation, which causes very high resistance to glyphosate [24,25]. Despite having the same mutation point, each resistant biotype has a variable amount of resistance, which can be attributed to the environment or other resistance mechanisms acting as a support [24,26]. The double TIPS mutation has very high resistance compared to the single EPSPS gene mutation at position 102 or 106 [27], but the results of this study yielded different results, so it needs to be studied further to be explained. The Thr102Iso and Pro106Ser mutations have been reported in *E. indica* and other weed populations, such as *Bidens pilosa* [15]. Other weed species, such as *Ecinochloa colona*, *E. indica*, and *Lolium rigidum*, have been shown to contain Pro106Thr, Ala, and Leu mutations in the EPSPS gene that function as glyphosate resistance mechanisms [15]. Additionally, it has been reported that *Amaranthus hybridus* develops glyphosate resistance when the amino acid alanine at position 103 is changed to valine (Ala103Val), together with the substitution of Thr102Iso and Pro106Ser [28,29].

Weed resistance to glyphosate is one of the largest cases of weed resistance in the world, demonstrating that weeds have a significant potential to acquire resistance to glyphosate [15]. This definitely needs to be managed correctly to prevent the emergence of multiple resistances to herbicides with different modes of action. In several regions of Brazil, resistance to glyphosate, paraquat, ACCase, and ALS inhibitors has been identified in *E. indica* populations [5,30]. This is similar to the findings of this study, which showed that glyphosate-resistant biotypes of *E. indica* also exhibited resistance to paraquat and

glufosinate but were susceptible to ACCase, PPO, and PSII inhibitors. Unfortunately, resistance of *E. indica* to ACCase, PPO, and PSII inhibitor herbicides has been reported in various countries [15]. Herbicide rotation must be performed very well because otherwise, *E. indica* could end up developing resistance to ACCase, PPO, and PSII inhibitors. This would be a major problem for managing glyphosate-resistant biotypes of *E. indica*.

The non-selective herbicides paraquat and ammonium glufosinate were supposed to be able to manage glyphosate-resistant biotypes of *E. indica*, but due to improper application, glyphosate-resistant biotypes of *E. indica* can develop resistance to paraquat and glufosinate. There have been several reported cases of *E. indica* developing resistance to glufosinate herbicides worldwide. In Malaysia, a study conducted in 2010 found that several *E. indica* populations had developed resistance to glufosinate, paraquat, ACCase inhibitors, and glyphosate [31]. The frequent use of the same herbicides over time is one of the main reasons why weed resistance to herbicides has become a global issue. When farmers rely on a single herbicide year after year, the surviving weed populations can develop resistance to the herbicide, making it less effective [32].

The prevalence of *E. indica* resistance to herbicides highlights the urgent need for effective weed management strategies that integrate diverse control methods and are tailored to specific local conditions. Adopting an integrated weed management strategy that includes diverse weed control techniques is crucial to managing *E. indica*'s resistance to herbicides [2,33]. This includes the use of a variety of herbicides with different modes of action, as well as cultural approaches such as crop rotation, tillage, and the use of cover crops to reduce weed pressure. The findings of this study showed that glyphosate-resistant *E. indica* biotypes could be managed by ACCase, PPO, and PSII inhibitors, while certain biotypes could be managed by ammonium glufosinate. This information can guide the selection of herbicide rotation patterns. Herbicide rotation is a key approach for weed resistance management. Rotating herbicides with diverse modes of action reduces selection pressure on the weed population, which might delay or prevent resistance development [32,34].

5. Conclusions

E. indica originating from Lampung, Belitung, West Kalimantan, North Sumatra, and Riau were identified as being resistant to glyphosate. The EPSPS gene sequence of *E. indica*-resistant biotypes underwent amino acid substitutions of Thr102Iso and pro106Ser, which resulted in resistance to glyphosate. In the North Sumatra biotype of *E. indica*, resistance to glyphosate, paraquat, and ammonium glufosinate was found. The glyphosate-resistant strain of *E. indica* could be controlled by the herbicides propaquizafop (ACCase inhibitor), ametryn (photosystem II inhibitor), and sulfentrazone (protoporfirinogen oksidase inhibitor).

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