



Similarity and Phylogenetic Analysis of Herbicide-Resistant Goosegrass (*Eleusine indica*) Biotypes

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Abstract

Molecular approach as a herbicide-resistant agent detector is an initial solution before controlling of *Eleusine indica* weed. This review article is aimed to obtain the basic data of similarity and phylogenetic values among herbicide-resistant *E. indica* biotypes. This research used a descriptive analytical method. The analysis involved 14 nucleotide sequences of herbicide-resistant *E. indica* biotypes obtained from the National Center for Biotechnology Information. The nucleotide alignment of herbicide-resistant *E. indica* biotypes was conducted with ClustaLW using the Molecular Evolutionary Genetics Analysis (MEGA) v. 5.05 software based on a method of neighbor-joining tree construct/test. The results showed that two nucleotides of 7,921 herbicide-resistant *E. indica* biotypes were homologous (sequence 1,231 and sequence 1,408). The similarity values among herbicide-resistant *E. indica* biotypes ranged from 0.00 to 1.19. The information of phylogenetic pattern is needed in the selection of the herbicides mode of action rotation in order to control herbicide-resistant *E. indica* biotypes.

Keywords: goosegrass, herbicides, phylogenetic pattern, resistant

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INTRODUCTION

Integrated weed management (IWM) for sustainable agriculture is seen as an approach in increasing the effectiveness of long-term weed suppression and in reducing environmental contamination (Harker et al., 2012; Shaner, 2014; Liebman et al., 2016) and IWM does not exclude the use of herbicide (Harker et al., 2012; Harker and O'Donovan, 2013). The use of herbicides has increased agricultural profitability, reduced tillage practices that contribute to soil and water

conservation, increased agricultural labor efficiency and improved farmer's quality of life (Gianessi, 2013; Zimdahl, 2013). The continuous use of similar herbicides in plantations is less effective in suppressing of resistant weeds and can damage soil fertility due to herbicides persistence. Colquhoun (2006) stated that several herbicide families, including imidazolinones, isoxazolidinone, nitriles, glyphosate, dinitroanilines, bipyridyliums, triazines and uracils have moderate to long persistence levels. Selection of the herbicide families that are not-persistent can

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support the management of resistant weeds (MRW) and sustainable agriculture.

The use of herbicides with similar mode of action in controlling weeds are repeated in long period of time resulting in weed resistance (Purba, 2009). Controlling weed populations especially goosegrass [*Eleusine indica* (L.) Gaertn] on food crops, horticulture and plantations using herbicides with similar mode of action inflicts new problems, like the presence of *E. indica* biotypes that have herbicide-resistant genes. Baerson et al. (2002) reported that the presence of glyphosate-resistant *E. indica* biotypes was 5-fold higher and amino acids changes occurred compared to susceptible populations in the Johor, Malaysia. Chong et al. (2008) stated that glyphosate-resistant *E. indica* biotypes in the Chaah area experienced substitution of Cytosine (C) to Adenine (A) resulting in the change of Threonine₁₀₆ to Proline₁₀₆ and substitution of Cytosine (C) with Thymine (T) resulting in the change of Proline₁₀₆ to Serine₁₀₆, in the Bidor area, Malaysia. Takano et al. (2018) stated that the 330-bp fragment sequencing of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) gene identified a single nucleotide polymorphism that causes the Pro-106-Ser amino acid substitution of glyphosate-resistant biotypes in South America. This mutation caused 3.8-fold increase in glyphosate to inhibit 50% EPSPS activity. The substitution confirms the occurrence of glyphosate resistance. Cha et al. (2014) stated that the target point of Asn-2097-Asp of fluazifop-resistant *E. indica* (biotype P4) in Malaysia experienced a change in nucleotide, in the codon AAT into GAT.

The management of herbicide-resistant *E. indica* biotypes can use the rotation mode of action of herbicides (Monaco et al., 2002). Rotation of herbicide's mode of action will result in genetic amino acids substitution. However, particularly in Indonesia, there has not been any study examining the genetic relationship among herbicide-resistant *E. indica* biotypes using similarity and phylogenetic values. The molecular approach as a detector of herbicide-resistant genes is an initial approach before controlling *E. indica* in the North Sumatra Province, Indonesia, specifically on the oil palm plantations with

glyphosate-resistance of 83.33% in the South Tapanuli and Batu Bara regency (Tampubolon and Purba, 2018a; Tampubolon et al., 2018a); 89.36% in the Serdang Bedagai regency (Tampubolon et al., 2018b); 56.62% in the Deli Serdang regency (Tampubolon et al., 2018c); 42.11% in the Langkat regency (Tampubolon and Purba, 2018b); 64.69%; 58.89%; 72.22%; 46.43% and 85.71%, respectively, in the Asahan, Simalungun, Labuhan Batu, North Labuhanbatu and South Labuhanbatu regencies (Tampubolon et al., 2019). The similarity and phylogenetic values of this review article are expected to be the basic strategies for effective and sustainable weed management in plantation companies. Moreover, the strategies can also be integrated with agronomic practices. This review article is aimed to obtain the basic data of similarity and phylogenetic values among herbicide-resistant *E. indica* biotypes.

MATERIALS AND METHOD

Nucleotide databases of herbicide-resistant *E. indica* biotypes

This research uses a descriptive analytical method. Database nucleotide from herbicide-resistant *E. indica* was used to produce important values of information on biotechnology. This study was carried by recording information of *E. indica* from all databases before 30 March 2019. The analysis involved 14 sequences of nucleotide from herbicide-resistant *E. indica* biotypes from National Center for Biotechnology Information (NCBI) (Table 1).

Similarity and phylogenetic analysis of herbicide-resistant *E. indica* biotypes

The nucleotide sequences were aligned and similarity scores were obtained using the FASTA format (Pearson and Lipman, 1988). The nucleotide alignment of herbicide-resistant *E. indica* biotypes was conducted with ClustalW progress (Thompson et al., 1994) using the Molecular Evolutionary Genetics Analysis (MEGA) v. 5.05 software based on a construct/-test neighbor-joining tree method. Bootstrap analysis with 1000 replications from neighbor-joining construct/test was used to assess the strength of the nodes in the tree (Tamura et al., 2011).

Table 1. The accession number of herbicides-resistant *E. indica* biotypes.

Herbicide site of action*	Active ingredients of herbicides*	Sample name	Accession number**	Region
5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	Glyphosate, Sulfosate	EPSPS1	AJ417033.1	Malaysia
		EPSPS2	KX018289.1	China
		EPSPS3	JN004269.1	Mississippi
		EPSPS4	AY395699.1	Taiwan
		EPSPS5	AY157643.1	Malaysia
		EPSPS6	HQ403647.1	China
Glutamine synthetase (GS)	Glufosinate Ammonium, Bialaphos, etc.	GS1	KX817292.1	China
Acetyl-CoA carboxylase (ACCase)	Sethoxydim, Fluazifop-P-butyl, Diclofop-methyl, Propaquizafop, etc.	ACCcase1	KC778421.1	United States
		ACCcase2	KF700369.1	United States
		ACCcase3	KF700368.1	United States
		ACCcase4	KC778420.1	United States
Acetolactate synthase (ALS) enzyme	Imazapic, Pyribenzoxim, Chlorsulfuron, Cloransulam-methyl, etc.	ALS1	KU720629.1	China
4-Hydroxyphenylpyruvate dioxygenase (HPPD)	Sulcotrione, Isoxaflutole, Pyrazoxyfen, etc.	HPPD1	AX458025.1	United Kingdom
Photosystem I Electron Diverter (PSI)	Paraquat, Diquat	NA	NA	NA
Photosystem II (PS II)	Atrazine, Metribuzin, Ametryn, Propazine, etc.	PSII-1	JX852705.1	United States

Note: NA (not available from NCBI).

Source: **International Survey of Herbicide-Resistant Weeds*

**National Center for Biotechnology Information (NCBI)

RESULTS AND DISCUSSION

The results of DNA base sequence alignment of herbicide-resistant *E. indica* biotypes from the NCBI database are shown in Figure 1. Based on the gene sequence analysis of 14 herbicide-resistant *E. indica* biotypes, there were differences in nucleotide sequences. The nucleotide sequences of herbicide-resistant *E. indica* biotypes resulted in 2 homologous nucleotides (sequence 1,231 and sequence 1,408) from 7,921 nucleotides.

Based on Figure 1, there are substitutions of *E. indica* biotype nucleotides at the same herbicides exposure (ACCcase1 to ACCcase2 and ACCcase3) in the same country (United States) from T to C in the sequence 1,230. According to Huffman et al. (2016) there were three polymorphisms from Tennessee, United States, that changed in the nucleotide sequences from the glyphosate-

susceptible *E. indica* into glyphosate-resistant, particularly from C to T, from A to G and from A to C. According to McCullough et al. (2016) there were nucleotide substitutions in codon Ser-1805 (ACT to TCT) and Asp-2078 (GAT to GGT) of ACCCase-resistant *E. indica* biotypes compared to susceptible populations in Georgia. Simarmata and Penner (2008) also reported that nucleotide substitution occurred in EPSPS-susceptible *Lolium rigidum* weed in California from C to T (EPSPS-resistant biotype) at sequence 301. Zhang et al. (2015) stated that the EPSPS enzyme in glyphosate-resistant *E. indica* biotypes quickly responded at 12 hours after being exposed to glyphosate from South China. The expression of mRNA and protein in the glyphosate-resistant *E. indica* biotype increased, particularly with the increasing of glyphosate dose. Chen et al. (2015) stated that glyphosate-resistant *E. indica* biotype has a small decrease in chlorophyll content at the glyphosate dose of 1,680 g a.i.ha⁻¹.

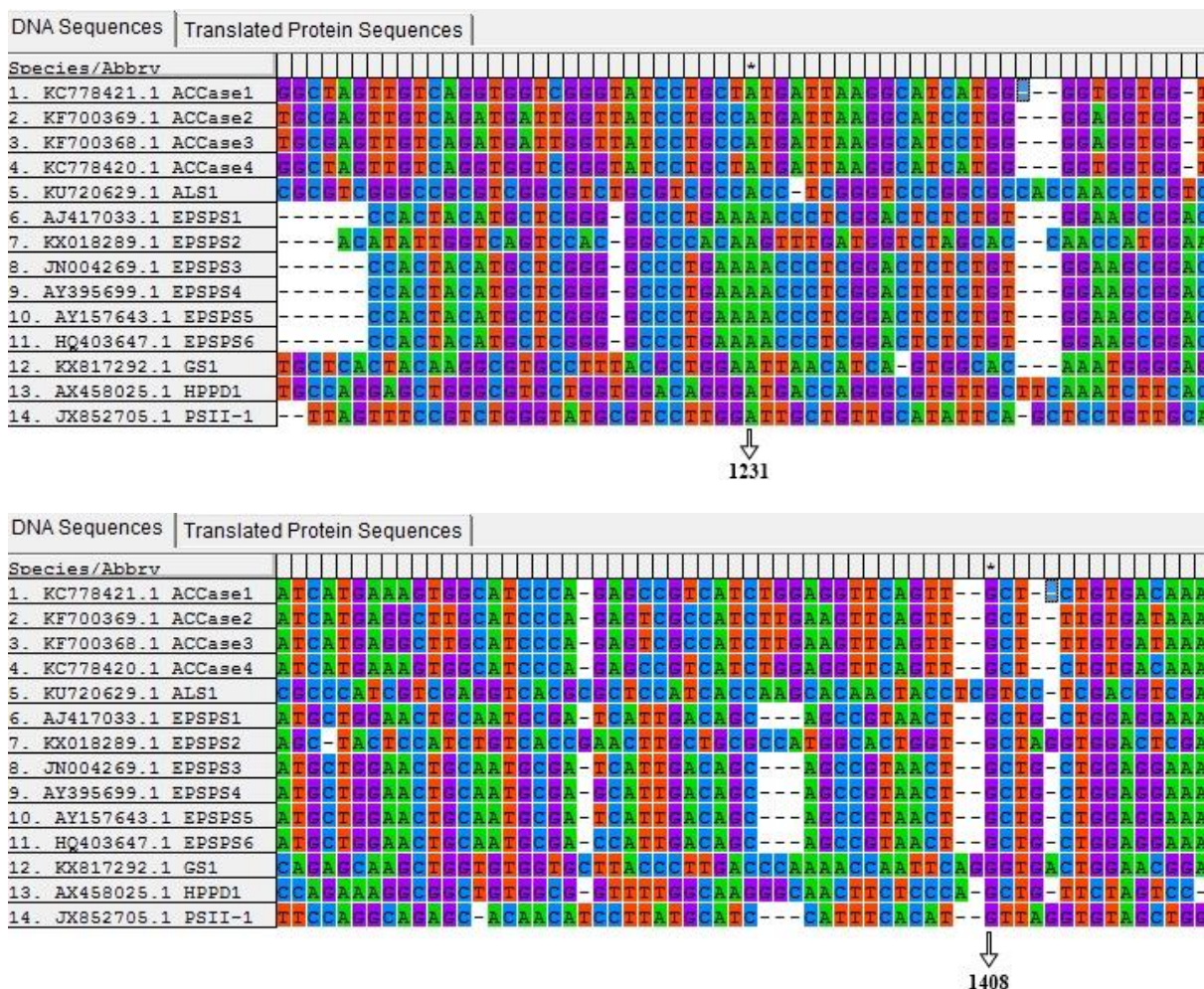


Figure 1. The results of DNA base sequence alignment of herbicide-resistant *E. indica* biotypes from the NCBI database.

The similarity value of herbicide-resistant *E. indica* biotypes ranged from 0.00 to 1.19 (Table 2). The *E. indica* biotypes showing the smallest genetic distance was found in the KF700369.1 (ACCcase2) and KF700368.1 (ACCcase3). This shows that there do not appear any different base pairs in the two accessions. The highest genetic distance was in accession AJ417033.1 (EPSPS1) and KX018289.1 (EPSPS2), meaning that these two accessions had many different base pairs. Chong et al. (2011) also stated that the genetic distance of 14 *E. indica* populations (6 glyphosate-resistant and 8 glyphosate-susceptible) in the Peninsular Malaysia ranged from 0.000-0.122. The highest

genetic distance value was found in the glyphosate-susceptible *E. indica* from Temerloh and glyphosate-resistant *E. indica* from Bidor area. Saidi et al. (2016) stated that the genetic similarity value of *E. indica* population from 29 locations in Malaysia was 74% with a genetic distance of 0.37. Galeano et al. (2016) also reported that nine biotypes of glyphosate-resistant *Digitaria insularis* and six glyphosate-susceptible populations from the State of Sao Paulo, Brazil, had DNA similarity sequences ranging from 74-82% (high), whereas cDNA sequences (RNA) showed similarity, ranging from 78-94% (high) with grasses weeds. The highest similarity for cDNA was found in *E. indica* by 94%.

Table 2. The genetic similarity distances of herbicide-resistant *E.indica* biotypes.

No	Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	KC778421.1 (ACCCase1)														
2	KF700369.1 (ACCCase2)	0.16													
3	KF700368.1 (ACCCase3)	0.16	0.00												
4	KC778420.1 (ACCCase4)	0.00	0.16	0.16											
5	KU720629.1 (ALS1)	1.16	1.16	1.16	1.16										
6	AJ417033.1 (EPSPS1)	0.82	0.85	0.85	0.82	1.02									
7	KX018289.1 (EPSPS2)	1.06	1.08	1.08	1.06	1.06	1.19								
8	JN004269.1 (EPSPS3)	0.88	0.90	0.90	0.88	1.11	0.17	1.15							
9	AY395699.1 (EPSPS4)	0.81	0.84	0.84	0.81	1.01	0.00	1.19	0.17						
10	AY157643.1 (EPSPS5)	0.87	0.90	0.90	0.87	1.11	0.16	1.14	0.00	0.17					
11	HQ403647.1 (EPSPS6)	0.82	0.85	0.85	0.82	1.03	0.00	1.19	0.17	0.00	0.17				
12	KX817292.1 (GS1)	1.16	1.12	1.12	1.16	1.13	1.04	1.11	1.07	1.05	1.07	1.05			
13	AX458025.1 (HPPD1)	1.00	0.97	0.97	1.00	0.74	0.90	1.02	0.93	0.90	0.93	0.91	0.95		
14	JX852705.1 (PSII-1)	1.14	1.10	1.10	1.14	1.30	1.19	1.21	1.07	1.19	1.07	1.18	1.19	1.09	

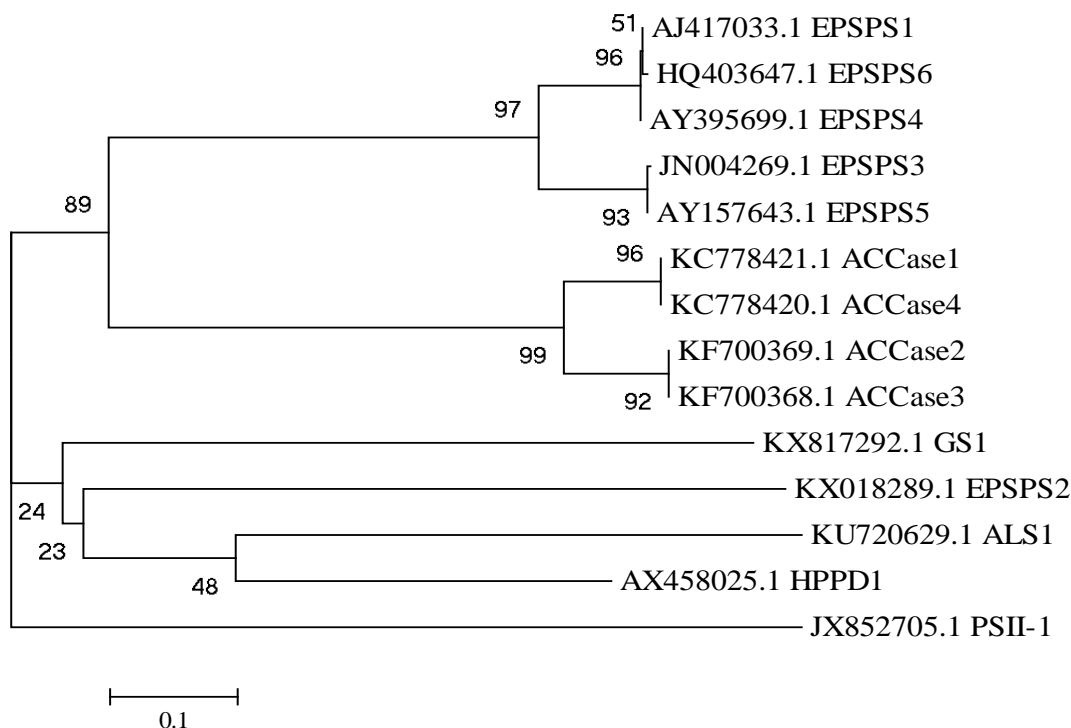


Figure 2. The phylogenetic tree of herbicide-resistant *E. indica* biotypes. The phylogenetic tree was constructed with the neighbor-joining method of the ClustaIW using Mega v. 5.05 Software. Numbers indicate bootstrap value from 1000 replications.

The phylogenetic pattern among herbicide-resistant *E. indica* biotypes deriving from the NCBI database (Figure 2). Based on the phylogenetic patterns, three clusters was formed between the biotypes of herbicide-resistant *E. indica*. Cluster 1 consists of the ACCCase-resistant *E. indica* biotypes (KC778421, KF700369, KF700368, KC778420) and EPSPS-resistant *E. indica* biotypes (AJ417033, JN004269, AY395699, AY157643, HQ403647) except KX018289 (EPSPS2) accession. Cluster 2 consists of *E. indica* biotypes with accession of

KX817292 (GS1), KX018289 (EPSPS2), KU720629 (ALS1) and AX458025 (HPPD1). Cluster 3 only consists of JX852705 (PSII-1) accession. There are differences of clusters between accession KX018289 (EPSPS2) and accession HQ403647 (EPSPS6), even though they are originated from the same country (China). This cluster difference is caused by higher genetic diversity in the two accessions (Table 2). There is not any positive relationship among the location of *E. indica* accession in influencing genetic diversity. This is linier with

Chong et al. (2011) stated that the genetic distance of 14 *E. indica* populations from Peninsular, Malaysia does not correlate with geography situation of the sample, but is attributable to human activities when moving seeds from one to another region. In addition, factors that influence other genetic diversity are genetic drift, inbreeding, and others.

The phylogenetic pattern confirm that the rotational mode of action of the herbicide is able to control of herbicide-resistant *E. indica* biotypes. This phylogenetic information is expected to be able to help farmers and oil palm plantations experiencing problems with herbicide-resistant *E. indica* biotypes in North Sumatra Indonesia, and reporting about the case. Phylogenetic pattern indicates that similar herbicide cannot control *E. indica* which has been confirmed resistant to similar herbicides. This is linear with Jalaludin et al. (2015) stated that the survival of the glufosinate-resistant *E. indica* biotypes decreased from the dose of 1,278 g a.i.ha⁻¹ glufosinate ammonium to 292 g a.i.ha⁻¹ paraquat. Simarmata (2009) stated that quizalofop herbicide with the dose of 0.06 kg a.i.ha⁻¹ effectively (100%) controlled of glyphosate-resistant rigid ryegrass (*Lolium rigidum*) compared to glyphosate at the dose of 4.5 kg a.i.ha⁻¹ (only 8%) at 3 weeks after treatment. Molin et al. (2013) stated that clethodim, fluazifop-P, paraquat and glufosinate herbicides effectively (95%, 98%, 100% and 80%) controlled of glyphosate-resistant *E. indica* biotype in Washington County, Mississippi. Hambali et al. (2015) confirmed that diuron at the dose of 1,500 g a.i.ha⁻¹ and ametryn at the dose of 1,000 g a.i.ha⁻¹ effectively (100%) controlled of paraquat-resistant *E. indica* biotype at Afdeling III of Adolina Estate compared to paraquat. In addition, Yulivi et al. (2014) also stated that glyphosate dose of 480 g a.i.ha⁻¹ and glufosinate ammonium dose of 110 g a.i.ha⁻¹ effectively (100%) controlled of paraquat-resistant *E. indica* biotype from Balai Benih Induk (BBI) Tanjung Selamat compared to paraquat.

CONCLUSIONS

The results of this study show that two nucleotides were homologous (sequence 1,231 and sequence 1,408) of 7,921 nucleotides in the herbicide-resistant *E. indica* biotypes. Similarity values among herbicide-resistant *E. indica* biotypes were ranged from 0.00 to 1.19.

Phylogenetics pattern confirms that the rotation of mode of action herbicide is able to control of herbicide-resistant *E. indica* biotypes.

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