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Genetic Diversity of *Echinochloa crus-galli* Var. *crus-galli* (L.) Beauv (Barnyardgrass: Poaceae) Ecotypes in Malaysia and Indonesia as Revealed by RAPD Markers

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Abstract: Fourty ecotypes of *Echinochloa crus-galli* Var. *crus-galli* (Barnyardgrass: Poaceae) collected from Malaysian (26 ecotypes) and Indonesian (14 ecotypes) rice fields were studied using Random Amplified Polymorphic DNA (RAPD) markers. The ecotypes were collected randomly from 11 locations in Malaysia and 6 locations in Indonesia. Individual ecotypes from all the sites were clearly distinguished from each other by RAPD-PCR polymorphisms. The 26 individual ecotypes of *E. crus-galli* Var. *crus-galli* were clustered into four groups at a similarity level of 60% in UPGMA (unweighted pair group method with arithmetic averages). Four groups were identified of the 14 barnyardgrass ecotypes from Indonesia with similarity level of 64%. Six groups have been classified among Malaysian and Indonesian ecotypes of barnyardgrass. Group one had the highest number in the phylogenetic majority, while group 4 and 6 had one ecotype, respectively. The results showed that the Malaysian ecotypes were more variable than Indonesia ecotypes. Three Malaysian ecotypes (in group five and six) formed different clusters. Two Malaysia ecotypes had genetic relationship with 4 Indonesia ecotypes (group two), while the rest of the ecotypes correlated with the geographic distribution. RAPD-PCR markers revealed relatively low genetic variation between barnyardgrass ecotypes. Thus, the genetic diversity observed might be attributed to the diversity among individual ecotypes from divergent locations that may affect weed management systems especially herbicide application. The genetic differences within and among barnyardgrass ecotypes were not sufficient enough for biocontrol implications.

Key words: Poaceae, barnyardgrass, *Echinochloa*, diversity, RAPD markers

INTRODUCTION

Barnyardgrass (*Echinochloa crus-galli* Var. *crus-galli* (L.) Beauv) is an annual weed native to Asia and at present it can be found throughout the world^[1]. The most widespread and economically important member of the genus is the annual *E. crus-galli*, which can be found within 50°N latitude and Longitude 40°S^[2]. Barnyardgrass is a noxious weed in rice fields and has been reported to reduce rice yield by 21 to 40% annually in Malaysia and Indonesia^[3,4].

The increasing trend of direct seeding practice for the last 20 years in several countries including Malaysia and Indonesia, grassy weeds especially barnyardgrass is becoming a serious problem in rice fields^[5,6]. As results, the use of herbicides for controlling barnyardgrass has been dramatically increased^[7]. Biotype of barnyardgrass has been found to be resistant to herbicides such as propanil and quinclorac^[8-10]. Klingaman and Oliver^[11]

defined ecotypes as plants genetically adapted to the habitat they colonized and biotypes as a plant showing a random genetic variation within an ecotype.

A range of plant characters are currently available in distinguishing between related individuals of barnyardgrass. Classical phenotypic feature, such as morphological traits, are still useful, but can some times be influenced by environmental condition^[12]. DNA-based marker clearly allows the direct comparison of the genetic materials of two individual plants with little or no environmental influences on gene expression.

Randomly Amplified Polymorphic DNA (RAPD) analysis involves the amplification of small sequences of target DNA using random primer. The strength of RAPD analysis is its ability to assess genetic variation of characters for evaluation^[13]. This makes RAPD analysis a powerful tool for assessing genetic variation within and among weed populations. RAPD analysis has been used to analyze different populations of leafy spurge

(*Euphorbia esula* L.) and may be important for determining the susceptibility of leafy spurge population to biocontrol agent^[3]. It is also used to study among ecotypes of *Imperata cylindrica*^[4]. Moreover, RAPD analysis has been successfully used to determine genetic variability among resistant and susceptible barnyardgrass (*Echinochloa crus-galli*) population in rice fields^[9,10]. Thus, the objective of the study was to analyze the level of molecular diversity between and within Malaysian and Indonesian barnyardgrass ecotypes.

MATERIALS AND METHODS

Study area and sampling: Information on identity and origin of the ecotypes is presented in Fig. 1 and Table 1. Forty selected individual ecotypes of barnyardgrass from rice fields were used in this study. Seeds were collected from parental populations. The seeds were grown in uniform environmental glass house conditions up to maturity. Once mother plants started heading, the inflorescences was covered by nylon paper to prevent cross-pollination among other ecotypes. Seeds were then grown in small pots in glasshouse up to four-leaf stage^[5].

DNA extraction and purifications: DNA was extracted from about 1 g of fresh leaves (at 4 leaf stage) and stem of single barnyardgrass. A modified CTAB extraction procedure, described by Martines *et al.*^[9] and Rutledge *et al.*^[10] was adopted for DNA extraction using 600 µl pre-warmed CTAB extraction buffer (2% (w/v) CTAB; 0.7 M NaCl; 50 mM Tris-HCl pH 8.0 and 0.1% (v/v) 2-mercaptoethanol (Sigma) and 10 mM EDTA). The homogenate was then incubated at 60°C for 1 h and allowed to cool at room temperature for 10 min. The homogenate was emulsified with equal volume of chloroform:isoamyl alcohol mixture (24:1) mixed by inversion and centrifuged at 12,000 rpm for 10 min. An equal volume of cool (-20°C) iso-propanol was centrifuged at 12,000 rpm for 10 min. The precipitated DNA was removed, rinsed several times in 90 and 70% ethanol, air-dried and redissolved at 60°C in 50 µl TE buffer (10 mM Tris-HCL; 1 mM EDTA pH 8.0). The quantity of extracted DNA was determined by ultraviolet absorbance. The DNA samples were adjusted to 0.5 µg µL⁻¹ by diluting with TE buffer.

RAPD amplifications: DNA variations were assessed using four decamer oligonucleotide primer: A-07 (5'-GAAACGGGTG), OPAE-12 (GTTGCGATCC), OPG-06 (GTGCCTAACC) and A-20 (CCGAGCAATC) (Alpha Montral, Quebec, CA). Approximately, 25 ng of DNA was used in a 50 µL amplification reaction mixture containing

Table 1: Origin and number of barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) ecotypes used in the study

Origin	Study label	Number of ecotypes	Latitude (°S)	Longitude (°N)
Malaysia				
Perlis	P	2	6.25	100.16
Kedah	K	4	6.12	100.24
Penang	PN	3	5.28	100.23
Perak	PK	3	4.34	101.36
Kelantan	KN	2	6.10	102.17
Terengganu	T	3	5.23	103.06
Pahang	PH	2	3.58	102.21
Selangor	S	2	2.44	101.42
Negeri Sembilan	NS	1	2.45	102.10
Melaka	M	2	2.16	102.15
Johor	J	1	1.52	102.59
Indonesia				
Lampung	L	2	5.45	105.50
Banten	B	3	6.37	106.08
West Java	WJ	2	6.14	108.48
Central Java	CJ	1	8.30	110.31
East Java	EJ	2	8.40	114.28
South Sulawesi	SS	4	0.12	112.30
Total	-	40	-	-



Fig. 1: Sampling sites in Malaysia (1:Perlis; 2: Kedah; 3: Penang; 4: Perak; 5: Kelantan; 6: Terengganu; 7: Pahang; 8: Selangor; 9: Negeri Sembilan; 10; Melaka; 11: Johor) and Indonesia (12: Lampung; 13: Banten; 14: West Java; 15: Central Java; 16: East Java; 17: South Sulawesi)

2.5 µl 10 X PCR Buffer (Promega Technologies); 2.5 mM MgCl₂, 25 pmole of primer, 10 µM each of dCTP, dATP, dTTP and dGTP, 1 unit of DNA *Thermus aquaticus* (Taq) polymerase (Fermentas). The reaction mixture was overlaid with 20 µl of mineral oil (Sigma). There was only one primer in each reaction. The polymerase chain reaction (PCR) was carried out in a Perkin Elmer thermocycler (Model PTC-200) with the following amplification conditions: Initial denaturation cycle at 94°C for 2 min followed by 45 cycles of 45 sec at 94°C, 5 min at 38°C and 2 min at 72°C and a final extension cycle at 72°C for 7 min.

RAPD-PCR analysis: The RAPD-PCR products were analyzed by horizontal electrophoresis in 1.5% agarose

gel (Sigma) with 1.0 X TBE buffer (89 mM Tris-HCl, 89 mM Boric acid, 2 mM EDTA pH 8.0). Electrophoresis was performed for 50 to 60 min at 90 volts, using 1 kb DNA ladder (Fermentas) as a molecular standard. The gels were then stained in a bath containing ethidium bromide ($0.5 \mu\text{g mL}^{-1}$), destained in distilled water and visualized and photographed under an ultraviolet transilluminator (Gel Documentation System).

The genetic variability patterns were scored on the basis of the presence or absence of amplified products. For each primer, the presence (1) or absence (0) of amplified DNA bands was scored using only those major bands whose presence or absence could be distinguished. As RAPD products are dominant, a locus was considered to be polymorphic if the presence of bands were observed in only some of the individuals. Band sharing analysis was conducted based on Jaccard's coefficient similarity (Jaccard, 1908 cited in Ramisah^[25]). Unweight pair group arithmetic average was used to plot a dendrogram using NTSYS-pc program Version 2.1^[16].

RESULTS

DNA extraction and purifications: The contamination of DNA with compound inhibiting the PCR reaction was a serious problem at the very beginning of this study. Independently of the arbitrary primer used, the PCR reaction, when applied to unpurified DNA, resulted in one light band of high molecular weight or no bands at all, while the purified DNA were more reproducible results.

Prior to the actual experimentation, a trial experiment to explore the possibility of using the primers was carried out. A Total of 18 ten-mer primers from Alpha sets primer were tested and only four were found to produce amplification product. The four of these primers constantly produced polymorphic banding patterns with multiple fragments and good reproducibility (Table 2 and 3).

Genetic diversity within Malaysian ecotypes: A total of 533 fragment bands were amplified using the four primers (Table 2). The four primers yielded 3 to 10 amplification products. The detectable fragments ranged between 62.5 to 3000 bp in size. Primer A 07, A 20, OPAE 12 and OPG 06 were the best primers that produced 86 to 89% of polymorphic fragments (Table 2). OPAE 12 fragments size ranged from 62.5 to 2500 bp with polymorphic fragments of 89%.

The genetic similarity within Malaysian ecotypes ranged from 14.29 to 100% (data not shown). Out of 26 ecotypes, 10 ecotypes (38.5%) i.e. Melaka, Selangor, Penang, Perlis and Terengganu ecotypes were found to

Table 2: RAPD analysis on 26 ecotypes of barnyardgrass (*E. crus-galli* Var. *crus galli*)

Primer	Sequences	Fragment size range (bp)	Number of amplified fragments	Percent of polymorphic fragments
A 07	GAAACGGGTG	125-2500	111	85.7
OPAE 12	GTTGCGATCC	62.5-2500	177	89.0
OPG 06	GTGCCTAACC	125-2500	130	87.5
A 20	CCGAGCAATC	250-3000	115	85.7
Total	-	-	533	-

Table 3: RAPD analysis on 14 ecotypes of barnyardgrass (*E. crus-galli* Var. *crus galli*)

Primer	Sequences	Fragment size range (bp)	Number of amplified fragments	Percent of polymorphic fragments
A 07	GAAACGGGTG	125-2500	59	67
OPAE 12	GTTGCGATCC	62.5-2500	70	80
OPG 06	GTGCCTAACC	125-2500	67	79
A 20	CCGAGCAATC	250-3000	70	80
Total	-	-	266	-

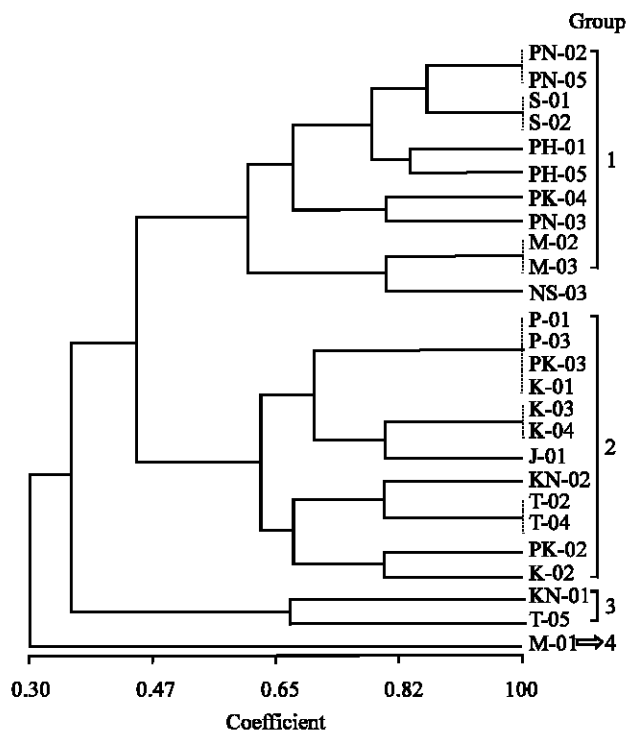


Fig. 2: Dendrogram based on Jaccards genetic distance within 26 Malaysian barnyardgrass ecotypes generated by UPGMA cluster analysis.

Note: PN=Penang; S=Selangor; PH=Pahang; PK=Perak; M=Melaka; NS=Negeri Sembilan; P=Perlis; K=Kedah; Johor; KN=Kelantan; T=Terengganu

be identical. However, 11 pair wise ecotypes (42%) were observed to have high diversity. Cluster analysis using RAPD markers of the Malaysian ecotypes is presented in Fig. 2 and 3. Genetic similarity ranged from 30 to 100%. At the genetic similarity of 47%, four groups of ecotypes were identified. The first group comprised 11 ecotypes

(Penang, Selangor, Pahang, Melaka, Perak and Negeri Sembilan, respectively). Ecotypes of Penang, Selangor, Pahang and Melaka were found to be linked together and have closer relationship with each other within group 1 (Fig. 2). The second group comprised of 12 ecotypes (Perlis, Kedah, Perak, Terengganu, Kelantan and Johor). Ecotypes of Penang, Kedah and Terengganu in group 2 were also found to be closely related. The third group consisted of Kelantan and Terengganu ecotypes and group 4 was only Melaka ecotype (Fig. 2). Primer A 20 generated a total of 115 fragment bands ranging from 250 to 3000 bp in size with 85.7% polymorphic bands from 26 Malaysian barnyardgrass ecotypes (Fig. 3).

Genetic diversity within Indonesian ecotypes: A total of 266 of fragment bands were amplified using the four primers (Table 3). The four primers yielded 4 to 10 amplification products. The detectable fragments ranged from 62.5 to 3000 bp in size. Primer A 07, A 20, OPAE 12 and OPG 06 were the best primers that produced 67 to 80% of polymorphic fragments (Table 3). Primer OPAE 12 and A 20 had fragments size of 62.5 to 2500 bp and 250 to 3000 bp, respectively. These primers produced polymorphic fragments of 80%, respectively. Pair-wise ecotypes i.e. East Java, Banten and South Sulawesi ecotypes were found to be identical. However, pair-wise ecotypes such as West Java, East Java and Lampung were observed to have high diversity (data not shown). Cluster analysis of the Indonesian ecotypes is presented in Fig. 4 and 5. Genetic distance similarity ranged from 27 to 100%. At the genetic similarity of 64%, four groups were identified. The first group comprised Central Java and East Java ecotypes. Group 2 consisted of only Lampung ecotype. Group 3 comprised Banten, West Java and Lampung ecotypes. While, group 4 consisted of South Sulawesi ecotypes. Primer A 20 generated a total of 62 fragment bands ranging from 250 to 3000 bp in size with 80% polymorphic bands from 14 the Indonesian ecotypes (Fig. 5).

Genetic diversity between the Malaysian and the Indonesian ecotypes: Dendrogram was constructed using UPGMA clustering to show the relationship among the 40 ecotypes (Fig. 6). At 54% genetic similarity, the ecotypes were classified into six distinct groups. Fifteen ecotypes were found in group 1, which consisted of 9 Malaysian ecotypes and 6 Indonesian ecotypes. Group 3 consisted of 15 ecotypes (12 Malaysian and 3 Indonesian ecotypes). Only three Malaysian ecotypes were found in groups 5 and 6. Two Malaysian ecotypes (Terengganu and Kelantan ecotypes) were found in group 5. Only Malaysian ecotype was identified in group 6 and only Indonesian ecotype was found group 4. Approximately

40% of the Indonesian (6 ecotypes) were observed to be highly related with 35% of the Malaysian (9 ecotypes). Of the size Indonesian ecotypes, five were from Java Island and 1 ecotype was from Sumatera Island. Interestingly, in group 2, four South Sulawesi ecotypes (Indonesia) had closely related with 2 Malaysia ecotypes (Perak and Penang).

DISCUSSION

Diversity within Malaysian and Indonesian ecotypes: The study showed that the presence of substantial amount of diversity in both of Malaysian and Indonesian ecotypes (Fig. 2 and 4; Table 2 and 3). Of the 18 primers assayed, four primers were polymorphic to Malaysian and Indonesian ecotypes (Table 2 and 3). The size of these primers ranged from 125 to 3000 bp. Out of these, the number of amplified fragments ranged from 111 to 177 with 85.7 to 89% of polymorphic fragments. OPAE 12 was found the highest for fragments amplification with 89% polymorphic fragments for Malaysian ecotypes (Table 2). The four primers used for assessment of the Indonesian ecotypes were found to be variable within the regions. OPAE 12 and A 20 primers showed much higher fragment amplification and polymorphic fragments of 80%, respectively (Table 3). The differences in number of amplified fragments and percent polymorphic fragments between Malaysian and Indonesian ecotypes is due to the difference in number of ecotypes.

The genetic similarity within Malaysian ecotypes ranged from 30 to 100%. On the other hands, 30% molecular diversity among 26 ecotypes has been observed (Fig. 2). While the genetic distance within 14 Indonesian ecotypes ranged from 27 to 100%. On the other hands, 33% genetic diversity within Indonesian ecotypes have been observed (Fig. 4). This genetic similarity was slightly similar with genetic distances among barnyardgrass population in Arkansas^[10] or genetic similarity among barnyardgrass species in Spain^[9]. However, relatively lower than genetic variability among the regions of barnyardgrass population^[13]. This kind of genetic diversity was expected because of the breeding systems of barnyardgrass (i.e. self pollinated, small-seeded and capability of colonizing a broad spectrum of both agricultural and ruderal wet disturbed habitat).

Four groups (sub-clusters) of both the Malaysian and Indonesian ecotypes were identified (Fig. 2 and 4). However, genetic diversity was also present within Malaysian and Indonesian barnyardgrass ecotypes. The clustering of the barnyardgrass ecotypes within the region (Malaysia or Indonesia) may be closely related to other barnyardgrass ecotypes. Instead of geographic



Fig. 3: Genomic DNA fragments within 26 Malaysian ecotypes of barnyardgrass amplified with A 20 primer. M=1 kb DNA ladder

Note: 1: Penang (PN-02); 2: Perlis (P-01); 3: Melaka (M-01); 4: Pahang (PH-01); 5-6: Melaka (M-02 and M-03); 7: Pahang (PH-05); 8: Perlis (P-03); 9: Perak (PK-03); 10: Negeri Sembilan (NS-03); 11-12: Kedah (K-03 and K-04); 13: Perak (PK-04); 14: Penang (PN-03); 15: Kelantan (KN-01); 16: Penang (PN-05); 17: Selangor (S-02); 18: Perak (PK-02); 19: Kelantan (KN-02); 20: Kedah (K-02); 21: Terengganu (T-02); 22: Kedah (K-01); 23: Johor (J-01); 24: Terengganu (T-05); 25: Selangor (S-03); 26: Terengganu (T-04)

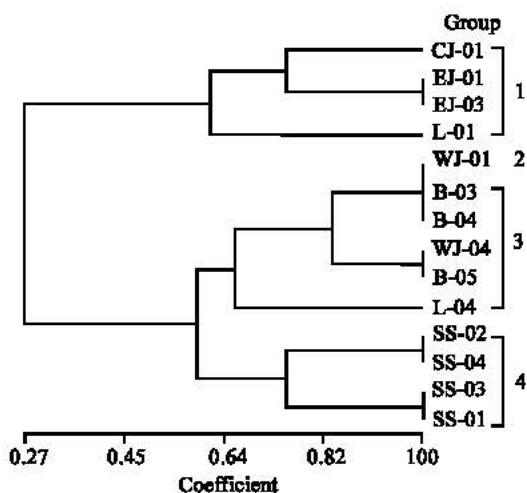


Fig. 4: Dendrogram based on Jaccards genetic distance within 14 Indonesian ecotypes of barnyardgrass generated by UPGMA cluster analysis.

Note: CJ=Central Java; EJ=East Java; L=Lampung; WJ=West Java; B=Banten; and SS=South Sulawesi

origin and weed management systems, the diversity in the barnyardgrass ecotypes may be partially due to out-crossing within or among closely related species, which could be account for the morphological variability observed among individuals^[2,17].

Burtos *et al.*^[18] found the level of intra and inter-population variation detected with RAPD markers to be different from each taxonomic unit, partially depending on the reproductive system of each of the genus *Hordeum* population. The genetic similarity of the Malaysian and Indonesian ecotypes slightly correlated with their close geographic locations. This phenomenon

was also observed in the morphological characteristics of barnyardgrass ecotypes^[19]. In general, some of the Malaysian ecotypes were found to be closely correlated with the distance of geographical locations, for example ecotypes within groups 1 and 3 (Fig. 2), exception for Johor ecotype (J-01) which was found to be closely related with Kedah ecotype (K-02 and K-3) in group 2. This may be due to seed dispersal through seed contamination by rice seed movement. Ash *et al.*^[20] concluded that there is distinct genetic variability within the *Cartahmus lanatus* population in Australia. Vellend and Waterway^[21] also observed the genetic diversity within *Carex rariflora* population form different habitat and locations in Canada.

Close similarity within Malaysian ecotypes (Kedah, Penang to Perak or Kelantan to Terengganu) and Indonesia ecotypes (Banten, West Java, Central Java to East Java) may be due to historical factors. The samples collected from these locations were within 100 km. In these areas, there is considerable movement of agricultural machinery for cultivation, transportation and harvesting. The opportunity for accidental introduction of seed might have therefore occurred and there may have been rapid transport of the seeds between farms, leading their closed relatedness. Interestingly, group 4 which consisted of South Sulawesi ecotypes uniquely found in this area and not in the other areas studied. These observations may be due to different ecotypes in different regions or they might disturb ecotypes adapted to each region. Similar results were found for the genetic differentiation within and among population of *Aster miyagii* in the Ryukyu Islands of Japan^[22]. Danquah *et al.*^[17] have confirmed the genetic variability within and among barnyardgrass population from different geographic origin. They found that the range of

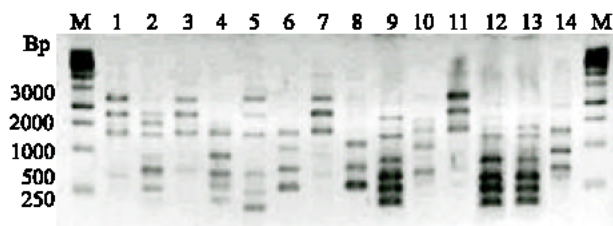


Fig. 5: Genomic DNA fragments within 14 Indonesian ecotypes of barnyardgrass amplified with A 20 primer. M:1 kb DNA ladder

Note: 1: Central Java (CJ-01); 2: West Java (WJ-01); 3: East Java (EJ-01); 4: West Java (WJ-04); 5: Lampung (L-04); 6: South Sulawesi (SS-02); 7: Lampung (L-01); 8: South Sulawesi (SS-04); 9: Banten (B-03); 10: South Sulawesi (SS-03); 11: East Java (EJ-03); 12-13: Banten (B-05 and B-04); 14: South Sulawesi (SS-01)

genetic distance among barnyardgrass population ranged from 20 to 100%. Chou *et al.*^[23] have also confirmed the occurrence of genetic variability and phylogeography of *Miscanthus chinensis* var. *condensatus* among islands of Taiwan and Japan. Dekker^[24] reported that studies of genetic diversity in weeds that rely on molecular polymorphism provide new insight into the heterogeneity within weedy populations. In this study, RAPD fingerprints revealed substantial amounts of polymorphism even within some locations of barnyardgrass ecotypes. Rutledge *et al.*^[10] who studied several populations of barnyardgrass on the basis of their resistance or susceptibility to propanil, found evidence to suggest that cluster in some ways relates to the location of barnyardgrass samples. Roy *et al.*^[15] has found that genetic variability of cold-adaptive barnyardgrass population in Quebec (Canada) were higher than those of European and the United States.

Variability between Malaysian and Indonesian ecotypes:

The dendrogram produced by the UPGMA of the Jaccard similarity of the Malaysian and Indonesian ecotypes is shown in Fig. 6. Six major groups were identified with genetic similarity ranged from 32 to 100%. This genetic similarity resulted 28% diversity between the Malaysian and the Indonesian ecotypes. This genetic similarity was slightly similar with the genetic diversity among the regions of barnyardgrass^[7].

Generally, most of the Indonesian ecotypes were found closely related with some of the Malaysian ecotypes (Fig. 6). Danquah *et al.*^[7] found that some barnyardgrass populations were found to be close relationship among the region, however they were distinctly separated within sub-clustering. Ramisah^[25]

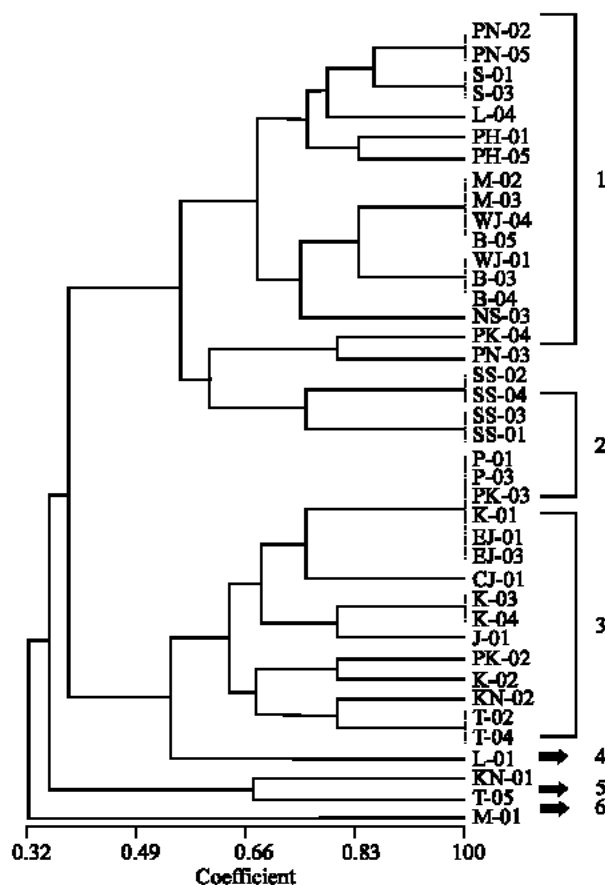


Fig. 6: Dendrogram based on Jaccards genetic distance between 40 Malaysian and Indonesian ecotypes of barnyardgrass generated by UPGMA cluster analysis

Note: PN=Penang; S=Selangor; PH=Pahang; PK=Perak; M=Melaka; NS=Negeri Sembilan; P=Perlis; K=Kedah; J=Johor; KN=Kelantan; T=terengganu; L=Lampung; WJ=West Java; B=Banten; EJ=East Java; CJ=Central Java; SS=South Sulawesi

clearly showed the separation of the genetic diversity of sweet potato (*Ipomoea batatas*) accessions between Malaysia and Indonesia. In this study showed that ecotypes from Indonesia such as West Java, Banten, East Java and South Sulawesi ecotypes highly varied with ecotypes of Perlis, Kedah, Pahang, Melaka, Perak and Kelantan (Malaysia).

Baltazar and Smith^[8], Martines *et al.*^[9] found variation of barnyardgrass susceptibility to herbicides such as propanil and quinclorac. Moreover, some genotypes might have emerged at different times in the crop phenology and so could escape intervention for weed control. Where genetic diversity exists, under strong selection pressure of application, the population is likely

to shift to constitute individuals that survive prevailing weed control strategies^[17]. The diversity of barnyardgrass ecotypes could also be due in part to the genetic drift within small groups of individuals in disturbed habitat. This could produce genetically different subgroups, which can be easily detected by RAPD-PCR. This hypothesis might explain why geographically distant samples of barnyardgrass ecotypes from Indonesia and Malaysia were separated by cluster analysis.

In this study, the common problem of non-reproducibility of RAPD-PCR banding pattern in multiple run was experienced and it was also reported by Patwary *et al.*^[26]. Part of the molecular variability and non-reproducibility of banding patterns observed among the barnyardgrass ecotypes could be attributed to the 'noise' of RAPD analysis. However, the reliability of RAPD-PCR analysis for studying genetic relationship has been investigated, especially for ecotype and strain levels^[9,23].

In the present study, the RAPD-PCR results are mainly based on the phenotypic UPGMA and only individual ecotypes differences were examined, which this modified method suitable for the analysis of molecular diversity among barnyardgrass ecotypes. The application of random amplified polymorphic DNA (RAPD) has provided a good basis for studies of genetic variation on barnyardgrass ecotypes. This technique was useful for characterization and grouping barnyardgrass ecotypes. Closely related ecotypes as well as very divergent ecotypes sources can be identified.

Exploration and application of potential fungal pathogen for barnyardgrass as bioherbicide is another approach that needs to be taken into account for sustainable agriculture practices in rice ecosystems. However, the large genetic differences within and among barnyardgrass ecotypes is noteworthy from its implications for biocontrol. The pattern of variability among barnyardgrass ecotypes possible relates to the geographic locations. A range of variability was found and it could be expected that the disease caused by biocontrol agent would not be the same across different ecotypes. The genetic variability of the barnyardgrass may be the cause of its variable response to herbicide or agricultural practices across Malaysian and Indonesian rice growing areas.

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