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MORPHOLOGICAL VARIATION OF THE ECOTYPES OF *Echinochloa crus-galli var crus-galli* (L). Beauv (Barnyard grass: Poaceae) IN MALAYSIA and INDONESIA

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ABSTRACT

Greenhouse experiments were conducted to examine the morphological traits of barnyard grass ecotypes from diverse geographic origin. Seeds (caryopsis) were collected from 17 locations of rice fields throughout Malaysia (11 states) and Indonesia (six provinces) and were grown in pots each containing 10 kg of paddy field soil. The experiments were arranged using completely randomized design (CRD) with five replicates. Mean separation was calculated using Duncan multiple range test at 5% probability level. Unweighted pair-group method of arithmetic averages (UPGMA) was performed to determine the individual relationship within ecotypes of barnyard grass. Twelve morphological traits such as culm, panicle, leaf, and spikelet traits were measured. The growth characters such as emergence date, heading time, and growth duration were also evaluated.

The average of emergence date, heading time, and growth duration of barnyard grass collected from Perils, Kedah, Penang, and Johor were relatively earlier than other ecotypes. Six groups were classified based on the cluster analysis of Malaysian ecotypes of barnyard grass. Principal component indicated that group six was found to be highly variable compared to others. While three groups were identified in Indonesian ecotypes of barnyard grass. Group one was observed to be highly variable. Results demonstrated that morphological variation among ecotypes of barnyard grass showing differences between the two regions illustrate the role of geographic variation.

Key words : Variation / ecotypes / paddy field weeds / barnyard grass.

INTRODUCTION

The barnyard grass *(Echinochloa crus-galli* var *crus-galli L.* Beauv.) is an annual weed native to Asia and presently can be found throughout the world (Holm *et at.* 1977). 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 latitude 40°S. (Holm *at al.* 1977; Maun and Barret 1985). Barnyard grass is a noxious weed in rice field and have been shown to reduce rice yield by 40% in direct seeded rice in Malaysia (Azmi 1988) and about 21 % annually in Indonesia (Tjitrosemito 1994).

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This weed belongs to the tribe Paniceae, family Gramineae. The genus may include 20 to 50 species that are widely represented in tropical and warm temperate regions of the world (Clayton and Ronvoize 1986; Martines *et al.* 1999; Michael 1983). With an increase in direct seeding rice practices, barnyard grass is becoming a greater problem in rice fields (Azmi and Baki 2002; Loux and Barry 1991). In addition, continuous chemical control of barnyard grass in rice plant has caused herbicide resistance to barnyard grass (Martines *et al.* 1999; Rutledge *et al.* 2000).

Klingaman and Oliver (1996) defined ecotypes as plants genetically adapted to the habitat they colonized and biotypes as plants showing a random genetic variant within an ecotype. Individual plants of barnyard grass can produce thousands of seeds, but seed germination declines rapidly over time, and sometimes they have long dormancy (Martinkova 1989). Barnyard grass typically grows 1 to 1.5 m tall and is capable of producing a large number of seeds (Azmi and Baki 1995; Itoh 1991). In certain conditions, it may grow up to 2 m tall (Anonim 2001). In addition, the plant can grow in diverse environment (Yabuno 1983; Yamasue 1997), and its growth habit is strongly affected by environmental conditions. Studies have shown that soil type, fertility level, and cultural regimes affect barnyard grass of some morphological traits. The production of a large number of easily dispersed seeds and the ability to flower under a wide range of photo periods contribute to the success of this weed (Holm *et al.* 1977).

Phenotypic and genetic variation among barnyard grass ecotypes would be divergent resulting from the selection pressure imposed by agricultural practices, crop characteristic, geographic origin, and herbicide application. Studies have shown that soil type and fertility level affect morphological characters of barnyard grass in rice field (Martines *et al.* 1999; Yamasue 1997). Recent evidence has shown that substantial variation exists among seed size within plant ecotypes (Marshal *et al.* 1986) and between geographical regions (Ransom *et al.* 1998; Sterling *et al.* 2000).

Phenotypic variability has been observed in numerous weed species, comprising half of the species reported as having biotypes or ecotypes. Weeds with biotypes differing in growth and morphological characteristics include Canada thistle *(Circium arvense L.* Scop) (Hodgson 1964), field bindweed *(Convolvulus arvensis* L.) (Degennaro and Weller 1984, quack grass *(Eltrygia repens* L.) (Westra and Wyse 1981), Johnson grass *(Sorghum helepense* L.) (McWhorter and Jordan 1976), yellow nutsedge *(Cyperus esculentus* L.) (Holt 1994), leafy spurge *(Euphorbia esula* L.) (Harvey *et al.* 1988), and hemp dogbane *(Apocynum cannabinum L.)* (Ransom *et al.* 1998). Differences between weed population can influence the competitive nature of weed species and may affect response to chemical or cultural control strategies. Most biotype studies previously reported have been conducted under different environment. It is very important to study variation in morphological characters among ecotypes to determine how plant genotype and diverse environmental conditions could influence the plant morphology of barnyard grass under uniform conditions. Thus, the objective of this study is to determine if barnyard grass seeds collected from geographically divergent ecotypes exhibit morphological variation when grown in uniform environmental glass house conditions.

MATERIALS AND METHODS

Seed collection

From December 2000 to March 2001, barnyard grass seeds were collected from 11 rice field locations across Peninsular Malaysia (100 - 119° E and 7°N) and 6 locations across Indonesia (94 - 141° Band 6° 8' - 11° 5' S) (Figure 1, Table 1). At each location, seeds from a single plant were randomly collected from different sites in[1] rice growing areas, so that the collected seeds would be genetically identical.

Figure 1. Location map of 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).

Table 1. Coordinates of the collection site of the barnyard grass seeds from 17 rice growing areas.

No.	Location	Latitude (0'N)	Longitude (0'E)	Altitude (ASL:M)
Malaysia				
1	Perlis	6 25	100 16	21.7
2	Kedah	6 12	100 24	3.9
3	Penang	5 28	100 23	2.8
4	Perak	4 34	101 06	40.1
5	Kelantan	6 10	102 17	4.6
6	Terengganu	5 23	103 06	5.2
7	Pahang	3 58	102 21	69.3
8	Selangor	2 44	101 42	16.3
9	Negeri Sembilan	2 45	102 10	60.0
10	Melaka	2 16	102 15	8.5
11	Johor	1 52	102 59	6.3
Indonesia				
12	Lampung	5 45	105 50	28.0
13	Banten	6 37	106 08	25.3
14	West Java	6 14	108 48	45.0
15	Central Java	8 30	110 31	42.0
16	East Java	8 40	114 28	38.0
17	South Sulawesi	0 12	112 30	35.5

ASL : above sea level (meter)

The seeds were chosen to represent the populations from which they were collected. Since seeds were collected from geographically divergent locations, they can be described as ecotypes (Klingaman and Oliver 1996). At each location, five sampling sites were randomly selected. From each of the five sampling sites, one individual ecotype per site, but more than one ecotype were collected when morphologically different types were present at each site using the nearest-neighbor techniques (Solbrig 1960) and the distance between sampling site of each location was more than 10 km (Barret 1982).

Propagation of the ecotypes

A total of 85 ecotypes of *E. cruss-galli* collected from the 17 locations were grown under uniform glass house conditions. Before planting, seeds were soaked in tap water for 24 hours at room temperature. Seeds were grown in each pot size containing 10 kg of paddy soil. Basal fertilizer (0.4 g N/pot, 0.4 g P/pot, and 0.2g K/pot or equal to 40 kg N/ha, 45 kg P/ha, and 30 kg K/ha) were applied directly in each pot, while one third of nitrogen were applied at five to six weeks after seedling emergence (Pane and Mansor 1997). Two weeks after emergence, seedlings were thinned to one plant per pot, leaving the tallest emergence individual in all replications. The pots were flooded shortly after thinning and five cm of water level of the soil surface were maintained throughout the study. Environmental glasshouse conditions such as RH, temperature, and light intensity (Li-Cor Model 185-B) were recorded and soil chemical contents were analyzed (Page 1982).

Morphological trait assessments

Traits such as plant type, plant height (cm), flag leaf length (cm) and width (cm), culm diameter (cm), panicle length (cm), empty glume length (mm), spikelet length (mm), spikelet width (mm), spikelet weight (fresh weight of 100 seeds in gram), panicle awn, and number of tiller were assessed at the maturity stages. Plant type (the degree of opening at the first node) and panicle awn were considered as qualitative characters visually rated on a scale from none (0), moderate (1) to severe (2) (Park *et al.* 1995; Yamasue 1997). In addition, growth characters such as emergence date, heading time and growth duration were also evaluated.

Specimen identification and statistical analysis

Specimen identification was carried out following the classification prepared by Yabuno (1983), Michael (1983), Itoh (1991), and Park *et al.* (1995) and compared with the specimens of the Faculty of Agriculture's herbarium of University Putra Malaysia. Morphological descriptors were subjected to cluster analysis using Euclidean distance coefficient of UPGMA analysis and associated dendrogram using NTSYS-pc package version 2.1 (Rohlf 2000). Principal component analysis (PCA) for step-wise observations among the groups of barnyard grass ecotypes was also analyzed. Square root transformation was used to transform the raw data before analysis. The SHAN clustering program of the NTSYS-pc was used to group the ecotype on the bases of those matrices. ANOVA was used to determine significant difference in growth character of each location of barnyard grass ecotypes with five replications. Means were separated using Duncan's multiple range test (DMRT) at the 5% probability level using SAS version 6.0 (SAS 1996).

RESULTS AND DISCUSSION

Soil analysis and environmental conditions of the glasshouse

A paddy field soil was taken from MARDI Research Station, Tanjong Karang, Selangor. The soil physical and chemical properties were as follows (sand 2%, clay 72%, dust 28%), C 3.13%, N 0.35%, pH 4.8, CEC (mg/lOOg 29.74), C/N ratio 10 Ca (bpj/lOOg = 8.37), and Mg (bpj/lOOg =1.94).

Relative humidity of the glasshouse during experimental period ranged from 68% to 90%, and temperature ranged from 24°C to 34°C, while light intensity ranged from 660 to 930 uM M^sec. These conditions were conducive for growth of barnyard grass.

Variability in growth characteristics among barnyard grass ecotypes

Variability in growth characteristics among barnyard grass ecotypes is presented in Table 2. The range of emergence was from 2.8 days to 7.2 days, that of heading time was from 42 to 56 days. While, the growth duration ranged from 89 days to 98.2 days. For emergence date, Perlis and Kedah ecotypes were the earliest (2.8 days to 3.0 days) and significantly different ($P<0.05$) from the rest of the ecotypes. The heading time (42 days and 44 days) was significantly earliest for Perlis, Kedah, Penang, Perak, Kelantan and Johor ecotypes. South Sulawesi was the latest besides having the longest growth period of 98.2 days. While Perlis, Kedah, Penang, Perak, Kelantan and Johor growth were the shortest and significantly different from the rest of the ecotypes.

Table 2. Growth characteristics of *E. crus-galli* var *crus-galli* ecotypes.

Ecotype location	Emergence	Heading time	Growth duration
		Days	
Perlis	2.8 g	43.0 ef	89.0 f
Kedah	3.0 g	44.0 ef	90.2 f
Penang	4.2 ef	45.0 de	90.2 f
Perak	4.8 def	44.0 ef	90.2 f
Kelantan	5.0 de	42.0 f	95.8 bc
Terengganu	5.6 cd	52.0 b	92.2 e
Pahang	7.2 a	48.0 c	95.8 bc
Selangor	4.8 def	48.4 c	95.2 c
Neg. Sembilan	6.0 bc	47.0 cd	94.4 cd
Melaka	6.8 ab	47.0 c	95.8 bc
Johor	4.0 f	43.0 ef	90.2 f
Lampung	7.2 a	47.2 c	97.8 a
Banten	4.8 def	49.0 c	97.8 a
West Java	5.6 cd	47.0 cd	95.6 bc
Central Java	6.8 ab	48.0 c	94.4 cd
East Java	5.6 cd	47.0 cd	93.2 de
South Sulawesi	6.8 bc	56.0 a	98.2 a

Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 95% confidence level

Results of the growth characteristics among barnyard grass ecotypes collected from Malaysian or Indonesian rice growing areas showed distinct variability. Growth characteristics of the ecotypes such as heading time (42 days to 56 days) and growth duration (89 days to 98 days) were slightly similar to the growth duration of barnyard grass (44 DAE to 45 DAE and 90 DAE to 95 DAE) as reported by Azmi *et al.* (1997) and Itoh (1991). However, the values were slightly lower than the growth duration of barnyard grass in Arkansas (Barret and Wilson 1983).

In general, growth characteristics variability among barnyard grass ecotypes might be affected by geographic origin. This observation was in line with the work of Johnson *et al.* (1989) who reported that even though ecotype of some Sweetvetch *(Hedysarum boreale* Nutt.) differed in their taxonomic distance values, this difference is not always closely related to specific characteristics of the collection sites. Widespread species tend to have more genetic variability than close relation with narrow distribution (Hamrick and Godt 1990; Karron 1987; Maki *et al.* 1999).

Pylogenetic relationship within individual barnyard grass ecotypes

Within Malaysian ecotypes

Dendrogram using UPGMA clustering methods from morphological traits is presented in Figure 2. Six groups were identified at the taxonomic distance of 0.016. The first group comprised 21 ecotypes mainly from Perils, Kedah, and Johor. The 3rd and 4th groups comprised 14 ecotypes, respectively. Groups 2, 5 and 6 consisted of 2, 3 and 1 ecotypes, respectively. Two individualecotypes belonged to group 2. The only ecotype from Selangor (S-02) was found in Group 6. Ecotypes from Perlis, kedah, Perak, and Johor were found to be linked together and have closer relation widi each other. Ecotypes from Perlis and Johor fell within Group 1, while those of Kelantan and Negeri Sembilan mostly belonged to Group 3. Penang, Pahang, and Selangor were dominant in Group 4.

Principal component analysis of barnyard grass was applied to differentiate variability among the existing groups. The proportion of the first and second components were 88.76% and 7.01%, respectively (Table 3). The first component was largely contributed by the size of characters such as plant height, plant type, and number of tillers. The second component was due to the shape and size characters of panicle awn, flag leaf width and spikelet length.

Scatter diagram between the first and second component showed that the individual plants present in different groups had characters different from the others (Figure 4.A). Most of them were separately distributed in the quadrants of the diagram. On the contrary, individual barnyard grass in Group 6 were farther apart than the other groups, indicating that they vary largely.

Within Indonesian ecotypes

At the taxonomic distance of 0.08, three groups were classified from six locations representing three different islands. Group 1 consisted of 19 ecotypes representing five locations (two islands), except for ecotype from Lampung. Eight ecotypes were obtained in Group 2, except for Lampung ecotype. In contrast, only three South Sulawesi ecotypes (SS-1, SS-2, SS-4) were found in Group 3 (Figure 3).

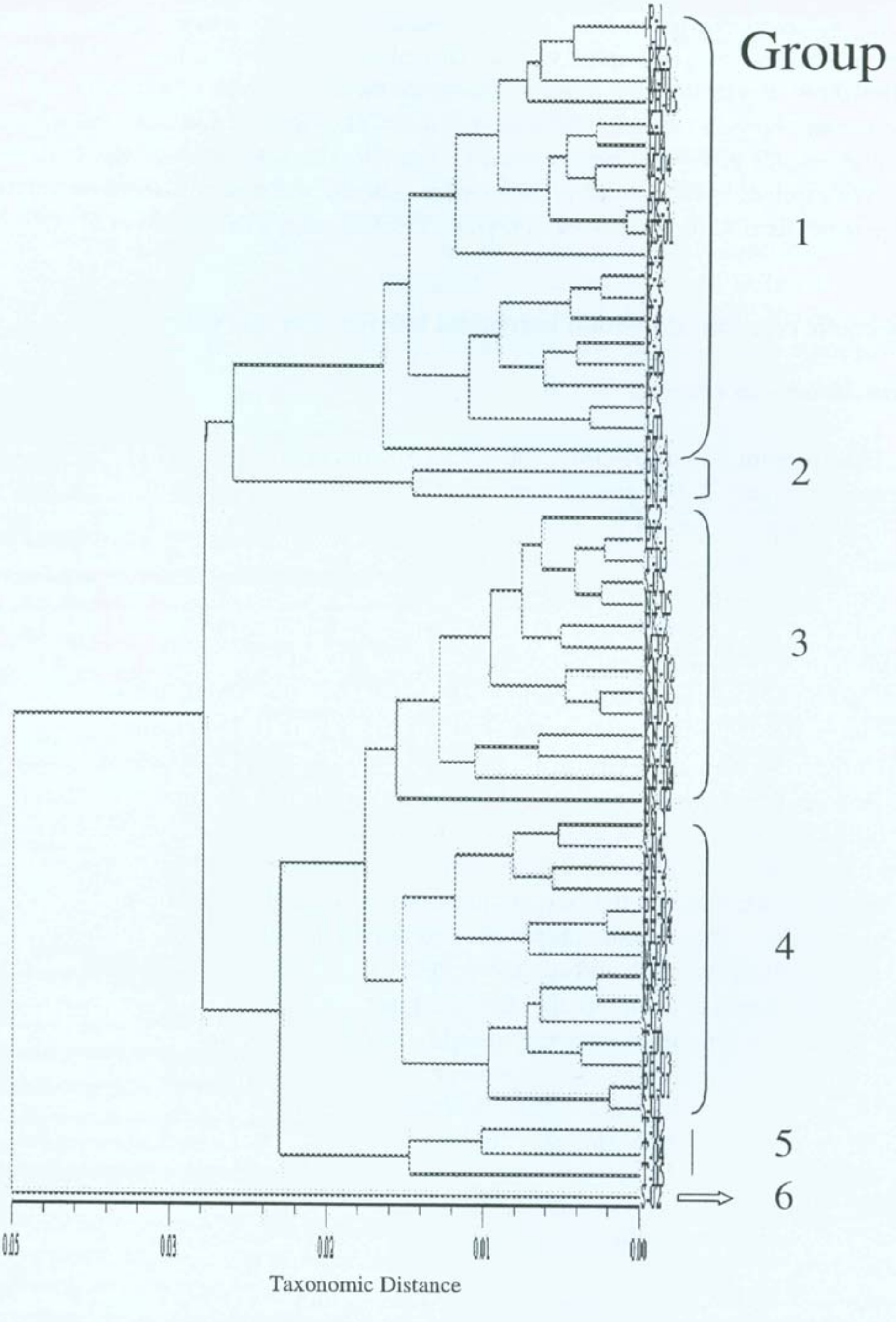

Figure 2. Dendrogram from UPGMA clustering analysis using Euclidian Distance coefficient of dissimilarity on 55 barnyard grass ecotypes from Malaysia. Note (P:Perlis; K:Kedah; PN:Penang; PK:Perak; KN:Kelantan; T:Terengganu; PH:Pahang; S:Selangor; NS: Negeri Sembilan; M:Melaka; J:Johor)

Table 3. Eigen values and proportion of the first two principal component

Characters	Malaysia		Indonesia	
	PC1	PC 2	PC 1	PC2
Plant height	0.9617	–0.1189	0.5720	0.1552
Plant type	0.0152	0.0010	0.0000	0.0000
Flag leaf length	–0.2480	–0.5469	–0.7125	–0.1930
Flag leaf width	–0.0081	0.0167	–0.0125	0.0044
Culm diameter	–0.0005	–0.0004	0.0106	–0.0393
Panicle awn	–0.0282	0.0601	0.3939	–0.0393
Panicle length	–0.0316	0.8240	0.3938	–0.3959
Spikelet weight	0.0000	–0.0020	0.0026	–0.0115
Spikelet width	–0.0018	–0.0021	–0.0025	0.0078
Glume length	–0.0039	–0.0052	–0.0058	–0.0319
Spikelet length	–0.0015	0.0024	–0.0088	0.0346
Number of tiller	0.1065	0.0613	–0.593	0.8470
Eigen value	68.45	5.41	12.19	0.61
Proportion (%)	88.76	7.01	95.22	61.12
Cumulative (%)	88.76	95.77	95.22	4.77

Principal component analysis among groups of barnyard grass is presented in Table 3. The proportion of the first and second components were 95.22% and 61.22%, respectively. The first component was largely due to shape and size of characters such as plant height, panicle awn, and panicle length, respectively. The second component was mainly due to plant height and number of tillers. Scatter diagram between the first and second components showed that plants in different groups had characters different from the others (Figure 4.B). Most of them were distributed in the first two components. However, ecotypes belonging to Group 1 were farther distributed than other Groups, indicating that Group 1 had wide variation.

The results of this study indicated that individual ecotype of Malaysian and Indonesian barnyard grass were found to be more variable (Tables 2 and 3; Figures 2 and 3). In general, ecotype of barnyard grass from Malaysia and Indonesia are separated by larger geographical distance and is expected to be different than those from the same locations. Yamasue (1997) reported that variation in morphological characters were significantly different among barnyard grass collected from different habitats, even though they are from the same locality. On the contrary, weed population which are continuously associated with specific agricultural systems may evolve phonological patterns which optimize survival within the most favorable growing areas (Barret 1983). The results demonstrated that genetic differentiations among ecotypes were apparently an important factor of the variation in weed management practices. The differentiation among local ecotypes was probably encouraged by self-pollinating reproduction of the barnyard grass ecotypes (Honek and Martinkova 1996). On the contrary, phenotypic variations in barnyard grass ecotypes from Indonesia and Malaysia affected seedling emergence and growth characteristics.

Interestingly, South Sulawesi ecotypes seemed to be specific in different habitats of different islands (Figure 3). This finding is to be similar with the work of Maki *et al.* (1999) who found that highly phenotypic and genetic differentiation of *Aster miyagii* (Asteraceae) existed among islands in Japan, or high levels of genetic diversity among island populations of *Suzukia luchunsis* (Labiatae) in Japan (Maki *et al.* 2003).

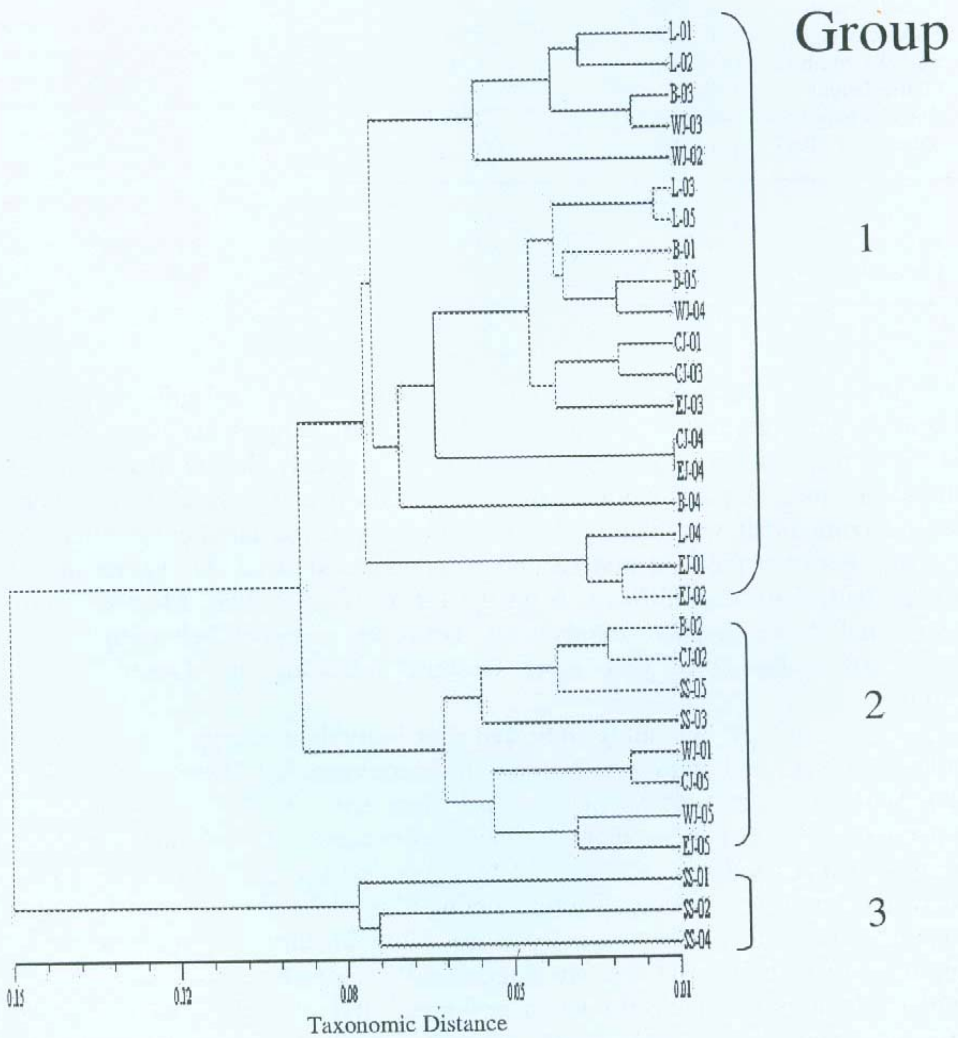

Figure 3. Dendrogram from UPGMA clustering analysis using Euclidian Distance coefficient of dissimilarity on 55 barnyard grass ecotypes from Indonesia. Note (L:Lampung; B:Banten; WJ:West Java; CJ: Central Java; EJ:East Java; SS: South Sulawesi).

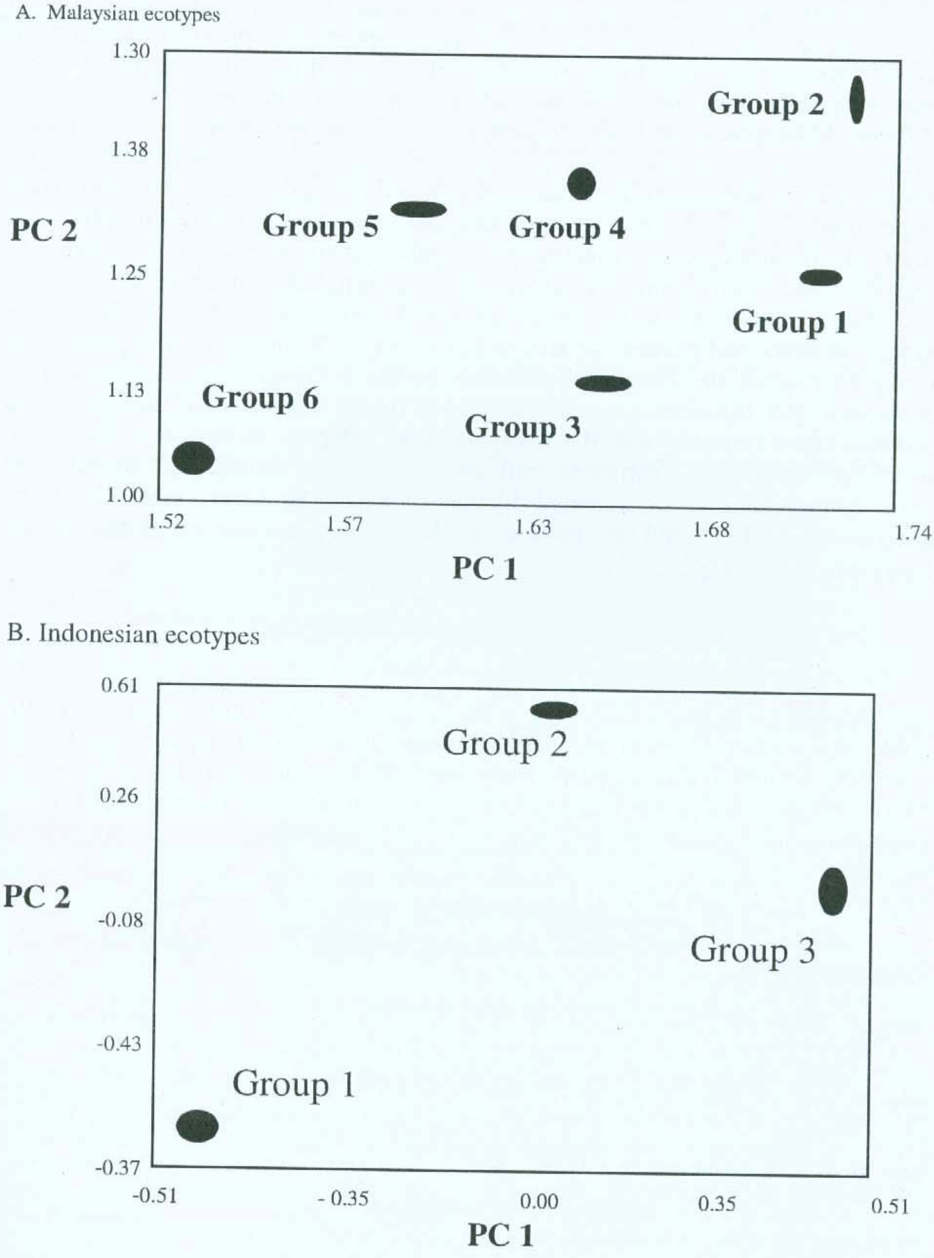

Figure 4. The first two components of *E. crus-galli* var. *crus-galli* ecotypes (A: Malaysian ecotypes; and B: Indonesian ecotypes)

Morphological variations among barnyard grass ecotypes in Malaysia and Indonesia are not very high at present. If newer strains (biotypes) of the barnyard grass migrate from one to other regions and cross-pollinate, the genetic diversity may increase, and the control of barnyard grass in rice production may become more difficult. Both genetic and environment play a role in the expression of barnyard grass phenotype. However, barnyard grass genotype maintained their general populations when grown in common site. Interestingly, when barnyard grass population was grown at the uniform sites (glasshouse conditions) ecotypes from the most Malaysian regions, maintained the highest plant size at short periods to reproductive maturity compared to barnyard grass from Indonesian regions.

The variability could be affected by local environmental heterogeneity, geographic distance and possibly agricultural practices. This finding is of importance in trying to explain the ecotype distribution among barnyard grass ecotypes from Malaysian and Indonesian rice fields. These results suggest that barnyard grass ecotypes have formed, and the ecotypes have adapted to specific geographic locations. The ecotype variations will affect successful management of barnyard grass using chemical or potential biocontrol agent. However, molecular traits analysis would be useful to characterize the level of genetic variability among barnyard grass ecotypes.

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