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Genetic Diversity and Morphological Variations of Goosegrass [*Eleusine indica* (L.) *Gaertn*] Ecotypes in Malaysia

Nazreen Saidi*, Jugah Kadir, and Lau Wei Hong

Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia

ABSTRACT. Goosegrass [*Eleusine indica* (L.) *Gaertn*] has been a nuisance to growers in Malaysia due to its increased resistance to commercial herbicides, rapid growth and dissemination, and interference with agricultural practices. In the course of developing an apt integrated management to control goosegrass, more information of this weed is needed. The aim of this study was to look into variations among the goosegrass ecotypes sampled throughout Malaysia from the aspects of genotype and phenotype. Sequence-related amplified polymorphism (SRAP) markers were employed in investigating the genetic diversity and relationships among the 18 goosegrass ecotypes. Consequently, 5 primer combinations amplified 13 fragments with the polymorphism rate of 69.23%. At 74% similarity, the ecotypes were clustered into 6 groups. Phenotypic variability of the goosegrass ecotypes was assessed by observing their morphology, growth and seed traits. Goosegrass ecotypes were sorted into 3 major groups at the genetic distance (DIST) of 0.37. Concurrences of the evaluated genetic distance, ecotypes with the closest and most distant relationships were assembled together in Group I which showed high variation even among ecotypes in the same group. Results obtained thus implied high molecular and morphological variations of the goosegrass ecotypes in Malaysia.

***Key words*:** Ecotypes, *Eleusine indica*, Goosegrass, Genetic diversity, Morphology

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***Corresponding author:** Phone) +60-38-947-4845, Fax) +60-38-938-1014; E-mail) nazreensaidi@live.com

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Introduction

Goosegrass is an annual grass weed that was ranked as the fifth worst weed in the world (Holm et al., 1977) and South East Asia (Waterhouse, 1993). In the tropical climate with high temperature and humidity like Malaysia, goosegrass grows and disperses all year round making it a troublesome pest to control in fruit orchards, plantations and farms. A survey done in the Peninsular Malaysia and Southern Thai pointed out that goosegrass is a bothering weed which interfered with field management practices, reduced crop growth and increased pest and disease problems (Miyaura and Ito, 1994). Little is known on the diversification of goosegrass ecotypes in Malaysia. The weed influences effort on composing the best integrated pest management for weed control.

Turesson first proposed the term ecotype in 1922 as an ecological unit resulted from the genotypical response of a population to a particular habitat. As a result of ecotype, populations change to adapt to new habitat and surrounding conditions. One of the ways to observe these variations are by looking at the phenotype, observable characteristics that resulted from the interaction between its genes and the environment. Phenotypic variation within species was found to be either as large as or larger than one observed among species (Bangert et al., 2006; Shuster et al., 2006). As long as variation prevails in ecologically important traits, the amount of diversity at any level can have important ecological effects. While numerous studies have been done on factors affecting germination of goosegrass, there seems to be a lack of evaluation on its morphology among ecotypes. Comparative assessment of quantitative and qualitative morphological characters was done in order to further understand how ecotypically different are populations of goosegrass in Malaysia.

Li and Quiros (2001) have successfully developed a DNA marker technique called sequence-related amplified polymorphism (SRAP). SRAP has been used for mapping and gene tagging in Brassica (Li and Quiros, 2001) and Cucurbita (Ferriol et al., 2003). Budak et al. (2004) reported SRAP markers were the only marker system among ISSR, SSR,

RAPD and SRAP that differentiated all genotypes studied including those with a similar pedigree. Genotypes from different geographical regions were grouped together, although there was a less-clear pattern. The values of average discriminating power followed the pattern SRAP > SSR > ISSR > RAPD, as a direct consequence of their confusion probabilities. SRAP had the highest polymorphic and discriminating fragments compared to all marker systems tested. Presence of co-dominant in SRAP would make it the marker of choice for segregation studies and genome mapping. This technique equals or exceeds that of the more labour intensive processes involved in the use of restriction fragment length polymorphism (RFLP) markers (Budak et al., 2004). For these reasons, we chose SRAP to differentiate the goosegrass ecotypes.

There will usually be a range of genetically diverse biotypes within a population of plant species (Burdon, 1987) which may include some resistant types. There is a widespread concern that weed species with higher levels of genetic diversity will exhibit considerable potential for weed adaptation and, therefore, may be able to reduce the effectiveness of weed control (Dekker, 1997; Holt and Hochberg, 1997). As such, determining how diversified weedy species is considered a high priority. Despite these efforts, there is a lack of evidence correlating genetic diversity with physiological, morphological or other ecological adaptations, such as adaptation to biocontrol agents (Chabondez and Sheppard, 1995). Thus, the objective of this study was to look into molecular and morphological variations among the goosegrass ecotypes in Malaysia and the correlation between said divergences.

Materials and Method

Seed collection and cultivation

Inflorescences of goosegrass were collected from random matured plants at 29 locations in Malaysia (18 areas in Peninsular Malaysia and 11 areas in East Malaysia), air-dried, hulled and stored at 4°C (Table 1). Prior to sowing, seeds were soaked in 5 ml of 0.2% potassium nitrate, dark condition overnight at 25°C. Seeds from each ecotype were sowed on top of the medium in seedling trays in the glasshouse. Throughout the experiment period, temperature ranged between 27°C to 38°C and relative humidity of 87% to 90%. Peat moss (Peatgro™ multipurpose soil conditioner) was used as the germinating medium. Planted seeds were observed every day and watered as needed. Water soluble fertilizer (Trio® Equal NPK 21:21:21:TE) was applied once the true leaves emerged. On day 20 after sowing, seedlings were transplanted into polybags filled with 3:2:1 mixture of top soil:sand:organic matter (chicken manure). Few young leaves were picked for DNA extraction 3 weeks after transplanting date. We planned to let the plants grow older for growth and morphological observations but they were heavily infested by the rusty plum aphid, *Hysteroneura setariae* (Thomas) that we had to remove and re-plant. No pesticide was applied to eliminate any effect it may have on the result of genetic variation. Similar steps of soaking, sowing and transplanting were done for the second batch. A week after transplanting, seedlings were thinned to 1 seedling per polybag and left to grow for growth and morphological observations. Three replications were used in both experiments.

DNA extraction

Out of 29 seeds of different goosegrass ecotypes, 18 of them germinated and grew in the glasshouse (K1, K2, B2, N1, M1, J1, D1, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10 and SW). Young leaf tissue was harvested and stored in liquid nitrogen at −70°C before use. A modified cetyltrimethylammonium bromide (CTAB) protocol was followed for plant DNA extraction (Xu et al., 2003). Plant tissue was ground to powder. 5 ml of extraction buffer [1% (w v^{-1}) CTAB, 0.1M Tris-Hcl, pH 7.5, 0.7 M NaCl, 0.1 M EDTA, 1% (v v^{-1}) β-mercaptoethanol] was added into tubes of 200 mg tissues. The tubes were inverted gently a few times while being incubated at 65°C for 1 hour. Subsequently, the tubes were left to cool down at room temperature for 10 minutes. The homogenate was extracted twice with 2.5 ml chloroform-octanol (24:1) followed by centrifugation at 2,000 g for 10 minutes. The aqueous phase was removed and the suspension was then treated with 50 μg ml^{-1} RNAse at room temperature for 30 minutes. 3 ml of isopropanol was added for DNA precipitation and the tubes were inverted for 5 times. DNA pellet was rinsed with 76% (v v^{-1}) absolute ethanol, 0.2 M sodium acetate for 20 minutes. A second rinse of DNA pellet was done by using 76% (v v^{-1}) absolute ethanol, 0.01 M ammonium acetate for 20 minutes. The DNA pellet was diluted in 0.5 ml Tris-EDTA solution and stored at 4°C.

Primer selection

Preliminary tests were done beforehand where 90 combinations of SRAP primers were screened for a few DNA samples (Table 2). 5 SRAP primer pairs were chosen for producing the clearest bands.

Polymerase chain reaction (PCR)

Each 25 μL PCR reaction mixture consisted of 0.2 μL DNA template, 0.2 mM DNTPs, 2.0 mM $MgCl_2$, 0.2 μM forward primer, 0.2 μM reverse primer, 1x ViBuffer A, and 1.5 unit Taq polymerase (My PCR Kit 3, Vivantis Technologies, Subang Jaya, Malaysia). PCR cycles followed the steps testified by Li and Quiros (2001).

Gel electrophoresis

The PCR products (25 μl) were fractionated on 2% agarose

Table 1. Goosegrass ecotypes sampled in Malaysia.

Ecotype	Area	State	Location	Herbicide history
K1	Sungai Petani	Kedah	Residential area	–[u]
K2	Padang Buluh	Kedah	Palm oil plantation	Glyphosate[v] Ammonium-glufosinate[w]
K3	Padang Serai	Kedah	Palm oil plantation	Glyphosate[v] Sodium chlorate[x]
K4	Bukit Selambau	Kedah	Palm oil plantation	Glyphosate[v] Ammonium-glufosinate[w]
A1	Bagan Datoh	Perak	Palm oil plantation	Glyphosate[v] Ammonium-glufosinate[w] Metsulfuron-methyl[y] 2,4-D amine[z]
A2	Teluk Intan	Perak	Palm oil plantation	Glyphosate[v] 2,4-D amine[f]
A3	Ulu Bernam	Perak	Palm oil plantation	Glyphosate[v] Ammonium-glufosinate[w]
A4	Tanjung Malim	Perak	Palm oil plantation	–[u]
B1	Sabak Bernam	Selangor	Palm oil plantation	Glyphosate[v] 2,4-D amine[f]
B2	Kapar	Selangor	Palm oil plantation	Glyphosate[v] Ammonium-glufosinate[w]
B3	Serdang	Selangor	Roadside	–[u]
N1	Jelebu	Negeri Sembilan	Roadside	–[u]
M1	Kesang	Melaka	Vegetable farm	Glyphosate[v] Ammonium-glufosinate[w]
J1	Ayer Hitam	Johor	Roadside	–[u]
D1	Bachok	Kelantan	Roadside	–[u]
D2	Pasir Puteh	Kelantan	Roadside	–[u]
T1	Kerteh	Terengganu	Roadside	–[u]
C1	Kuala Lipis	Pahang	Residential area	–[u]
S1	Telupid-Sandakan Road	Sabah	Vegetable farm	–[u]
S2	Telupid-Sandakan Road	Sabah	Oil palm plantation	–[u]
S3	Ranau-Telupid Road	Sabah	Banana farm	–[u]
S4	Ranau-Telupid Road	Sabah	Vegetable farm	–[u]
S5	Tambunan-Ranau Road	Sabah	Oil palm plantation	–[u]
S6	Tambunan-Ranau Road	Sabah	Vegetable farm	–[u]
S7	Kota Kinabalu-Tambunan Road	Sabah	Oil palm plantation	–[u]
S8	Penampang-Tambunan Road	Sabah	Oil palm plantation	–[u]
S9	Penampang	Sabah	Vegetable farm	–[u]
S10	Ranau-Telupid Road	Sabah	Vegetable farm	–[u]
SW	Bintulu	Sarawak	Vegetable farm	–[u]

[u]Data not available.
[v]Glyphosate-isopropylammonium 41% concentration soluble concentrate.
[w]Ammonium-glufosinate 13.5% concentration soluble concentrate.
[x]Sodium chlorate 99% concentration soluble powder.
[y]Metusulfuron-methyl 20% concentration wettable powder.
[z]2,4-D dimethylamine 48-69.5% concentration soluble concentrate.

Table 2. The forward and reverse SRAP primer pairs and their sequences.

Primer combination	Sequence (5'-3')
me 5-em1	TGA GTC CAA ACC GGA AC GAC TGC GTA CGA ATT AAT
me1-em5	TGA GTC CAA ACC GGA GC GAC TGC GTA CGA ATT AAC
me9-em5	TGA GTC CAA ACC GGA TC GAC TGC GTA CGA ATT AAC
me5-em6	TGA GTC CAA ACC GGA AC GAC TGC GTA CGA ATT TGA
me4-em4	TGA GTC CAA ACC GGA CA GAC TGC GTA CGA ATT CTG

gels in TAE buffer (40 mM Tris-acetate, 20 mM sodium acetate, 1 mM EDTA; pH 7.7) with electrophoresis condition held at 70 V for 1 hour at room temperature. Gel Doc^{TM} XR was used to photograph gel.

Data analysis

DNA fragments were scored as '1' and '0' where '1' stands for the presence and '0' stands for the absence of each SRAP fragment. A dendrogram was constructed based on the Jaccard's genetic distance by using the unweighted pair-group method with arithmetic (UPGMA) cluster analysis with NTSYS. Data of growth and morphological variations were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) tests. Means for each morphological character were standardized (STAND) and product-moment correlation (CORR) coefficients were generated using interval data (SIMINT) procedure from NTSYS. Principal Component Analysis (PCA) was performed with CORR and EIGEN programmes. A dendrogram was constructed based on the average taxonomic distance (DIST).

Results

Genetic diversity analysis

The five primer combinations amplified 13 fragments ranging in size between 50 to 400 base pairs, of which 9 (69.23%) were polymorphic (Table 3). The highest number of amplification products was obtained with the primer combination me5-em9 and the lowest with me4-em4 while the average number of bands among the five primer pairs was 2.6 (Fig. 1). The number of polymorphic fragments for each primer combination varied from 0 to 4 with the average number 1.8.

The goosegrass ecotypes can be divided into 6 groups at 74% genetic similarity (Fig. 2). Jaccard's genetic similarity coefficients ranged from 0.30 to 1.00 with the lowest similarity

Table 3. Polymorphism of goosegrass ecotypes.

Primer combination	Number of amplified bands	Number of polymorphic bands	Percentage of polymorphic bands	Range of product (bp)
me5-em1	3	1	33.3%	50-200
me1-em5	3	2	66.7%	150-400
me5-em9	4	4	100.0%	50-280
me6-em5	2	2	100.0%	50-180
me4-em4	1	0	0%	50
Total	13	9		50-400

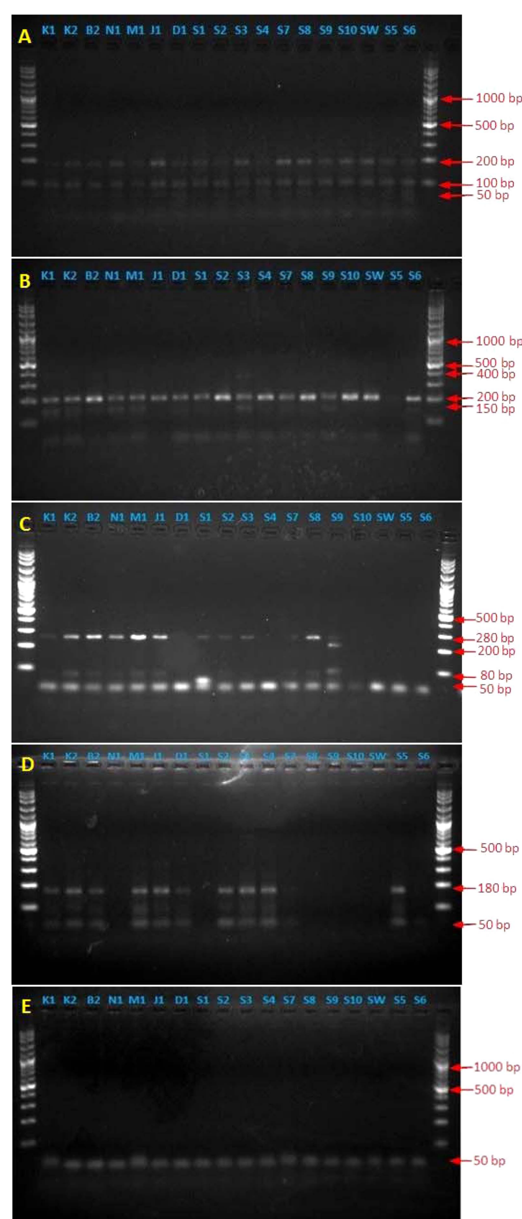

Fig. 1. SRAP-PCR banding patterns produced by the primer pair (A): me5-em1; (B): me1-em5; (C): me5-em9; (D): me6-em5; (E): me4-em4. The molecular weight standard at the last lane consisted of 100 bp DNA ladder (Vivantis Technologies, Subang Jaya, Malaysia).

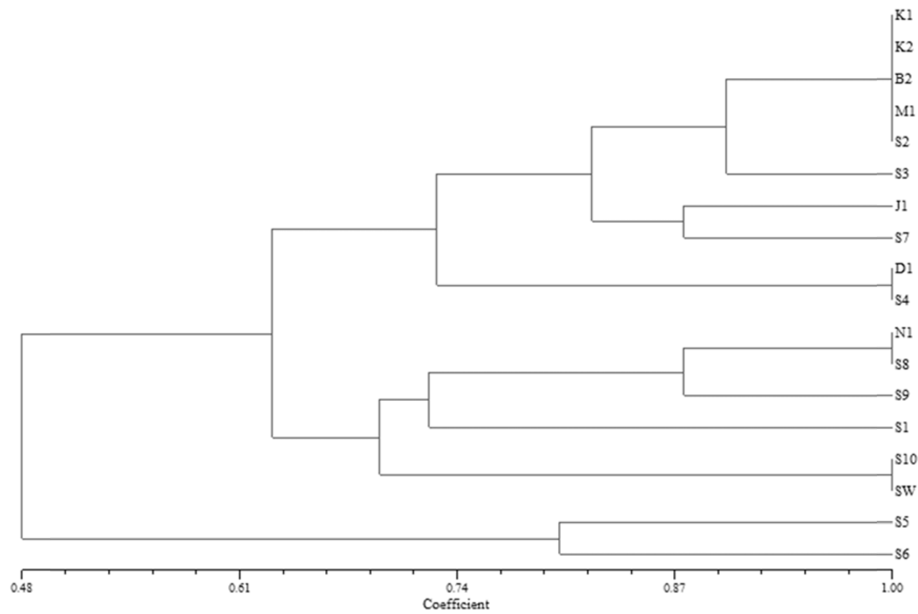

Fig. 2. Dendrogram of goosegrass ecotypes from cluster analysis (UPGMA) based on Jaccard's genetic distance from the SRAP marker analysis.

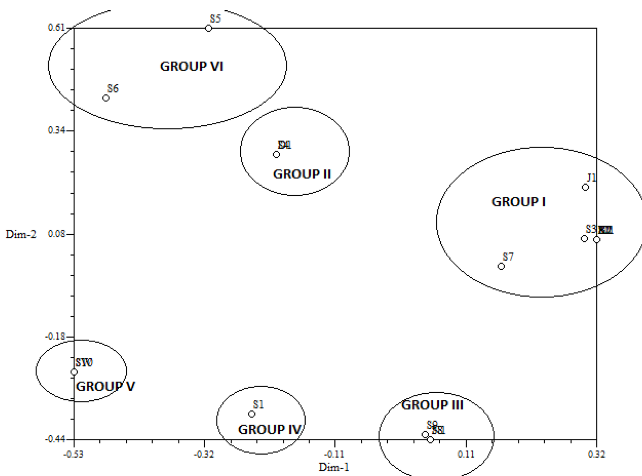

Fig. 3. Principal component analysis of the goosegrass clustering according to UPGMA.

was between ecotype S5 (Group III) and S9 (Group VI). Group I is the largest group consisted of 8 ecotypes K1, K2, B2, M1, S2, S3, J1, and S7, mainly from West of Peninsular Malaysia and Sabah. Only 2 ecotypes were grouped together in Group II: D1 from Kelantan and S4 from Sabah. The two ecotypes were not geographically in close proximity to each other as the two states are separated by the South China Sea. The same circumstance applied to Group III (N1, S8, and S9) where two of the ecotypes were from Sabah and one from Negeri Sembilan. Group IV contained sole ecotype from Sabah, S1. S1 has distinct genetic variation that separated it from the other ecotypes. However, the degree of variation is not high enough resulting S1 to remain close to Group III and V. Group V had samples S10 and SW from Sabah and

Table 4. Summary statistics of morphological traits measured for 22 goosegrass ecotypes.

Character	Mean	Standard deviation	Minimum	Maximum
Number of tillers	4.51	0.90	2.67	6.00
Plant height (cm)	45.07	10.11	24.76	64.47
Flag leaf length (cm)	28.06	4.32	21.60	35.60
Flag leaf width (cm)	0.84	0.08	0.70	1.03
Panicle length (cm)	9.05	1.81	6.14	13.11
Number of panicles	8.09	1.93	5.50	12.33

Table 5. Results of ANOVA performed on 22 ecotypes of goosegrass.

Parameter	F	Sig.
Number of tillers	1.640	0.083
Plant height (cm)	1.750	0.059
Flag leaf length (cm)	2.540	0.006*
Flag leaf width (cm)	1.739	0.080
Panicle length (cm)	3.536	0.000*
Number of panicles	6.218	0.000*
Heading time (days)	8.638	0.000*

*denotes significant differences among ecotypes at $\alpha = 0.05$.

Table 6. Morphological characteristics of goosegrass ecotypes.

Ecotype	Number of tillers	Plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Number of panicles
K1	5.3	36.4	22.4[a]	0.8	6.5[ab]	10.0[ef]
K2	6.0	38.8	22.8[a]	0.9	8.8[abcdefg]	7.7[abcde]
K3	4.7	32.7	23.1[a]	0.7	6.9[abcd]	7.3[abcd]
B1	4.7	47.0	25.4[ab]	0.8	9.5[bcdefg]	6.3[abc]
B2	5.0	48.2	24.2[ab]	0.8	7.5[abcde]	7.7[abcde]
N1	4.7	53.8	34.8[cd]	0.9	8.3[abcdef]	6.7[abcd]
M1	4.3	64.5	31.0[abcd]	0.9	9.3[abcdefg]	8.3[bcde]
J1	5.7	51.5	21.6[a]	0.7	8.3[abcdef]	6.3[abc]
D1	3.3	48.9	29.1[abcd]	0.8	10.2[efgh]	5.5[a]
D2	3.7	53.6	27.1[abcd]	0.8	9.6[bcdefg]	7.0[abcd]
T1	4.7	24.8	30.7[abcd]	0.8	7.9[abcdef]	6.0[ab]
S1	3.7	52.1	33.4[bcd]	0.9	11.7[gh]	9.0[de]
S2	5.3	41.2	30.6[abcd]	0.9	6.8[abc]	9.0[de]
S3	4.7	42.0	28.3[abcd]	1.0	10.2[defgh]	6.0[ab]
S4	5.0	53.3	24.7[ab]	0.8	10.0[cdefgh]	7.0[abcd]
S5	2.7	48.4	35.6[d]	0.9	13.1[h]	9.0[de]
S6*	4.3	27.7	–	–	–	–
S7*	4.3	39.5	–	–	–	–
S8	6.0	48.2	26.6[abc]	0.9	9.7[bcdefg]	8.7[cde]
S9	4.7	30.6	26.0[abc]	0.8	6.1[a]	10.0[ef]
S10	3.0	52.6	30.9[abcd]	1.0	11.1[fgh]	12.3[f]
SW	3.7	55.7	33.2[bcd]	1.0	9.3[abcdefg]	12.0[f]

*denotes S6 and S7 did not head resulting no flag leaf and panicles formed.
[a]means followed by the same letter in each column are not significantly different at $P < 0.05$ (DMRT).

Sarawak, correspondingly. S5 and S6 were clustered into Group VI.

Principal component analysis successfully revealed a pattern of distribution of the 6 groups obtained from the cluster analysis above (Fig. 3). The goosegrass samples were segregated throughout the dimensions according to its similarities in the polymorphism.

Phenotypic variation analysis

Out of 29 goosegrass ecotypes, 22 of them were successfully cultivated in the glasshouse (K1, K2, K3, B1, B2, N1, M1, J1, D1, D2, T1, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10 and SW). Six morphological parameters were observed (Table 4). There was no significant difference among ecotypes for average number of tillers, plant height and flag leaf width (Table 5). However, flag leaf length, number of panicles and panicle length showed significant differences between ecotypes (Table 6).

As in Table 7, the average heading time was 64.5 days from sowing date for all ecotypes with the earliest ecotype to produce flowers was M1 (47 days) and the ecotype which delayed the longest in producing flowers was S6 (83 days). Goosegrass germination was rapid with the average seedling emergence of 1.8 day. All ecotypes were found to germinate within 4 days except K3, K4, A1, A2, A3, A4 and B1 (Fig. 4).

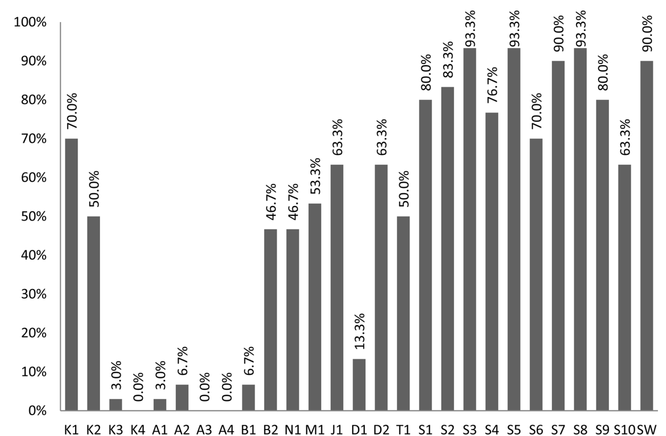

Fig. 4. Germination rate of goosegrass ecotypes.

Twenty two goosegrass ecotypes were clustered into 3

Table 7. Seed and growth characteristics of goosegrass ecotypes.

Ecotype	Average seed weight (g 100 seeds^{-1})	Seed colour	Emergence time (days)	Heading time (days)	Germination rate (%)
K1	0.031	Black	1	52.7^{abc}	70.0
K2	0.021	Black	1	55.7^{abc}	50.0
K3	0.020	Black	4	57.3^{abc}	3.0
B1	0.029	Reddish black	3	63.7^{bcde}	6.7
B2	0.027	Reddish black	3	51.3^{abc}	46.7
N1	0.027	Reddish black	1	50.3^{ab}	46.7
M1	0.022	Reddish black	3	59.0^{abcd}	53.3
J1	0.026	Reddish black	1	47.3^{a}	63.3
D1	0.021	Black	2	57.5^{abcd}	13.3
D2	0.024	Black	4	62.0^{bcde}	63.3
T1	0.032	Black	2	54.7^{abc}	50.0
S1	0.027	Reddish black	1	73.0^{efg}	80.0
S2	0.027	Reddish black	3	63.7^{bcde}	83.3
S3	0.027	Reddish black	2	63.0^{bcde}	93.3
S4	0.020	Reddish black	1	81.7^{fg}	76.7
S5	0.029	Reddish black	1	82.0^{fg}	93.3
S6	0.027	Reddish black	1	83.0^{g}	70.0
S7	0.019	Reddish black	1	82.5^{g}	90.0
S8	0.025	Reddish black	1	70.0^{def}	93.3
S9	0.034	Reddish black	1	81.7^{fg}	80.0
S10	0.035	Reddish black	1	62.7^{bcde}	63.3
SW	0.027	Black	1	64.0^{cde}	90.0

[a]means followed by the same letter in each column are not significantly different at P < 0.05 (DMRT).

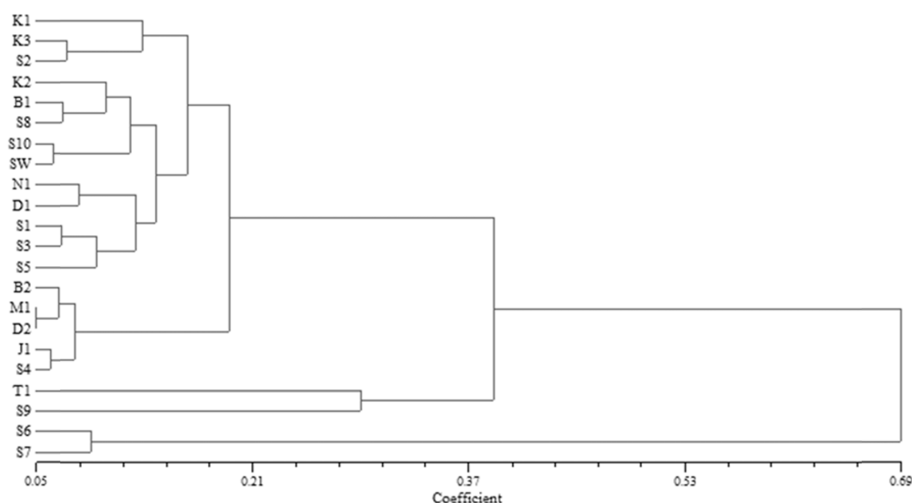

Fig. 5. Dendrogram from UPGMA clustering using average taxonomic distance (DIST).

groups based on UPGMA clustering using DIST coefficient at 0.37 similarity (Fig. 5). The genetic distance based on the morphological characters was reported to be in the range of 1.03 and 9.88 with the shortest distance between ecotype M1 and B1 while the longest one between ecotype S8 and SW. Group I is the largest group with a total of 18 ecotypes from Peninsular and West Malaysia. Two ecotypes were grouped together in Group II (T1 and S9) and Group III (S6 and S7),

Table 8. Means of morphological traits of the 3 goosegrass groups.

Traits	Group I	Group II	Group III
Number of ecotypes	18	2	2
Number of tillers	4.5	4.7	4.3
Plant height (cm)	48.3	27.7	33.6
Flag leaf length (cm)	26.3	28.4	–*
Flag leaf width (cm)	0.8	0.8	–*
Panicle length (cm)	8.7	7.0	–*
Number of panicles	7.5	8.0	–*
Average 100-seed weight (g)	0.028	0.033	0.023
Seed colour	Reddish black to black	Reddish black to black	Reddish black
Emergence time (days)	1.9	1.5	1.0
Heading time (days)	78.8	68.2	82.8

*Data not available.

Table 9. Eigenvector coefficients of principal component analysis. Character loading values are for the first three Principal Component (PC) axes.

Character	PC1	PC2	PC3
Number of tillers	0.7790	0.1687	0.4482
Plant height	–0.5850	–0.3641	0.6791
Flag leaf length	–0.8321	–0.0197	–0.2889
Flag leaf width	–0.7638	0.4548	0.1265
Panicle length	–0.7687	–0.4745	0.0260
Number of panicles	–0.4866	0.7771	0.1554
Eigenvalue	3.0525	1.1972	0.7864
Percentage	50.8757	19.9533	13.1062
Cumulative percentage	50.8757	70.8290	83.9353

respectively. No data of flag leaf and panicle characters for Group III was obtained as none of the plants produce inflorescences until the end of the growth experiment (Table 8). Under the same glasshouse conditions, S6 and S7 were found to be very late in heading and took longer than 3 months to produce inflorescences. Number of tillers ranged from 4.3 to 4.7 per plant. Goosegrass in Group I were the tallest in growth although they were the slowest to germinate. In contrast, Group II had the shortest plant growth among all. Group I had shorter flag leaves than those of Group II but there was no difference in the width of flag leaves. Group II produced shorter and more panicles per plant compared to Group I.

The reddish black seeds of Group III were the lightest among the three groups. Apparently in our preliminary study

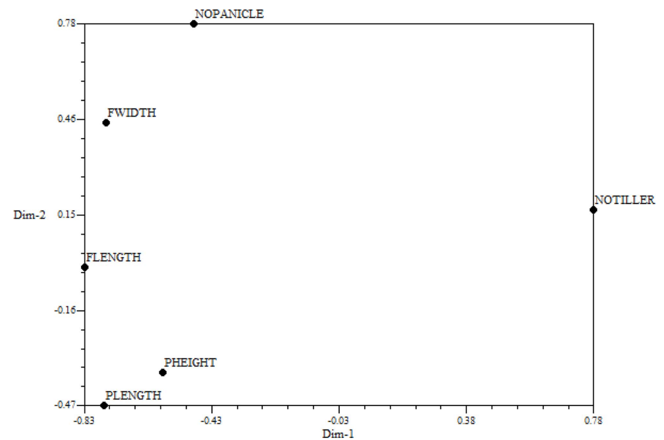

Fig. 6. Principal component analysis of the observed goosegrass morphological characters. (NOPANICLE: number of panicles; FWIDTH: flag leaf width; FLENGTH: flag leaf length; PHEIGHT: plant height; PLENGTH: average panicle length; NOTILLER: number of tillers).

we found out that yellow and light brown seeds failed to germinate. The non-viable seeds might be naturally infertile and carried no embryo (empty seeds). Seedling emergence was relatively quick for all groups with days ranging from 1.0 to 1.9. Group II was the quickest to develop inflorescence (68.2 days) followed by Group I (78.8 days) and Group III (82.8 days).

The first principal components (PC1) made up 50.88% of the total variation with the highest contributions from the flag leaf length, number of tillers, panicle length and flag leaf width (Table 9). Meanwhile the second principal components (PC2) and third principal components (PC3) explained 19.95% and 13.11%, respectively, of the total variation. These showed that the main morphological traits for grouping of goosegrass ecotypes were the number of tillers, flag leaf length, flag leaf width and panicle length. Number of goosegrass tillers was the major outlier judging by the distance of it to the other parameters (Fig. 6). Ecotypes that were clustered together have somewhat similar features of these 4 traits.

Genotype and phenotype relationship

2-way Mantel test was done using standardized CORR matrices. Genetic and morphological distances were not significantly correlated ($r = -0.1706$; $p = 0.1132$) (Fig. 7). Even though both markers indicated the changes on goosegrass ecotypes, we could not justify the connection between the genotypes and their phenotypes. Molecular markers are not affected by environmental conditions unlike morphological markers. Epistasis, the interaction between genes with one gene expression is affected by other independently inherited gene(s) expression, is often observed between morphological marker loci and the genetic background and prevents characterization of genotypes associated with morphological

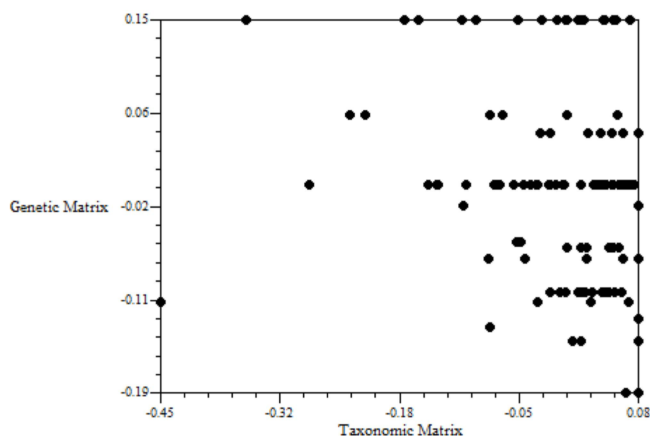

Fig. 7. Scatter plot of goosegrass ecotypes correlating their genetic diversity and morphological variations.

markers. Quantitative morphological markers are more affected by environment than molecular markers, therefore morphologically similar plants may contain different gene pools.

Discussion

K1, K2, B2, M1 and S2 in Group I were found to be closely related. M1 was sampled from a vegetable farm in Malacca and known to have developed twofold resistance to herbicide ammonium-glufosinate based on the previous research by Adam et al. (2010). Likewise, K2, B2 and J1 were collected from oil palm plantations which were heavily applied with glyphosate and ammonium-glufosinate for weed control. Detection of genes involved in herbicide resistance by SRAP markers may have influenced grouping of these ecotypes.

All ten ecotypes from Sabah were divided into the six groups. Since genetic differentiation in resistance gene might have affected the groupings, we supposed to possess variations of alleles that are suited for the management practices or they had different genetic background than when they were first introduced in Sabah. Other possible reason of this high genetic variation among all ecotypes is due to the gene flow between populations of goosegrass in different areas. Dissemination of tiny goosegrass seeds over a vast area is facilitated by wind. Viability of seed is longer than of pollen making dispersal of goosegrass even more thriving. Moving equipments and human activities in and out of the fields lead to high likelihood of seed escape as well (Kausch et al., 2010). Wang et al. (2011) suggested three other possible reasons: genetic overlap between goosegrass ecotypes, exchange of germplasm resources and ploidy. Goosegrass is of six diploid (2n = 16, 18 and 20) and three tetraploid (2n = 36 or 38) taxa (Phillips, 1995).

In this study, low number of bands per primer combination was detected which is fairly similar to that of its family *Eleusine coracana* using RAPD analysis (2 to 9 bands/primer) as tested by Das et al. (2009). The SRAP random primers target the open reading frame regions of genome to detect mutations arising from variations in introns, promoters and spacers. Ferriol et al. (2003) reported that the information acquired from SRAP markers was more concordant with the morphological variations. Therefore, despite the number of DNA fragments obtained was small, SRAP markers have the potential to strengthen the link between DNA polymorphisms and morphological traits. Scores of genetic loci can be accurately screened with 6% denaturing acrylamide gel but due to several constrains, it cannot be done in our lab. Li et al. (2014) suggested the usage of 2-2.5% agarose gels as an alternative to resolve multiple DNA fragments of sizes varying from 100 bp up to 1.5 kb. Amplified DNA products resolved on 1.5-2% agarose gels gave the average of 3.64 bands per RAPD primer, 5.30 and 5.60 bands per SRAP primer combinations on elephantgrass (Passos et al., 2005), banana (Pinar et al., 2015) and *Butea monosperma* (Vashishta et al., 2013), a little bit higher than our result (2.60 bands/primer pairs). Nevertheless, SRAP is a useful tool for characterization and genetic diversity purposes as it had detected high polymorphism in goosegrass accessions. SRAP targets the open reading frame regions of genome with unanimous primers to reveal mutations across whole genome. Genetic heterogeneity instigates varied response to herbicides thus it is vital to study plant diversity at genetic level prior to developing potential biocontrol agent particularly genes that confer herbicide resistance and their expressions.

Goosegrass congregated in Group I seemed to share the habitual traits of herbicide-resistant strain with tall growth and late heading. Muona et al. (1984) reported that resistant barley differed from the susceptible ones in heading date and plant height where the resistant type was taller and later than the susceptible families. The study showed goosegrass ecotypes with prolonged exposure to glyphosate and ammonium-glufosinate were grouped together in Group I by our separation methodology. This could have impelled some changes in the phenotypic traits of goosegrass in Group I that differentiated them from the other 2 groups. Herbicide-resistant late watergrass *Echinochloa phyllopogon* was reported to be shorter, had narrower, shorter flag leaves, thinner culms, smaller and slender spikelets compared to the susceptible biotype (Tsuji et al., 2003). With the exception of the plant height and unstudied characters, goosegrass ecotypes in Group I had similar narrow and short flag leaves similar to traits shown in the resistant late watergrass.

Findings in this study have shown that goosegrass ecotypes exhibited significant difference in heading date, flag leaf length and panicle characters but comparable in other traits. Dinitroaniline-resistant and dinitroaniline-susceptible goosegrass populations were observed to be similar in growth

and development except for the inflorescence dry weight (Murphy et al., 1986). Underlying basis for robustness of herbicide-resistant goosegrass and its association with plant maturity may be a great help in developing potential biocontrol agent.

A weak insignificant correlation between the molecular and morphological distances of goosegrass (Fig. 11) follows similar findings in studies of other plants; a tropical tree species *Paramichelia baillonii* (Li et al., 2008), barley (Lund, 2002) and potato (Vetelainen et al., 2005). Molecular markers might have detected variations in parts of the genome that do not appear to influence the phenotype. Genes revealed by the molecular markers are not necessarily expressed nor do they reflect the diversity in functional characters. Thus both molecular and morphological assays are equally essential in diversity study. Three other possible reasons that could have led to non-significant relationship between both markers are phenotypic plasticity, local adaptation and neutral polymorphism (Duminil and Di Michele, 2009). Plants are able to change their phenotypes in response to changes in environment, be it biotic or abiotic factors. Different temperature, humidity, light intensity, water availability, and soil conditions in the glasshouse as compared to their native habitats could have evoked the plasticity. Local adaptation led to evolution of intraspecific plants following selective pressure. Genetic changes as a result of natural selection allow individuals to have higher relative fitness in their natural territory than non-native individuals of the same species. Last but not least, neutral polymorphism is a variation in DNA sequences (mutation of an initial codon that does not affect it to become codon of different amino acid) that do not have any phenotypic effect. Morphologically similar ecotypes could arise from diverse genotypes as a result of this polymorphism, resulting in an insignificant relationship between the molecular and morphological markers.

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