Malays. Appl. Biol. (2011) 40(2): 27–36

GENETIC DIVERSITY OF GLYPHOSATE-RESISTANT AND GLYPHOSATE-SUSCEPTIBLE *Eleusine indica* (L.) Gaertn (POACEAE) POPULATIONS FROM PENINSULAR MALAYSIA

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ABSTRACT

Genetic diversity within and among six glyphosate-resistant (R) and eight glyphosate-susceptible (S) *Eleusine indica* populations was determined using isozyme markers. Genetic variations at 13 enzyme loci from 8 enzyme systems were determined in a total of 840 accessions. Mean percentage of polymorphic loci (P = 23.08 %), mean number of alleles per locus (A = 1.2) and effective number of alleles per locus (A_e = 1.1) were similar for both R and S populations. Levels of expected heterozygosity (H_e) were also low and not significantly different (P> 0.10) between the R (H_e = 0.067) and S (H_e = 0.069) biotypes but levels of observed heterozygosity (H_o) were significantly lower (P< 0.10) in the S populations (H_o = 0.003) than in the R populations (H_o = 0.014). However, the overall degree of genetic differentiation for the 14 populations was high (F_{ST} = 0.53), indicating high genetic divergence among the populations surveyed which was mainly contributed by the S populations (F_{ST} = 0.622). The total gene flow was low (N_m = 0.225) with mean genetic distance of 0.046, which is consistent with high F_{ST} values. UPGMA clustering analysis revealed two main clusters: cluster I consisting of the S populations from Kuala Selangor, and Sungai Tangkas, Selangor; Jasin, Melaka; Pulau Tikus, Pulau Pinang; Bidor, Perak and Chaah, Johor while cluster II consists of all the R populations and the S populations from Temerloh, Pahang and Lenggeng, Negeri Sembilan.

ABSTRAK

Kepelbagaian genetik di dalam dan antara enam populasi rintang-glifosat (R) dan lapan populasi rentan-glifosat (S) telah ditentukan menggunakan penanda isozim. Variasi genetik pada 13 lokus enzim daripada 8 sistem enzim ditentukan dalam sejumlah 840 aksesi. Purata peratus lokus polimorfik (P = 23.08 %), purata alel setiap lokus (A = 1.2) dan bilangan alel berkesan setiap lokus (A_e = 1.1) adalah agak serupa untuk kedua-dua populasi R dan S. Keheterozigotan dijangka (H_e) juga rendah dan tidak berbeza secara signifikan (P> 0.10) antara biotip R (H_e = 0.067) dan S (H_e = 0.069) tetapi keheterozigotan dicerap (H_o) rendah secara signifikan (P< 0.10) dalam populasi S (H_o = 0.003) berbanding populasi R (H_o =0.014). Namun, perbezaan genetik keseluruhan untuk 14 populasi adalah tinggi (F_{ST} = 0.53), menunjukkan kepelbagaian genetik tinggi antara populasi yang dikaji yang sebahagian besarnya disumbang oleh populasi S (F_{ST} = 0.622). Jumlah aliran gen adalah rendah (N_m = 0.225) dengan purata jarak genetik 0.046, konsisten dengan nilai F_{ST} yang tinggi. Analisis UPGMA menunjukkan dua kumpulan utama: kumpulan I terdiri daripada semua populasi S dari Kuala Selangor dan Sungai Tangkas, Selangor; Jasin, Melaka; Pulau Tikus, Pulau Pinang; Bidor, Perak dan Chaah, Johor manakala kumpulan II mewakili semua populasi R dan populasi S Temerloh, Pahang dan Lenggeng, Negeri Sembilan.

Key words: genetic diversity, glyphosate, resistance, *Eleusine indica,* isozyme markers

INTRODUCTION

Eleusine indica (L.) Gaertn or more commonly known as goosegrass is an annual, self-pollinating cosmopolitan diploid grass of the Poaceae (Gramineae) family (Barnes & Chan 1990). In Malaysia, it is one of the most serious weeds in vegetable farms, orchards, oil palm and rubber plantations, as well as in wasteland and along roadsides (Holm *et al*., 1977).

Compounding this problem is the fact that the species has demonstrated the capacity to evolve

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resistance against several herbicides (Doll 1999). In Malaysia, *E. indica* biotypes that are confirmed to be resistant to glyphosate were first reported in 1997 (Lim & Ngim 2000). After glyphosate [N-(phosphonomethyl)glycine] was introduced as a commercial herbicide in 1974 (Duke & Powles 2008), it has been used widely throughout this region during the past 8 – 10 years, resulting in intense selection pressure and the potential for evolution of glyphosate resistance. The resistant populations evolved after an estimated 10 years of selection pressure attributable to repeated applications of glyphosate, and demonstrated a 2- to 4-fold resistance as compared to the sensitive biotypes (Teng & Teo 1999).

Control efforts of the R biotype of *E. indica* have focused on eradication with limited success as the main herbicide strategy practiced by farmers and growers was to include application of herbicides utilizing other modes of action and with soil-residual activity together with glyphosate (Beckie 2011). To date, there have been no investigations on the levels of genetic variation within and among the different glyphosate-resistant (R) and glyphosate-susceptible (S) populations of *E. indica*. A recent analysis of the variation among *Eleusine* species using isozyme markers (Werth *et al.,* 1994) suggested substantial genetic variability among the diploid *Eleusine* species. However the study was oriented towards understanding the origin of finger millet (*E. coracana*) and differentiation among the *Eleusine* species. Thus, this study aims to analyze the genetic diversity within and among different populations of glyphosate-resistant and glyphosate-susceptible populations of *E. indica* from Peninsular Malaysia using isozyme markers and to determine if the populations originated from genetically different source populations.

MATERIALS AND METHODS

Plant materials

Mature *E. indica* seeds were collected from areas where populations of glyphosate-resistant and glyphosate-susceptible *E. indica* were reported in Peninsular Malaysia namely Bidor, Perak; Chaah, Johor; Lenggeng, Negeri Sembilan; Temerloh, Pahang; Kuala Selangor, Selangor and Jasin, Melaka (Figure 1). In addition, seeds were also collected from two other susceptible populations namely Sungai Tangkas, Selangor and Pulau Tikus, Pulau Pinang. Screening of the R and S biotypes were conducted by applying glyphosate at the

Fig. 1. Map indicating the *E. indica* sampling sites.

recommended dosage of 1.08 kg ae/ha on the seedlings. For R biotypes, seedlings that survived were allowed to reach maturity and inflorescences of each mature individual were collected and kept separately. A number of 20 families each from Bidor, Chaah, Lenggeng and Temerloh were used for this study. From each family, 1 or 2 seedlings were randomly selected as representatives making a total of 30 samples taken from each respective population and biotype. As for the Kuala Selangor, Jasin, Sungai Tangkas and Pulau Tikus populations, a total of 35 families were used for germinating where 30 samples were randomly picked to represent 30 families from each population and biotype (Table 1). After one week, the seedlings were transferred to black polybags (measuring 8 cm across) containing the Right Grow ® commercial potting mix (distributed by Kosas Profile ® Sdn. Bhd.) and grown in the greenhouse at 29 ± 4ºC, with the light intensity of 800 μE m^{-2} s^{-1} and a 12-hour photoperiod. The plants were watered twice daily by an automated sprinkler system.

Enzyme extraction

At two months of age, young leaves were harvested for enzyme extraction. Crude extracts for the electrophoresis were prepared from fresh young leaf samples with a ratio of 1:1 of leaf weight to the leaf extraction buffer (Wickneswari & Norwati 1991). Leaf samples were excised and ground into powder in liquid nitrogen before the extraction buffer was added. Extracts were absorbed into paper wicks and kept in -20ºC freezer until further use. For this study, 14 populations were tested namely Bidor R and S, Chaah R and S, Lenggeng R and S, Temerloh R and S, Kuala Selangor R and S, Jasin R and S, Sungai Tangkas S and Pulau Tikus S.

Table 1. Number of families and samples per *E. indica* population used

Region	Biotype	Number of families	Number of samples
Bidor	Susceptible	20	30
	Resistant	20	30
Chaah	Susceptible	20	30
	Resistant	20	30
Lenggeng	Susceptible	20	30
	Resistant	20	30
Temerloh	Susceptible	20	30
	Resistant	20	30
Kuala	Susceptible	35	30
Selangor	Resistant	35	30
Melaka	Susceptible	35	30
	Resistant	35	30
Sungai Tangkas	Susceptible	35	30
Pulau Pinang	Susceptible	35	30
TOTAL		370	420

Electrophoresis and staining for enzyme systems

11% starch gel was used in the electrophoresis. After electrophoresis, the gel was sliced horizontally and assayed for 25 enzymes. Four electrophoretic buffer systems were used namely lithium-hydroxide (Selander *et al.,* 1971), morpholine-citrate system pH 8.2 (Wickneswari & Norwati 1991), histidine-citrate (Wickneswari & Norwati 1991), and discontinous tris-citrate pH 8.6 (Wickneswari & Norwati 1991). The enzyme staining methods were modified from Wickneswari and Norwati (1991) and Soltis *et al.* (1983).

Data analysis

Scoring of putative loci was carried out on the basis of the observed profiles and of earlier literature (Werth *et al.,* 1994). For enzymes with more than one putative locus, the staining zones were labeled alphabetically starting from the anodal end. A maximum of two isozymes were detected at each locus; with loci labeled alphabetically consecutively beginning with the most anodal form. Due to the lack of consistent activity, only 8 enzyme systems were used for this study namely glutamate dehydrogenase (*Gdh*), glucose-6-phosphate isomerase (*Pgi* or *Gpi*), phosphoglucomutase (*Pgm*), acid phosphatase (*Acp*), isocitrate dehydrogenase (*Idh*), glycerate dehydrogenase (*Gly*), uridine diphosphogluconate pyrophosphate (*Ugp*) and malate dehydrogenase (*Mdh*). Full-sib progeny arrays were used to infer the genetic basis of complex banding patterns such as *Mdh* and *Acp*.

To assess genetic diversity within populations, the following parameters were used, calculated from the frequencies of alleles and genotypes namely the proportion of polymorphic loci (P), the average number of alleles per locus (A); mean effective number of alleles (A_e), the average observed heterozygosity (H_o) and the average expected heterozygosity (H_e) where H_e is an unbiased estimate (Nei 1978). Genetic differentiation among populations was estimated using the gene diversity (G_{ST}) of Nei (1973) and the unbiased genetic distance (D) of Nei (1978). The amount of gene flow among populations (N_m) was estimated by using the method of Slatkin (1985), based on private alleles with an adjustment for sample size. Total genetic diversity was partitioned within and among populations to access genetic differentiation. The data were analyzed using the computer programme POPGENE version 1.32 (Yeh & Boyle 1999) and BIOSYS-2 (Swofford & Selander 1997). Parameters of genetic diversity estimated using POPGENE included allelic frequencies, mean number of alleles per locus (A_e) which was calculated according to Crow & Kimura (1970), percentage of polymorphic loci (0.99 criterion) (P), expected (H_e) and observed (H_o) proportion of heterozygosities, genetic

differentiation (F_{st}) and fixation indices (F_{is}) and their variances. BIOSYS-2 was used to test for deviation from Hardy-Weinberg equilibrium and to calculate genetic distance (Nei 1978) and also to visualize the relationships among the populations through the construction of a dendrogram by the unweighted pair-group method with arithmetic average (UPGMA; Sneath & Sokal 1973).

RESULTS AND DISCUSSION

Genetic diversity

Preliminary results of electrophoresis showed that only three buffer systems were essential for the study of enzyme systems namely the lithium-hydroxide, morpholine-citrate and discontinous tris-citrate. From a total of 25 enzymes tested, only 14 at the initial stage showed activity in the gels. These enzymes were *Aat*, *Est*, *Pgi* and *Per* for the lithium-hydroxide system; *Acp*, *Ald*, *Idh*, *Mdh* and *Skdh* for the morpholine-citrate and *Gdh*, *Gly*, *Pgm*, *Tpi* and *Ugp* for the discontinous tris-citrate system. This was consistent with the findings of Werth *et al.* (1994) who reported that the enzymes *Acp*, *Ald*, *Aat*, *Idh*, *Mdh*, *Pgi*, *Pgm*, *Skdh* and *Tpi* were suitable for use in his study on the origin of finger millet (*E. coracana*).

However as further testing was conducted, it was found that only *Acp*, *Gdh*, *Gly*, *Pgi*, *Idh*, *Mdh*, *Pgm* and *Ugp* could yield clear and consistent banding patterns. As for *Aat*, *Ald*, *Skdh*, *Est*, *Per* and *Tpi*, these enzymes showed faint banding patterns which were difficult to document and score.

All other enzyme systems namely *Gdh*, *Gly*, *Pgi*, *Idh*, *Ugp* and *Pgm* produced banding phenotypes that were consistent with homozygous loci. As for *Mdh*, five clear different banding patterns were exhibited among the samples assayed. *Acp* exhibited two different banding patterns, one for the resistant and the other for the susceptible samples.

To facilitate scoring of genotypes for estimation of genetic diversity parameters, full sib progeny array analysis was conducted and used to determine the genetics of loci of the eight enzyme systems.

Allele frequencies

A total of 8 enzyme systems were assayed, in which 13 loci were detected, which included *Gdh-1*, *Gly-1*, *Ugp-1*, *Acp-1*, *Pgi-1*, *Pgi-2*, *Idh-1*, *Idh-2*, *Idh-3*, *Pgm-1*, *Pgm-2*, *Mdh-1* and *Mdh-2* (Table 2). From these, a total of three loci were found to be polymorphic namely *Acp-1*, *Mdh-1* and *Mdh-2*.

This lack of diversity may be a reflection that over such an extensive geographic range, a general-purpose variant is adaptable in many ecosystems, until the herbicide selection pressure is exerted over time. For *Mdh-1*, the most frequent allele was the *b* allele while the most frequent allele for *Mdh-2* differed from population to population. As for *Acp-1*, *a* allele was most frequently found in susceptible populations. Instead the *b* allele was more commonly found in resistant populations.

According to Ng *et al.* (2004), banding patterns of *Acp-1* can be used to identify glyphosate R and S *E. indica* plants as the *a* allele indicates susceptibility while the *b* allele shows resistance. This indicated that both resistant and susceptible

Table 2. Allele frequencies in the 14 *E. indica* populations surveyed from Peninsular Malaysia

Locus	Allele	Bidor		Chaah		Lenggeng		Temerloh		Kuala Selangor		Jasin		Sungai Tangkas	Pulau Tikus
		S	R	S	R	S	R	S	R	S	R	S	R	S	S
Gly-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gdh-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ugp-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-3	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Acp-1	A	0.967	0.033	0.633	0.467	0.400	0.433	0.833	0.133	0.933	0.167	0.967	0.133	0.967	0.967
	B	0.033	0.967	0.367	0.533	0.600	0.567	0.167	0.867	0.067	0.833	0.033	0.867	0.033	0.033
Mdh-1	A	–	–	–	–	0.100	0.033	0.333	–	–	–	–	–	–	–
	B	1.000	1.000	1.000	1.000	0.900	0.967	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	A	0.033	0.133	0.050	0.350	0.450	0.317	0.967	0.767	–	0.733	–	0.850	–	–
	B	0.967	0.867	0.950	0.650	0.550	0.683	0.033	0.233	1.000	0.267	1.000	0.150	1.000	1.000

S= susceptible population, R= resistant population. *Gly*: glycerate dehydrogenase; *Gdh:* glutamate dehydrogenase; *Ugp:* uridine diphosphogluconate pyrophosphate; *Pgi:* glucose-6-phosphate isomerase; *Pgm*: phosphoglucomutase; *Idh*: isocitrate dehydrogenase; *Acp*: acid phosphatase; *Mdh*: malate dehydrogenase

plants were present in all populations surveyed irrespective of whether the population was putatively resistant or susceptible to glyphosate. These results suggest that the frequency of the glyphosate-resistant biotype present within the weed populations might not be so low even before the herbicide selection pressure was applied (Prather *et al.,* 2000).

Allele frequencies by biotype was found to be similar for both the R and S populations with the mean percentage of polymorphic loci, P being 23.08%, mean number of alleles per locus, A of 1.2 and effective number of alleles per locus $A_{e,}$ was 1.1. Across the populations (Table 3), the mean number of allele per locus (A) was found to be small (1.2), while the mean effective number of alleles (A_e) was 1.1. The range of percentage of polymorphic loci (P) at 0.95 criterion was from 7.69 to 23.08% with a mean of 14.83%. The low genetic variation within populations is consistent with the autogamous reproduction in *E. indica*.

Heterozygosity

The heterozygosity measures per biotype showed that low levels of expected heterozygosity (H_e) and no significant difference ($P > 0.05$) between the R (H_e=0.067) and S (H_e= 0.050) biotypes but the levels of observed heterozygosity (H_o) was found to be significantly lower ($P<0.05$) in the S populations (H_o=0.003) than in R populations (H_o=0.014).

Additionally, analysis of genetic variation revealed a substantial heterozygosity deficiency in all analysed populations (Table 4). Genetic diversity in the *E. indica* populations examined (H_e= 0.0387) was less than the mean values reported for monocot species (H_e= 0.144; Hamrick & Godt 1989) and endemic species (H_e= 0.063; Hamrick & Godt 1991). Overall, very low genetic diversity was observed in the *E. indica* populations surveyed.

The mating system is one of the most important factors affecting both the level and contribution of genetic variation in plant species (Loveless & Hamrick 1984; Hamrick & Godt 1991). In general, predominantly autogamous species are characterized by substantially greater genetic differences among populations, and reduced genetic variability within populations (Hamrick & Godt 1991). However, the existence of higher levels of observed heterozygosity in the resistant biotype suggests that establishment of resistant populations is due to multiple founding events rather than to the spread of a single resistant allele.

Fixation indices

In divided populations, the genetic structure can be analyzed by F-statistics (Wright 1965). In a population, the degree of confirmatory to the Hardy-Weinberg equilibrium (HWE) throws light on evolutionary processes within that population.

Fixation index, F_{is} is the deviation of genotypic frequencies from HWE within a single population and it ranges from -1 (an excess of heterozygotes) to 1 (a deficiency of heterozygotes). As genotype frequencies in natural populations that are divided into sub-populations might deviate from HWE due to natural selection or in the case of small populations, random genetic drift; thus heterozygote genotype frequencies decrease while the

Table 3. Allele frequencies in the susceptible and resistant populations of *E. indica*

Region	Biotype	Mean number of alleles per locus (A)	Effective number of alleles per locus (A_e)	Mean percentage of polymorphic loci (P)
Bidor	Suceptible	1.2	1.0	15.38
	Resistant	1.2	1.0	15.38
Chaah	Susceptible	1.2	1.1	15.38
	Resistant	1.2	1.1	15.38
Lenggeng	Susceptible	1.2	1.2	23.08
	Resistant	1.2	1.1	23.08
Temerloh	Susceptible	1.2	1.1	23.08
	Resistant	1.2	1.1	15.38
Kuala	Susceptible	1.1	1.0	7.69
Selangor	Resistant	1.2	1.1	15.38
Jasin	Susceptible	1.1	1.0	7.69
	Resistant	1.2	1.0	15.38
Sungai Tangkas	Susceptible	1.1	1.0	7.69
Pulau Tikus	Susceptible	1.1	1.0	7.69
MEAN		1.2	1.1	14.83

Table 4. Observed (H_o) and expected (H_e) heterozygosity and fixation index (F_{is}) for all *E. indica* populations studied (standard errors in parentheses)

Region	Biotype	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Fixation index (F_{is})
Bidor	Susceptible	0.005 (0.019)	0.010 (0.025)	0.483
	Resistant	0.010 (0.037)	0.023 (0.066)	0.549
Chaah	Susceptible	0.008 (0.028)	0.044 (0.132)	0.821
	Resistant	0.028 (0.102)	0.075 (0.182)	0.615
Lenggeng	Susceptible	0.008 (0.028)	0.090 (0.187)	0.913
	Resistant	0.013 (0.046)	0.077 (0.176)	0.831
Temerloh	Susceptible	0.005 (0.019)	0.062 (0.141)	0.915
	Resistant	0.005 (0.019)	0.046 (0.116)	0.887
Kuala Selangor	Susceptible	0 (0.000)	0.010 (0.035)	1.000
	Resistant	0.010 (0.037)	0.052 (0.130)	0.800
Jasin	Susceptible	0 (0.000)	0.005 (0.018)	1.000
	Resistant	0.018 (0.065)	0.038 (0.093)	0.520
Sungai Tangkas	Susceptible	0 (0.000)	0.005 (0.005)	1.000
Pulau Tikus	Susceptible	0 (0.000)	0.005 (0.005)	1.000
MEAN		0.007	0.0387	0.810

Table 5. Summary of F-statistics and gene flow at all polymorphic loci

Loci	Sample Size	F_{is}	F_{it}	F_{st}	N_m*
Acp-1	840	1.0	1.0	0.499	0.251
Mdh-1	840	1.0	1.0	0.237	0.807
Mdh-2	840	0.496	0.769	0.543	0.211
MEAN	840	0.793	0.897	0.501	0.249

homozygous genotype frequency of the whole population is more than HWE ratios (Wahlund 1928, cited by Hedrick 2005).

In this study, F_{is} values were in the range from 0.496 to 1.0 with a mean of 0.793. The lowest F_{is} value was from *Mdh-2* while the other two loci, *Acp-1* and *Mdh-1* had a F_{is} value of 1.00 respectively (Table 5). All the populations surveyed had a positive value of F_{is}, indicating an excess of homozygotes, strongly suggesting that the considered species are in effect strictly autogamous or which may have been caused by small populations sizes, or inbreeding.

Population genetics theory predicts that, as a consequence of genetic drift and inbreeding, small populations will have decreased levels of genetic variation (Van Treuren *et al.,* 1991). Fragmentation isolating previously connecting populations could also have deleterious effects by disrupting gene flow among populations and could exacerbate loss of diversity through drift and further increase inbreeding in the remaining fragments (Templeton *et al*., 1990; Young *et al*., 1996).

Genetic differentiation among populations

Among the *Eleusine indica* populations surveyed, overall degree of genetic differentiation was 0.501 (Table 5). This indicates that there was high divergence among the populations. The partitioning of genetic diversity per biotype showed that the F_{st} values among the R populations were low (0.230) while the S populations had a high F_{st} value (0.433), which contributed to the high genetic differentiation among the 14 populations. Between loci, the F_{st} values were significantly different, ranging from 0.237 for *Mdh-1* to 0.543 for *Mdh-2*, as shown in Table 5.

According to Loveless and Hamrick (1994), genetic differentiation among populations is a function of gene flow among populations by pollen and seed dispersal. Isolated populations will experience less gene flow than contiguous or continuously distributed populations. Low levels of gene flow between populations will therefore be consistent with higher levels of genetic differentiation among populations.

For the populations surveyed, it was found that the total gene flow across the populations was low at N_m of 0.249 (Table 5). The consequences of gene flow and genetic bottlenecks on the genetic structures of populations include possible results such as decreased fitness through outbreeding or

inbreeding depression and reduction of local variation. The eventual outcome of this process will be homogenization of allele frequencies and a severe reduction in genetic diversity among populations.

As indicated earlier, populations of predominantly autogamous species will tend to be genetically uniform but highly differentiated from one another, whereas population differentiation in allogamous species is less apparent and most of the variation characteristic of the species is found in a given population (Warwick & Black 1993). As a result, the patterns of genetic variability predicted after founder events, or the genetic bottlenecks which are likely to have occurred with the selection of resistant genotypes and subsequent build-up of resistant populations are likely to be more severe in autogamous weedy species (Darmency & Gasquez 1990).

Genetic distance

Genetic distance among populations was very low and the mean genetic distance for the 14 *E. indica* populations surveyed was 0.046, with values ranging from 0.000 to 0.122 (Table 6). The lowest genetic distance value, 0.00 was observed was between fourteen pairs of populations.

The highest value of genetic distance was instead observed between Temerloh S and Bidor R. It is apparent that genetic distance between many pairs of *E. indica* populations did not correlate with the geography of the populations (Figure 1). It is possible that human activities transported seeds from one region to another, in addition to other genetic diversity influencing factors such as founder events, genetic drift and others.

The UPGMA dendrogram in Figure 2 shows how the populations are grouped on the basis of Nei's (1978) genetic distance. There are two main clusters; cluster I consisting of only S populations from Bidor, Chaah, Jasin, Sungai Tangkas, Pulau Tikus and Kuala Selangor while cluster II has Temerloh S, Lenggeng S and all the R populations.

CONCLUSION

As for genetic diversity among and within different populations of glyphosate-resistant and glyphosate-susceptible populations of *E. indica*, overall levels of isozyme diversity observed in R and S populations of *E. indica* were quite low and F_{is} values showed large heterozygote deficiencies. Isozyme variation between populations contributed the major proportion of total diversity of the variable detected isozymes, while the contribution of within-population variation was negligible. The low levels of within-population diversity match the predominantly selfing strategy of the populations examined.

The high levels of genetic divergence among populations suggest that these isolated populations have been isolated long enough to have caused population differentiation. Theoretically, there should have been an adequate amount of time for independent genetic mutations to occur and accumulate, with subsequent genetic drift in small populations resulting in fixation of unique alleles.

Table 6. Estimates of mean genetic distance of Nei (1978) between 14 populations of *E. indica*

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Bidor S	***													
2. Bidor R	.071	***												
3. Chaah S	.008	.029	***											
4. Chaah R	.027	.018	.008	***										
5. Lenggeng S	.024	.034	.013	.002	***									
6. Lenggeng R	.028	.015	.008	.000	.003	***								
7. Temerloh S	.082	.122	.083	.052	.030	.056	***							
8. Temerloh R	.102	.033	.063	.022	.026	.023	.052	***						
9. Kuala Selangor S	.000	.067	.007	.026	.025	.027	.087	.102	***					
10. Kuala Selangor R	.093	.030	.056	.018	.022	.019	.050	.000	.093	***				
11. Jasin S	.000	.072	.008	.029	.027	.030	.087	.106	.000	.097	***			
12. Jasin R	.113	.042	.073	.029	.031	.030	.050	.000	.113	.000	.118	***		
13. Sungai Tangkas S	.000	.072	.008	.029	.027	.030	.087	.106	.000	.097	.000	.118	***	
14. Pulau Tikus S	.000	.072	.008	.029	.027	.030	.087	.106	.000	.097	.000	.118	.000	***

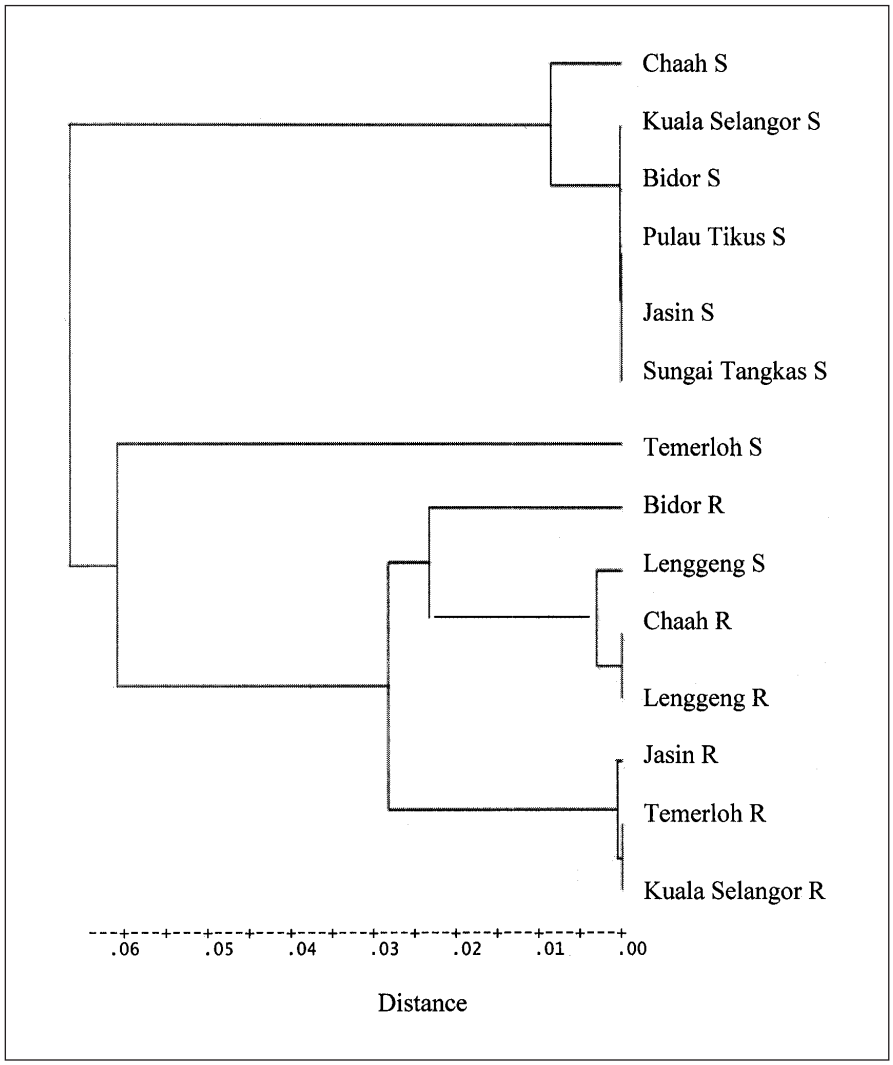

Fig. 2. Dendrogram based on UPGMA clustering of *E. indica* populations using Nei's (1978) genetic distance.

The small percentage of polymorphic loci and number of alleles per locus suggest that the populations have a history of significantly severe or long-lasting population bottlenecks that have eroded genetic diversity. For resistant populations, this would be consistent with the selection pressure exerted by herbicide use over time. But as the populations are genetically depauperate, there is a possibility that the source populations were also genetically homogeneous.

The genetic structure of *E. indica* populations appears to slightly differ with biotype. Thus, the number of years since resistance has appeared in a weed population and whether or not glyphosate has been used regularly after the resistance appeared could also affect the levels of genetic polymorphism in resistant populations. In addition, genetic diversity and distance derived from isozyme analyses were very low and, due to the small number of polymorphic alleles, any small difference had a strong influence on the distribution and grouping of accessions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Monsanto Grant D/8/2000 and the Malaysian Ministry of Science, Technology and Innovation (MOSTI) for the National Science Fellowship scholarship awarded to the first author.

REFERENCES

Barnes, D.E. & Chan, L.G. 1990. *Common Weeds of Malaysia and Their Control*. Kuala Lumpur: Ancom Berhad. 349 pp.

Beckie, H.J. 2011. Herbicide-resistant weed management: focus on glyphosate. *Pest Management Science* **67:** 1037–1048.

Crow, J.F. & Kimura, M. 1970. *An Introduction to Population Genetic Theory*. New York: Harper and Row. 656 pp.

Darmency, H. & Gasquez, J. 1990. Appearance and spread of triazine resistance in common lambsquarters (*Chenopodium album*). *Weed Technology* **4:** 173–177.

Doll, J. 1999. Glyphosate Resistance Updates: Glyphosate Resistance in Another Plant. http://www.tricity.wsu.edu/aenews/DecOOAENews/DecOOAENews.htm#anchor176277

Duke, S.O. & Powles, S.B. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Management Science* **64:** 319–325.

Hamrick, J.L. & Godt, M.J.W. 1989. Allozyme diversity in plants. In Brown, A.H.D., Clegg, M.T., Kahler, A.L. & Weir, B.S. (eds.). *Plant Population Genetics, Breeding and Genetic Resources*, pp 43-63. Sinderland: Sinauer Associates.

Hamrick, J.L., Gom, M.J.W., Murawski, D.A. & Loveless, M.D. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In Falk, D.A. & Holsinger, K.E. (eds.). *Genetics and Conservation of Rare Plants*, pp 75-86. New York: Oxford University Press.

Hedrick, P.W. 2005. *Genetic of Populations*. 3rd Ed. Sudbury: Jones and Bartlett Publishers. 737 pp.

Holm, L.G., Plucknett, D.L., Pancho, J.V. & Herberger, J.P. 1977. The weeds. In *The World's Worst Weeds: Distribution and Biology*, pp 47-53. Honolulu: The University Press of Hawaii.

Lim, J.L. & Ngim, J. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L.) Gaertn) in Malaysia. *Pest Management Science* **56:** 336–339.

Loveless, M.D. & Hamrick, J.L. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review Ecology and Systematics* **15:** 65–96.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* **70(12):** 3321–3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583–590.

Ng, C.H., Wickneswari, R., Salmijah, S. & Ismail, B.S. 2004. Inheritance of glyphosate resistance in goosegrass (*Eleusine indica*). *Weed Science* **52:** 564–570.

Prather, T.S., Ditomaso, J.M. & Holt, J.S. 2000. Herbicide Resistance: Definition and Management Strategies. *University of California Division of Agriculture and Natural Resources Publication* **8012:** 1–14.

Selander, R.K., Smith, M.J., Yang, S.Y., Johnson, W.E. & Gentry, J.B. 1971. Polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Studies in Genetics VI. *University of Texas Publications* **7103:** 49–90.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution* **39:** 53–65.

Sneath, P.H.A. & Sokal, R.R. 1973. *Numeral Taxonomy*. San Francisco: Freeman. 573 pp.

Soltis, D.E., Haufler, C.H., Darrow, D.C. & Gastony, G.J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* **73:** 9–27.

Swofford, P.L. & Selander, R.B. 1997. *BIOSYS-2: A computer program for the analysis of allelic variation in population genetic and biochemical systematics*. Release 1.7. Users' Manual. Illinois Natural History Survey, IL, USA.

Templeton, A.R., Shaw, K., Routman, E. & Davis, S.K. 1990. The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanic Garden* **77:** 13–27.

Teng, Y.T. & Teo, K.C. 1999. Weed control and management of resistant goosegrass (*Eleusine indica*) in Malaysia. *Proceedings of the 17th Asian-Pacific Weed Science Society Conference* **I (B):** 753–758.

Van Treuren, R., Bijlsma, R., Van Delden, W. & Ouberg, N.J. 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* **66:** 181–189.

Wahlund, S. 1928. Zusammensetzung von populationen und korrelationserscheinungen von stanpunkt der vererbungslehre aus betrachtet. *Heriditas* **11:** 65–106.

Warwick, S.I. & Black, L.D. 1993. Electrophoretic variation in triazine-resistant and – susceptible populations of the allogamous weed *Brassica rapa*. *Weed Research* **33:** 105–114.

Werth, C.R., Hilu, K.W. & Langner, C.A. 1994. Isozymes of *Eleusine* (Gramineae) and the origin of finger millet. *American Journal of Botany* **81:** 1186–1197.

Wickneswari, R. & Norwati, M. 1991. Techniques for starch gel electrophoresis of enzymes from acacias. In Carron, L.T. & Aken, K.M. (eds.). *Breeding Technologies for Tropical Acacias ACIAR Proceedings No.* **37:** 88–100.

Wright, S. 1965. The interpretation of genetic structure by F-statistics with special regard to systems of mating. *Evolution* **19:** 355–420.

Yeh, F.C. & Boyle, T. 1999. *POPGENE version 1.32. The user-friendly software for population genetic analysis*. University of Alberta and CIFOR, Calgary, Alta.

Young, A., Boyle, T. & Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11:** 413–418.