INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1082/2018/20–3–628–636
DOI: 10.17957/IJAB/15.0537
http://www.fspublishers.org

*Full Length Article*

# Determination of Morphological and Genetic Diversity of ALS (Acetolactate Synthase)-Herbicide-Resistant *Echinochloa oryzoides* Biotypes in Rice

**Emine Kaya Altop[1,*], Khawar Jabran[2] and Husrev Mennan[1]**
[1]*Faculty of Agriculture, Department of Plant Protection, Ondokuz Mayis University, Samsun, Turkey*
[2]*Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Duzce University, Duzce, Turkey*
[*]For correspondence: kayae@omu.edu.tr

## Abstract

This work aimed to evaluate the genetic and morphological diversity of 62 biotypes of *Echinochloa oryzoides* (Ard.) Fritsch. (early watergrass) that are resistant to ALS-inhibitor herbicides (bispyribac-sodium and penoxsulam), using the single sequence repeat (SSR) marker system. To determine the morphological diversity, the weed was grown (five seeds from each population) under screenhouse conditions and morphological characteristics were recorded. Parameter values subjected to a hierarchical clustering analysis showed significant variation among the populations. The genetic variation among populations was determined using the 5′-SSR primer. Populations were classified into two main groups according to the results obtained from the SSR alleles. The allele numbers ranged between 2–6 per locus, the diversity values ranged between 0.27–0.99. The morphological and molecular analyses revealed differences in terms of several quantitative characteristics among the populations examined. Similarities were also found among different *E. oryzoides* populations grown in different regions in terms of their morphological characteristics, the genetic diversity was found to be higher. There is a potential for the spread of resistance through gene flow owing to high genetic diversity and low differentiation among population of *E. oryzoides*. Under such situations to prevent further resistance spread, early watergrass management in this area should focus both on reducing seed movement among rice fields and the field management practices such as implementation of crop rotation, use of herbicides with new mode of actions or cultural methods. © 2018 Friends Science Publishers

**Keywords:** Early watergrass; Gene flow; Management strategies; Resistance; Single sequence repeat

## Introduction

Weed species of the genus *Echinochloa*, such as *Echinochloa crus-galli* (L.) P. Beauv., *Echinochloa colona* (L.) Link, and *Echinochloa oryzoides* (Ard) Fritsch pose serious challenges to rice farming, both in Turkey and around the world (Holm *et al*., 1977; Damalas *et al*., 2008; Park *et al*., 2010; Altop *et al*., 2014; Moon *et al*., 2014; Kraehmer *et al*., 2015; Heap, 2016). *E. oryzoides* is considered to have infiltrated Turkey and other countries through contaminated rice seeds. Traits that helped its spread and establishment include self-fertilization ability, high adaptation skills (the weed being a hexaploid) and high ecological tolerance levels (Barret, 1983). The seeds of this weed usually have high germination capacity, it may mimic rice and possess a great degree of ecological plasticity. Another important character of the weed is high seed production. It is among the weeds that are difficult to control and cause massive economic loss due to their wide ecological tolerance levels (Kraehmer *et al*., 2015; Whitney *et al*., 2017). During the recent past, the excessive use of herbicides has resulted in higher costs of weed control, disturbance of natural ecosystems and evolution of herbicide resistance in weeds (Owen and Zelaya, 2005). If rice weeds are not adequately controlled, the yield losses can be 50% or higher (Jabran *et al*., 2012; Mennan *et al*., 2012; Jabran and Chauhan, 2015).

Continuous use of herbicides with the same mechanisms of action leads to the evolution of herbicide-resistant weed populations (Holt *et al.*, 1993). At present, there are 252 resistant (R) weed species worldwide. Common weeds of rice represent a large number of the R biotypes, having been detected in at least 50 different species including several species of the *Echinochloa* genus (Heap, 2017). Similarly, the using of ALS and ACCase herbicides has caused the herbicide-resistant weed species to become increasingly dominant in rice fields in Turkey (Mennan *et al*., 2012; Altop *et al*., 2014). Especially, resistant biotypes of *E. crus-galli* and *E. colona* have been reported in many countries (Heap, 2017). Herbicide resistant *E. oryzoides* was reported in the USA and Turkey (Fischer *et al.*, 2000; Fischer *et al*., 2000a; Altop *et al.*, 2014).

**To cite this paper:** Altop, E.K., K. Jabran and H. Mennan, 2018. Determination of morphological and genetic diversity of ALS (acetolactate synthase)-herbicide-resistant *Echinochloa oryzoides* biotypes in rice. *Int. J. Agric. Biol*., 20: 628–636

Morphological characteristics are used to investigate the phylogenetic relationships among species, hybridization between closely related species, and genetic variation in species. The phenotypic characteristics of *E. oryzoides* may vary depending on the geographical location of the population (Tasrif *et al*., 2004). These morphological differences can have an impact on the competitiveness of species, and therefore, effective control strategies may be formulated after the levels of variation are determined.

In addition to quantitative characteristics, genetic diversity in weed populations help them adapting the environmental conditions and agricultural practices (Neve *et al*., 2009). Natural selection, genetic flow and genetic drift are the forces that cause an evolution in both the natural environments and weeds (Vigueira *et al*., 2013). Many weed species display differences in terms of their genetic characteristics due to different growth patterns and morphological characteristics, depending on the area in which they grow (Yabuno, 1996; Michishita and Yamaguchi, 2003; Claerhout *et al*., 2015; Karn and Jasieniuk, 2017). The sizes of resistant populations give rise to high genetic diversity within a species in a region by accumulating mutations over time (Karn and Jasieniuk, 2017). In addition, key occurrences during the propagation of resistance, such as local mutation and selection pressure, and intra-population pollen and seed dispersal, have led to the formation of regional genetic variations throughout the agricultural landscape. Herbicide-resistance evolution in the susceptible populations may be triggered due to high level of genetic diversity that supports the increase in the adaptation capacity of more durable species. Geographical location differences provide information on the pathway and mechanisms of resistance propagation (Menchari *et al*., 2007; Delye *et al*., 2010; Duhoux *et al*., 2017; Karn and Jasieniuk, 2017).

Even though *Echinochloa* spp. are some of the principal weeds causing an economic loss in rice fields of the world, its genetic diversity is not yet known (Lee *et al.*, 2015). Recent research shows that genetic variation in E. oryzoides populations has risen and it differentiated populations by geographical region, which was associated with resistance phenotype. Genetic diversity was slightly greater in the resistant group (0.387) than in the susceptible group (0.321) in *E. crus-galli* and *E. oryzicola* populations (Lee *et al.*, 2015). Osuna *et al*. (2011) detected low genetic variation within E. oryzoides populations, however, susceptible populations (0.262) tended to be more diverse than resistant populations (0.161). In addition, morphological variation of resistant *E. crus-galli* and *E. muricata* accessions were significantly correlated with genetic variation (Claerhout *et al.*, 2015). The spread of herbicide resistance among populations of other *Echinochloa* species has also been inferred using molecular markers (Rutledge *et al*., 2000).

Hence, this work was aimed at studying the genetic background and discriminating the morphological traits of *E. oryzoides* populations that had evolved a resistance against ALS inhibitor herbicides.

## Materials and Methods

### Plant Materials

This research has been conducted in screen house in Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey. Seeds from 62 population of *E. oryzoides* were used, whose resistance against ALS-inhibitor herbicides (bispyribac-sodium and penoxsulam) had been confirmed in our previous work (Mennan *et al.*, 2012; Altop *et al.*, 2014). Populations were collected from nine provinces from the Marmara and Black Sea regions, which correspond to 95% of Turkey's paddy fields (Fig. 1 and Table 1). The seeds were collected from fields with a long history of herbicide use and where control problems had been detected. In addition to those fields, seed samples were obtained randomly from rice fields of the regions. Seeds of *E. oryzoides* (500 g) were collected from each field and were cleaned and stored at room temperature until experiments were initiated.

### Morphological Studies

Morphological studies were conducted following the protocols provided by International Survey of Herbicide Resistant Weeds (http://www.weedscience.org/). Ten seeds of *E. oryzoides* (from each population) were planted in plastic pots (diameter 20 cm; height 25 cm) in 2011. The pots were filled with paddy soil and the experiments were set up with five replications following a randomized complete block design. For each population, the five *E. oryzoides* seedlings that germinated the earliest were cultivated after labelling, whereas the others were discarded. The biological cycles were monitored for each plant and the morphological characteristics were recorded. Harvest took place when more than 85% of panicles were mature. This varied between 92 and 116 DAS (days after sowing). The measurements were conducted on this seedling. The examined characteristics such as number of tillers (per plant), plant stem diameter (mm), spikelet diameter (mm), spike length (mm), awn length (cm), spikelet length (mm), number of spikelets (per plant), plant height (cm), above-ground biomass (g), and the flag leaf area ($cm^2$) were assessed at the maturity stages at postharvest. For biomass, the plants were harvested and then dried for three days at 70°C. In addition, growth characters such as germination speed (DAS) and seedling time (DAS) were also evaluated.

### Molecular Studies

Sixty-two samples of *E. oryzoides* from different locations were used, all representing different populations with varying degree of herbicide resistance.

**Table 1:** Labelling and geographical information of the populations

| Label | Origin | Coordinates | | Label | Origin | Coordinates | |
|---|---|---|---|---|---|---|---|
| EDİ58 | Edirne-Havsa | 41° 25.705′ | 26° 48.914′ | KAS1 | Kastamonu-Hanönü | 41° 37.248′ | 34° 28.703′ |
| EDİ63 | Edirne-Havsa | 41° 29.246′ | 26° 48.811′ | KAS6 | Kastamonu-Tosya | 40° 56.368′ | 33° 52.502′ |
| EDİ27 | Edirne-İpsala | 40° 51.748′ | 26° 20.546′ | KAS14 | Kastamonu-Tosya | 41° 02.654′ | 34° 11.373′ |
| EDİ68 | Edirne-İpsala | 40° 52.868′ | 26° 23.020′ | KAS16 | Kastamonu-Tosya | 41° 03.730′ | 34° 12.300′ |
| EDİ84 | Edirne-İpsala | 40° 55.921′ | 26° 24.520′ | TEK3 | Tekirdağ-Hayrabolu | 41° 03.275′ | 27° 03.625′ |
| EDİ92 | Edirne-İpsala | 40° 56.004′ | 26° 24.869′ | TEK6 | Tekirdağ-Hayrabolu | 41° 03.229′ | 27° 03.672′ |
| EDİ99 | Edirne-İpsala | 40° 53.652′ | 26° 21.898′ | TEK9 | Tekirdağ-Malkara | 40° 56.830′ | 27° 01.020′ |
| EDİ121 | Edirne-İpsala | 40° 53.353′ | 26° 21.493′ | BUR1 | Bursa-Centre | 40° 10.356′ | 28° 11.256′ |
| EDİ128 | Edirne-İpsala | 40° 53.390′ | 26° 21.121′ | BUR7 | Bursa-Centre | 40° 11.873′ | 28° 11.337′ |
| EDİ137 | Edirne-İpsala | 40° 50.381′ | 26° 17.704′ | BUR17 | Bursa-Centre | 40° 11.758′ | 28° 11.300′ |
| EDİ143 | Edirne-Keşan | 40° 44.591′ | 26° 25.653′ | SİN3 | Sinop-Saraydüzü | 41° 23.532′ | 34° 56.981′ |
| EDİ150 | Edirne-Keşan | 40° 46.678′ | 26° 41.873′ | SİN9 | Sinop-Boyabat | 41° 37.290′ | 34° 36.730′ |
| EDİ10 | Edirne-Meriç | 41° 05.458′ | 26° 22.215′ | SİN16 | Sinop-Boyabat | 41° 32.955′ | 34° 42.959′ |
| EDİ171 | Edirne-Meriç | 41° 06.386′ | 26° 20.595′ | SİN25 | Sinop-Durağan | 41° 26.722′ | 34° 54.735′ |
| EDİ174 | Edirne-Meriç | 41° 06.426′ | 26° 20.542′ | SİN32 | Sinop-Durağan | 41° 25.954′ | 34° 56.650′ |
| EDİ182 | Edirne-Meriç | 41° 03.192′ | 26° 21.810′ | BAL4 | Balıkesir-Gönen | 40° 07.161′ | 27° 43.387′ |
| EDİ196 | Edirne-Centre | 41° 30.844′ | 26° 36.642′ | BAL26 | Balıkesir-Gönen | 40° 07.056′ | 27° 42.101′ |
| EDİ203 | Edirne-Centre | 41° 29.712′ | 26° 37.067′ | BAL55 | Balıkesir-Manyas | 40° 04.680′ | 28° 02.410′ |
| SAM4 | Samsun-Alaçam | 41° 37.400′ | 35° 43.456′ | BAL77 | Balıkesir-Manyas | 40° 04.987′ | 28° 02.578′ |
| SAM12 | Samsun-Bafra | 41° 38.824′ | 35° 49.332′ | BAL81 | Balıkesir-Manyas | 40° 04.993′ | 28° 02.581′ |
| SAM30 | Samsun-Bafra | 41° 42.043′ | 35° 55.014′ | BAL86 | Balıkesir-Manyas | 40° 06.140′ | 28° 08.241′ |
| SAM45 | Samsun-Bafra | 41° 43.412′ | 35° 57.281′ | COR2 | Çorum-Kargı | 41° 06.098′ | 34° 24.910′ |
| SAM53 | Samsun-Çarşamba | 41° 16.568′ | 36° 44.104′ | COR9 | Çorum-Kargı | 41° 04.986′ | 34° 26.134′ |
| SAM58 | Samsun-Çarşamba | 41° 12.494′ | 36° 36.012′ | COR19 | Çorum-Kargı | 41° 07.123′ | 34° 25.272′ |
| SAM64 | Samsun-OndokuzMayıs | 41° 32.075′ | 36° 03.828′ | COR29 | Çorum-Osmancık | 40° 58.821′ | 34° 55.776′ |
| SAM68 | Samsun-Terme | 41° 13.500′ | 36° 58.096′ | COR42 | Çorum-Osmancık | 40° 57.726′ | 34° 50.011′ |
| SAM77 | Samsun-Terme | 41° 11.305′ | 36° 59.033′ | COR53 | Çorum-Osmancık | 40° 56.319′ | 34° 51.357′ |
| SAM89 | Samsun-Yakakent | 41° 37.656′ | 35° 33.829′ | COR57 | Çorum-Bayat | 40° 31.376′ | 34° 20.545′ |
| KIR2 | Kırklareli-Babaeski | 41° 20.940′ | 27° 07.340′ | COR67 | Çorum-Dodurga | 40° 49.609′ | 34° 51.519′ |
| KIR6 | Kırklareli-Babaeski | 41° 21.425′ | 27° 04.110′ | COR74 | Çorum-İskilip | 40° 36.055′ | 34° 28.523′ |
| KIR13 | Kırklareli-Pehlivanköy | 41° 22.044′ | 26° 52.956′ | COR82 | Çorum-Laçin | 40° 49.602′ | 34° 51.529′ |


**Fig. 1:** *Echinochloa oryzoides* populations in Marmara region (1: Balıkesir, 2: Bursa, 3: Edirne, 4: Kırklareli, 5: Tekirdağ) and Black Sea region (6: Çorum, 7: Kastamonu, 8: Samsun, 9: Sinop)

The seeds from each population were germinated in petri dishes. Seedlings were planted in pots and grown until two–four leaves stage in a screenhouse. The total genomic DNA was extracted when plants reached the six-leaves stage using DNeasy Plant Mini Kits (Qiagen, Qiagen GmbH, Hilden, Germany), according to the instructions provided. The concentration and relative purity of the isolated DNA were checked using Nanodrop ND-1000 (Agricultural Genomic Laboratory) and adjusted to 30 ng $\mu L^{-1}$ (Danquah *et al*., 2002; Santaella *et al*., 2006). A total of five simple sequence repeat (SSR) markers, including four *Echinochloa* spp. specific SSR markers developed by Danquah *et al*. (2002a), were used.

Microsatellite amplification was performed in a 25 µL reaction mixture that contained approximately 2 µL of 30 ng genomic DNA, 0.5 µL (50 pmol) of each primer, 3 µL of 300 µM dNTP mix (Sigma, St. Louis, MO, USA), 0.5 µL of 2.5 U/µL Taq DNA polymerase (Sigma), 2.5 µL of 2.5 mM $MgSO_4$, 2.5 µL of 1×PCR buffer, and 14 µL of $sdH_2O$.

The reaction proceeded in a thermal cycler (Rotor-Gene Q 5plex HRM) under the following conditions: an initial denaturation step at 94°C for one min, followed by 35 cycles of denaturation at 94°C for one min, annealing at 54°C for one min, and extension at 72°C for one min, followed by a final extension step of 10 min at 72°C. A two-percent agarose gel was used for the analysis of DNA fragments after PCR. Photographs of the DNA bands in the gel were taken using a gel-imaging device (Vilber Lourmat, France), with reference to a 1-Kb DNA marker (New England Biolabs).

**Data Analysis**

Dendrograms for morphological data were generated by subjecting the morphological data to a hierarchical cluster analysis using the SPSS 21.0 (IBM Crop. Armonk, NY: USA Released 2012) statistical package program. In addition, the principal component analysis (PCA) was performed to determine whether the variance of these

characteristics in the population could be explained with fewer variables without statistically significant loss of information. The genotypes were sorted by principal coordinates analysis (PCA), which was performed to show the distribution of the genotypes in a scatter plot.

For genetic data, only the clear and unambiguous bands were scored. Statistics, including the number of alleles (NA), gene diversity (GD), heterozygosity and polymorphism information content (PIC), were calculated using the genetic analysis program Power Marker v.3.25 (Liu and Muse, 2005). Markers (5′-SSR primers) were scored for the presence (1) and absence (0) of the corresponding band among the genotypes. Consequently, a data matrix comprising '1' and '0' was formed and subjected to further analysis. Further processing of data was done by carrying out sequential agglomerative hierarchical non-overlapping clustering (SAHN) on squared Euclidean distance matrix. Dissimilarity matrices were used to construct the UPGMA (Unweighted Pair Group Method with Arithmetic average) dendrogram. In addition, the genetic relationships among genotypes were represented using a PCA (Backhaus *et al.*, 1989) analysis with SPSS 21.0 software (IBM Crop. Armonk, NY: USA Released 2012) (Juraimi *et al.*, 2005; Vilatersana *et al.*, 2005; Santaella *et al.*, 2006; Lee *et al.*, 2014; Claerhout *et al.*, 2015; Karn and Jasieniuk, 2017).

## Results

### Morphological Studies

The correlation matrix of the morphological parameters for *E. oryzoides* population is provided in Table 2. Among the morphological characteristics examined, significant and positive statistical relationships were identified among the germination speed parameter and other traits, namely, seedling time (0.32**), flag leaf area (0.25**), plant stem diameter (0.23**), and plant height (0.21**) from all the morphological characteristics examined. The seedling time had a significant negative correlation with all other parameters, except spikelet diameter and awn length.

The length of the awn is known to have a significant negative correlation with the germination speed (-0.23**), number of tillers (-0.18**), plant stem diameter (-0.18**), number of spikelets (-0.17**), and biomass (-0.23**). The PC components obtained as a result of the Principal Component Analysis and the corresponding factor groups of these components are shown in Table 3. From the total of 12 characteristics examined, four PC componenets were obtained, representing 71.04% of the total variation. Among the morphological characteristics, the most important parameters, which constituted the first PC component (accounting for 33.41% of the total variation), were: above-ground biomass (0.429), plant stem diameter (0.428), plant height (0.410), and number of tillers (0.350).

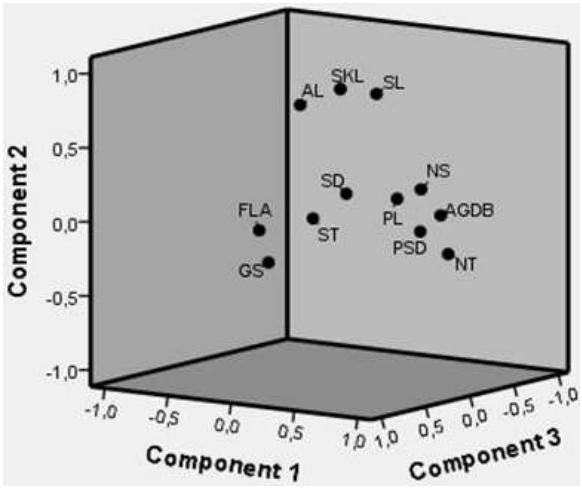


**Fig. 2:** The component plot formed by morphological characteristics

GS: Germination Speed, ST: Seedling Time, NT: Number of Tillers, PSD: Plant Stem Diameter, SD: Spikelet Diameter, SL: Spike Length, AL: Awn Length, SKL: Spikelet Length, NS: Number of Spikelets, PL: Plant Height, AGDB: Above-Ground Biomass, FLA: Flag Leaf Area

Fig. 2 shows the Principal component graph constructed using the first (PCA1), second (PCA2), and third component (PCA3), which account for 33.41%, 16.24%, and 11.86% of the total variation in the morphological characteristics of *E. oryzoides* populations, respectively. This chart summarizes the impact of the parameters on variation, as well as other necessary details about their interactions with each other. The points along the x-axis show differences in terms of the main effects, while the deviations from zero on the z- and y-axis represent the interactions. Here, parameters close to zero have a similar effect on variation.

Negative correlations were observed for the spikelet (-0.536) and spike length (-0.529) on the second PC axis (accounting for 16.24% variation), and they were the most significant parameters in this group. The flag leaf area (-0.538) accounted for the 11.86% variation in the PC3 axis, the 9.52% variation in the PC4 axis was found to be made up of values obtained from the spike diameter (-0.780) (Table 3).

Fig. 3 represents the dendrogram constructed according to the similarity levels when all the morphological characteristics of the populations were subjected to the hierarchical cluster analysis. It can be seen that, there are two main groups in the taxonomic range of 0–25%. The first main group is divided into two subgroups. The primary observation here is the representation of the first group with 58 genotypes, which rules out geographic isolation, while the second subgroup consists of the KIR1 population specific to the Marmara Region. The first subgroup of the second main group is formed of populations COR29 from the Central Black Sea Region and EDI182 from the Marmara Region.

**Table 2:** Correlation matrix of morphological parameters for *E. oryzoides* populations

| | GS | ST | NT | PSD | SD | SL | AL | SKL | NS | PL | AGDB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ST | 0.32** | | | | | | | | | | |
| NT | 0.05 | -0.26** | | | | | | | | | |
| PSD | 0.23** | -0.41** | 0.76** | | | | | | | | |
| SD | 0.15* | -0.03 | 0.04 | 0.46** | | | | | | | |
| SL | -0.15* | -0.24** | 0.05 | 0.21** | 0.19** | | | | | | |
| AL | -0.23** | 0.16* | -0.18** | -0.18** | 0.06 | 0.27** | | | | | |
| SKL | -0.01 | -0.25** | -0.05** | 0.17 | 0.18** | 0.61** | 0.36** | | | | |
| NS | 0.00 | -0.22** | 0.37** | 0.43** | 0.14* | 0.47** | -0.17** | 0.08 | | | |
| PL | 0.21** | -0.64** | 0.45** | 0.55** | 0.08 | 0.42** | -0.16* | 0.29** | 0.49** | | |
| AGDB | 0.10 | -0.37** | 0.73** | 0.75** | 0.25** | 0.27** | -0.23** | 0.22** | 0.49** | 0.63** | |
| FLA | 0.25** | -0.24** | -0.05** | 0.06 | -0.01 | 0.00 | -0.09 | 0.03 | 0.09 | 0.12 | 0.07 |

** Significant by 0.01, * Significant by 0.05

GS: Germination Speed, ST: Seedling Time, NT: Number of Tillers, PSD: Plant Stem Diameter, SD: Spikelet Diameter, SL: Spike Length, AL: Awn Length, SKL: Spikelet Length, NS: Number of Spikelets, PL: Plant Height, AGDB: Above-Ground Biomass, FLA: Flag Leaf Area

**Table 3:** Factor groups comprising the morphological parameters of *E. oryzoides* populations, and their corresponding PCA axes

| PCA axis | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Eigenvalues | 4.0097 | 1.9487 | 1.4241 | 1.1428 |
| Variation (%) | 33.41 | 16.24 | 11.86 | 9.52 |
| Cumulative variation (%) | 33.41 | 49.65 | 61.52 | 71.04 |
| Factor coefficients | | | | |
| Parameters | PCA1 | PCA2 | PCA3 | PCA4 |
| Germination speed (DAS) | 0.125 | 0.271 | -0.510 | -0.328 |
| Seedling time (DAS) | -0.312 | -0.028 | 0.414 | -0.181 |
| Number of tillers (per plant) | 0.350 | 0.223 | 0.359 | 0.051 |
| Plant stem diameter (mm) | 0.428 | 0.111 | 0.159 | -0.280 |
| Spikelet diameter (mm) | 0.163 | -0.117 | 0.055 | -0.780 |
| Spike length (mm) | 0.235 | -0.529 | -0.025 | 0.153 |
| Awn length (cm) | -0.105 | -0.511 | 0.030 | -0.143 |
| Spikelet length (mm) | 0.168 | -0.536 | -0.199 | -0.068 |
| Number of spikelets (per plant) | 0.321 | -0.054 | 0.153 | 0.250 |
| Plant height (cm) | 0.410 | -0.033 | -0.140 | 0.236 |
| Above-ground biomass (g) | 0.429 | 0.079 | 0.193 | -0.017 |
| Flag leaf area ($cm^2$) | 0.078 | 0.106 | -0.538 | 0.057 |

The geographical distance between these populations is 890 km. The second subgroup is represented by a single population (TEK9). TEK9 population differed significantly from all other populations with respect to number of tillers, plant height and above ground biomass and this parameter values were the highest values of all populations. Among those populations, TEK 9 had the fastest germination speed (9 DAS) so it may obtain competitive advantage than other population (data not presented here). In addition, this population had the shortest awn as well as lowest spikelet diameter when compared to other population. The fields from where these populations were obtained, rice is rotated with maize and sunflower. This may result in high adaptation of this population in different growing conditions and make some changes in its morphological parameters.

### Genetic Studies

All five microsatellites showed inter and/or intraspecific polymorphism, there were a total of 18 alleles, ranging between 2–6 alleles per locus. The gene diversity for five loci (EC1, EC2, EC3, EC4, and EC5) polymorphic within *E. oryzoides* ranged from 0.27–0.99. Loci EC1 and EC2 also gave unique alleles readings. The average gene diversity and PIC (polymorphism information content) values were 0.642 and 0.234, respectively with a range of 0.982. All the SSR markers used in this study showed very high heterozygosity, especially EC1 with 81% (Table 4).

According to the PCA analysis, 73.1% of the variation was explained using eight PC axes. The total variation represented by them was 42.1, 23.8, 7.2, 4.1, 3.4, 2.5, 2.3 and 1.8%, respectively. The greatest contribution to the 42.1% variation in the first basic coordinate was from the SAM89 population, whereas the highest contribution to the positive and negative multiple effects on the second coordinated axis with a variation of 23.8% was from the EDI137 population. As for the other axes (PCA3–PCA8), KIR2-COR67-BUR1-COR67-SAM53 and SAM45 populations were the major contributors to the variation (data not shown).

According to the UPGMA dendrogram generated using Average Linkage, it can be seen that, working with a genetic distance of 0.25, the populations are divided into two main groups (Fig. 4). The first main group comprised the Edirne and Samsun populations and the geographical isolation was clearly seen. However, the second group was characterised by a wide geographical distribution.

## Discussion

Studies on morphological and genetic diversity are important in terms of developing an understanding of how both herbicides and environmental influences affect weed species (Sterling *et al*., 2004; Claerhout *et al*., 2015; Karn and Jasieniuk, 2017). Through statistical evaluations based on population genetic structure and quantitative characteristics, the study clearly demonstrates morphological similarities and genetical distances of *E. oryzoides* populations. This means resistant populations are still evolving resistance. Resistance will be fixed and continue to spread over time without proper control measures (Lee *et al*., 2014, 2015, 2016; Karn and Jasieniuk, 2017).

**Table 4:** Genetic characterization of *E. oryzoides* populations

| Locus | Primer (5′-3′) | Size (bp) | No. of alleles | Gene diversity | Heterozygosity | Polymorphism information content |
|---|---|---|---|---|---|---|
| EC1 | F: ATTACTGGTCAGACGGAAAC R: GCAGTTATCTCCGTGGGCAC | 108–120 | 3 | 0.451 | 0.814 | 0.420 |
| EC2 | F: GGCTCCAAACAAGGCAATTC R: TTCAGGGAATTTAGTACAAG | 95–177 | 3 | 0.273 | 0.375 | 0.234 |
| EC3 | F: GAAAGGAAATGGGTTGGCTG R: CTTCGCACCATGATCTTCTC | 76–85 | 4 | 0.982 | 0.134 | 0.972 |
| EC4 | F: AGTAGAAGGCTGCAAGAAGG R: TCTCAGCCCACTTTGTATAG | 167–181 | 2 | 0.994 | 0.123 | 0.982 |
| EC5 | F: CAGAGCCTTCAATCATGGTG R: TGCTTCAAGTTCTAGGAGAC | 89–99 | 6 | 0.615 | 0.377 | 0.602 |
| Mean | | | 3.6 | 0.663 | 0.364 | 0.642 |

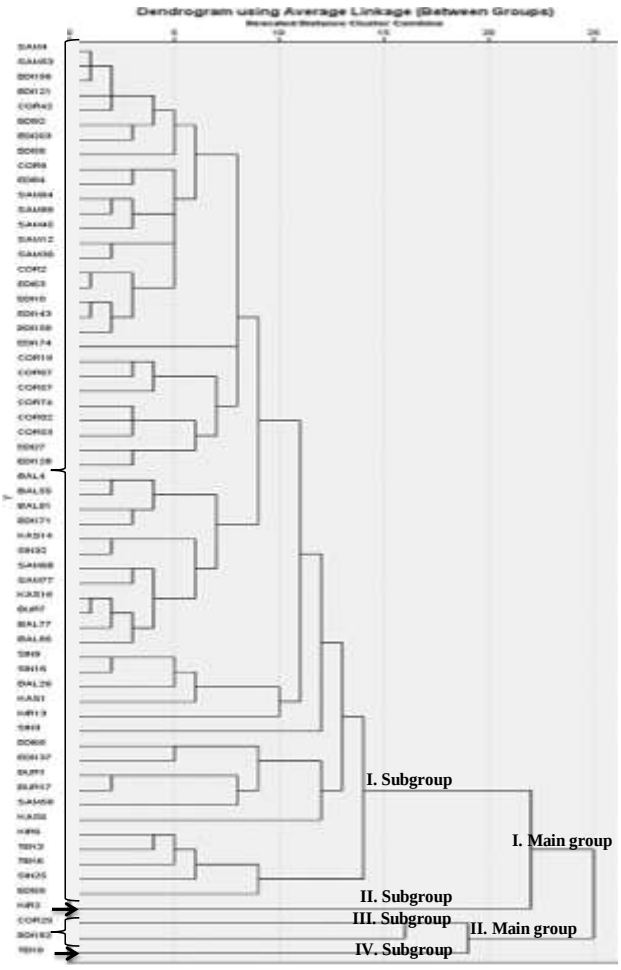


**Fig. 3:** Dendrogram generated from hierarchical clustering analysis of the morphological characteristics of different populations of *E. oryzoides*

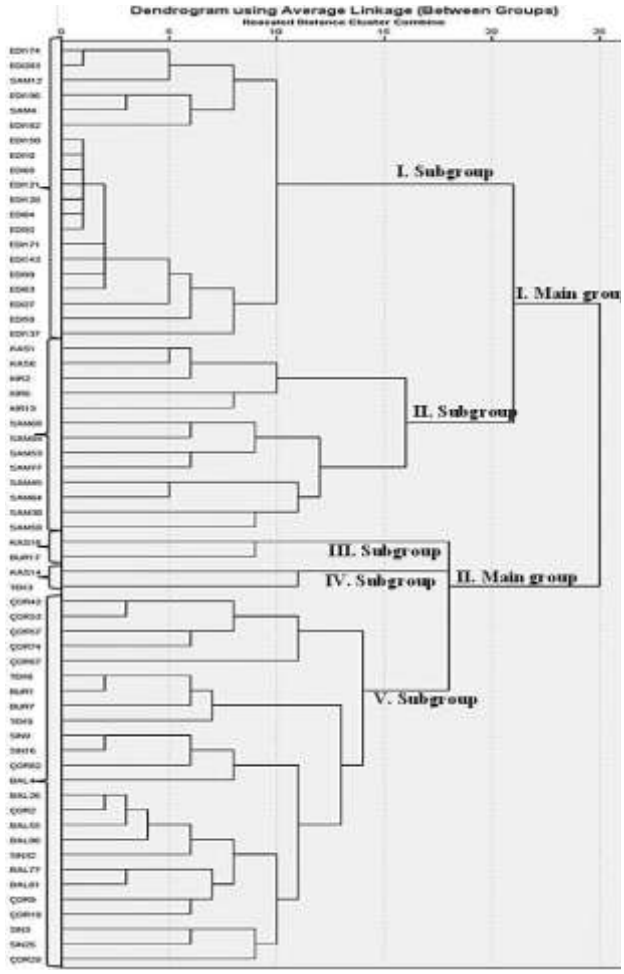


**Fig. 4:** Dendrogram generated from hierarchical clustering analysis based on SSR analysis of different populations of *E. oryzoides*

The principal component analysis for ten quantitative morphological parameters that showed significant variation indicated that the first four PCs for 71.7% of the total variations. Therefore, morphological similarity among populations ranged from 75% to 100%. In the literature, the presence or absence of awns is considered the first of the distinguishing features that differentiate *E. oryzoides* from other species of *Echinochloa* (Barret, 1983; Danquah *et al.*, 2002; Damalas *et al.*, 2008). In this study, length of the awn is known to have a significant negative correlation with the germination speed, number of tiller, plant stem diameter, number of spikelets and biomass parameters.

In genetic structure, the mean gene diversity using five SSR markers was 66.3%. The increased gene diversity found in this study compared to that (55.6%) by Danquah *et al.* (2002) and (37.4%) by Lee *et al.* (2015), was probably because of sampling from far wider areas and the rate of resistance being more dramatic. The molecular data obtained from the *E. oryzoides* populations identified a total of eight basic coordinates, and the proportion of genetic

variation described by these coordinates ranged between 42.1% and 1.75%, similar to the results of Danquah *et al.* (2002), in terms of the components and the variation. In addition, the genetic diversity ratios (0.22–0.99) obtained in our study could be confirmed by the same study (Danquah *et al*., 2002).

Geographic isolation is more visible in molecular dendrogram compared with morphological dendrogram. Population is geographically spread across wide swathes of land, although they are morphologically within the same group. Tillage and harvesting machines in paddy fields can be cited as reasons for this. Ecotypes displaying high levels of phenotypic similarity might not display genetic similarities (Vellend, 2005), as different gene pools can be formed. Phylogenetic data must be carefully examined to better understand the gene flow among populations, which occurs at varying degrees over time, with particular focus on the variation-related genes. In addition, genetic and morphological similarities can be explored together to make reliable interpretations (Bromham *et al*., 2002).

Genetic diversity is linked to the rate of gene transfer, which means that, higher the genetic diversity among populations, lesser is the gene transfer, and vice versa (Merotto *et al*., 2010). While gene transfer is linked to the distances among populations, breeding systems, pollination characteristics, vegetation, environmental conditions, and vectors (Lee *et al*., 2012, 2014), some studies have also found that genetic relationships among populations are not linked to geographical distances (Merotto *et al*., 2009). The current study not only reveals that genetic associations are not always related to geographical distances (subgroups 5), based on the interpretation of UPGMA dendrogram data, but also that there are populations (subgroups 1) where geographical isolation is quite visible. For example, although the geographical distance (approximately 800 km) between the BAL4 and COR2 populations in the same group in the genetic-relationship dendrogram is considerably large, they have close geographical distances (about 16 km) with BAL55 in the same group. In general terms, when genetic affinities, geographical locations, and herbicide activities are considered together, it appears that the three cannot be directly associated with one another.

The lack of very high genetic similarity in our study indicates a great potential for gene flow. At the same time, it also indicates that more durable species are rapidly spreading. It has been emphasized in various previous studies that resistance to herbicides among populations with high genetic similarities can spread by gene transfer (Rutledge *et al*., 2000; Stankiewicz *et al*., 2001; Tsuji *et al*., 2003; Merotto *et al*., 2010; Claerhout *et al*., 2015; Karn and Jasieniuk, 2017), and that gene transfer takes place more often through spreading of seeds rather than through pollens, taking into account the self-pollinating properties of *E. oryzoides* (Baker *et al*., 2007).

Differences were revealed when the groups formed by the populations were examined morphologically and genetically. The results obtained are consistent with previous research work. This is because many weed species of rice display differences in terms of their genetic and morphological characteristics. They have different growth patterns and morphological characteristics, depending on the area in which they grow (Yamaguchi *et al*., 1996; Yabuno, 1996; Michishita and Yamaguchi, 2003). Over recent years, there has been more emphasis on the need to study the genetic diversity of some species, as morphological studies cannot single-handedly account for the diversity among species (Roy *et al*., 2000; Rutledge *et al*., 2000; Dodet *et al*., 2008; Claerhout *et al*., 2015; Karn and Jasieniuk, 2017).

As mentioned by Roy *et al*. (2000), given the fact that non-certified seeds get transferred from one region to another, and the quick adaptation skills of this alien weed species to its environment, it is possible to form morphologically close groups that could be genetically different. Cross-pollination, strong clonal growth, sexual reproduction, and spreading through humans are the key factors giving rise to variation, as shown in various studies (Tayyar *et al*., 2003; Ren *et al*., 2005; Imaizumi *et al*., 2008; Claerhout *et al*., 2015).

## Conclusion

The ALS inhibitors herbicide resistant populations were genetically diverse but morphologically similar. Molecular analysis indicated that resistant alleles had a potential to spread. Susceptible seed migrants are usually wiped due to herbicide application, while the resistant ones spread out. This comes out a low genetic differentiation among resistant populations and sustention of herbicide-resistant genetic diversity. Weed populations had a high genetic diversity despite the consistent herbicide use over the past fortyr years; this will positively impact the adaptive capability of weeds to herbicide resistance (for new chemistries) or other crop management practices. The way forward is the implementation of integrated weed management that may help to decrease the selection pressure and may limit the dispersal of resistance through gene flow. The important and complex issue of herbicide resistance should be dealt with decisions based on sound policy, and an understanding of the biological cycles in the farming systems.

## Acknowledgments

The authors thank The Scientific and Technological Research Council of Turkey (TUBITAK) for supporting the Project (Number TOVAG 1080371).

## References

Altop, E.K., H. Mennan, J.C. Streibig, U. Budak and C. Ritz, 2014. Detecting ALS and ACCase herbicide tolerant accession of *Echinochloa oryzoides* (Ard.) Fritsch. in rice (*Oryza sativa* L.) fields. *Crop Prot*., 65: 202–206

Backhaus, R., H. Sax and K. Wanders, 1989. Status and perspectives of vegetation monitoring by remote sensing. *Space Tech.*, 4: 333–338

Baker, J., I. Hidayat and C. Preston, 2007. Molecular tools for understanding distribution and spread of weed genotypes. *Crop Prot.*, 26: 198–205

Barret, S.C.H., 1983. Crop mimicry in weeds. *Econ. Bot.*, 37: 255–282

Bromham, L., M. Woolfit, M.S.Y. Lee and A. Rambaut, 2002. Testing the relationship between morphological and molecular rates of change along phylogenies. *Evolution*, 56: 1921–1930

Claerhout, S., D. Reheul and B.D. Cauwer, 2015. Sensitivity of *Echinochloa crus-galli* populations to maize herbicides: a comparison between cropping systems. *Weed Sci*., 55: 470–481

Damalas, C.A., K.V. Dhima and I.G. Eleftherohorinos, 2008. Bispyribac-sodium efficacy on early watergrass (*Echinochloa oryzoides*) and late watergrass (*Echinochloa phyllopogon*) as affected by co application of selected rice herbicides and insecticides. *Weed Technol*., 22: 622–627

Danquah, E.Y., D.E. Johnson, C. Riches, G.M. Arnold and A. Karp, 2002. Genetic diversity in *Echinochloa* spp. collected from different geographic origins and within rice fields in Cote d'Ivoire. *Weed Res.*, 42: 394–405

Danquah, E.Y., S.J. Hanley, R.C. Brookes, C. Aldam and A. Karp, 2002a. Isolation and characterization of microsatellites in *Echinochloa* (L.) Beauv. spp. *Mol. Ecol. Notes*, 2: 54–56

Delye, C., S. Michel, A. Berard, J. Chauvel, D. Brunel, J.P. Guillemin, F. Dessaint and V.L. Corre, 2010. Geographical variation in resistance to acetyl-coenzyme a carboxylase a inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides* (blackgrass). *New Phytol*., 186: 1005–1017

Dodet, M., R.J. Petit and J. Gasquez, 2008. Local spread of the invasive *Cyperus esculentus* (Cyperaceae) inferred using molecular genetic markers. *Weed Res.*, 48: 19–27

Duhoux, A., S. Carrère, A. Duhoux and C. Délye, 2017. Transcriptional markers enable identification of rye-grass (*Lolium* spp.) plants with non-target-site-based resistance to herbicides inhibiting acetolactate-synthase. *Plant Sci*., 257: 22–36

Fischer, A.J., C.M. Ateh, D.E. Bayer and J.E. Hill, 2000. Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. *Weed Sci*., 48: 225–230

Fischer, A.J., D.E. Bayer, M.D. Carriere, C.M. Ateh and K.O. Yim, 2000a. Mechanisms of resistance to bispyribac-sodium in an *Echinochloa phyllopogon*. *Pestic. Biochem. Physiol*., 68: 156–165

Heap, I.M., 2017. *International Survey of Herbicide Resistant Weeds*. Annual Report Internet. Available at: www.weedscience.com [accessed November 14, 2017]

Heap, I.M., 2016. *International Survey of Herbicide Resistant Weeds*. Annual Report Internet. Available at: www.weedscience.com [accessed December 15, 2016]

Holm, L., D. Plucknett, J. Pancho and J. Herberger, 1977. *The World's Worst Weeds. Distribution and Biology*, p: 609. Honolulu, Hawaii, USA: University Press of Hawaii

Holt, J.S., J.A.M. Holtum and S.B. Powles, 1993. Mechanisms and agronomic aspects of herbicide resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 44: 203–229

Imaizumi, T., G. Wang, T. Ohsako and T. Tominaga, 2008. Genetic diversity of sulfonylurea-resistant and-susceptible *Monochoria vaginalis* populations in Japan. *Weed Res.*, 48: 187–196

Jabran, K. and B.S. Chauhan, 2015. Weed management in aerobic rice systems. *Crop Prot.*, 78: 151–163

Jabran, K., M. Farooq, M. Hussain, Ehsanullah, M.B. Khan, M. Shahid and D. Lee, 2012. Efficient weeds control with penoxsulam application ensures higher productivity and economic returns of direct seeded rice. *Int. J. Agric. Biol*., 14: 901–907

Juraimi, A.S., A. Tarif, J. Kadir, S. Sastroutomo and S. Napis, 2005. Morphological and RAPD variability among Malaysian ecotypes of barnyard grass (*Echinochloa crus-galli var. crus-galli* (L.) P. Beauv.). *Plant Prot. Q*., 20: 52–57

Karn, E. and M. Jasieniuk, 2017. Genetic diversity and structure of *Lolium perenne* ssp. *multiflorum* in California vineyards and orchards indicate potential for spread of herbicide resistance via gene flow. *Evol. Appl.*, 10: 616–629

Kraehmer, H., K. Jabran, H. Mennan and B.S. Chauhan, 2015. Global distribution of rice weeds–a review. *Crop Prot*., 80: 73–86

Lee, E.J., G. Nah, M.J. Yook, S.H. Lim, T.S. Park, D. Lee and D.S. Kim, 2016. Phylogenetic relationship of *Echinochloa* species based on simple sequence repeat and phenotypic marker analyses. *Weed Sci*., 64: 441–454

Lee, I.Y., H.G. Lee, C.S. Kim and J. Lee, 2012. Special lecture for weeds and herbicides. *Kor. J. Weed Sci*., 32: 24–27

Lee, J., K.W. Park, I.Y. Lee, C.S. Kim, O.D. Kown and T.S. Park, 2015. Simple sequence repeat analysis of genetic diversity among Acetyl-CoA carboxylase inhibitor-resistant and inhibitor-susceptible *Echinochloa crus-galli* and *E. oryzicola* populations in Korea. *Weed Res.*, 55: 90–100

Lee, J., C.S. Kim and I.L. Lee, 2014. Taxonomic review of the genus Echinochloa in Korea (II): Inferred from simple sequence repeats. *Weed Turf. Sci.*, 3: 190–195

Liu, K. and S.V. Muse, 2005. PowerMarker: Integrated analysis environment for genetic marker data. *Bioinformatics*, 21: 2128–2129

Menchari, Y., C. Delye and L.V. Corre, 2007. Genetic variation and population structure in blackgrass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. *Mol. Ecol.*, 16: 3161–3172

Mennan, H., M. Ngouajio, M. Sahin, D. Isık and E. Kaya Altop, 2012. Competitiveness of rice (*Oryza sativa* L.) cultivars against *Echinochloa crus-galli* (L.) Beauv. in water-seeded production systems. *Crop Prot*., 41: 1–9

Merotto, A.J.R., M. Jasieniuk and A.J. Fischer, 2010. Distribution and cross-resistance patterns of ALS-inhibiting herbicide resistance in small flower umbrella sedge (*Cyperus difformis*). *Weed Sci*., 58: 22–29

Merotto, A.J.R., M. Jasieniuk and A.J. Fischer, 2009. Estimating the out crossing rate of *Cyperus difformis* using resistance to ALS-inhibiting herbicides and molecular markers. *Weed Res*., 49: 29–36

Michishita, Y. and H. Yamaguchi, 2003. Unique forms of weeds and millets in East Asian annual *Echinochloa*. *In: Proc. 19thAsian-Pacific Weed Sci. Soc. Conf*., pp: 215–219. Vol. 1. *Manila, Philippines.*

Moon, B.C., J.W. Kim, S.H. Cho, J.E. Park, J.S. Song and D.S. Kim, 2014. Modelling the effects of herbicide dose and weed density on rice–weed competition. *Weed Res*., 54: 484–491

Neve, P., M. Vila-Aiub and F. Roux, 2009. Evolutionary thinking in agricultural weed management. *New Phytol*., 184: 783–793

Osuna, M.D., M. Okada, R. Ahmad, A.J. Fischer and M. Jasieniuk, 2011. Genetic diversity and spread of thiobencarb resistant early watergrass (*Echinochloa oryzoides*) in California. *Weed Sci.*, 59: 195–201

Owen, M.D. and I.A. Zelaya, 2005. Herbicideresistant crops and weed resistance to herbicides. *Pest Manag. Sci*., 61: 301–311

Park, T.S., B.I. Ku, S.K. Kang, M.K. Choi, H.K. Park, K.B. Lee and J.K. Ko, 2010. Response of the resistant biotype of *Echinochloa oryzoides* to ACCase and ALS inhibitors, and effect of alternative herbicides. *Kor. J. Weed Sci*., 30: 291–299

Ren, M.X., Q.G. Zhang and D.Y. Zhang, 2005. Random amplified polymorphic DNA markers reveal low genetic variation and a single dominant genotype in *Eichhornia crassipes* populations throughout China. *Weed Res.*, 45: 236–244

Roy, S., J.P. Simon and F.J. Lapointe, 2000. Determination of the origin of the cold-adapted populations of baryard grass (*Echinochloa crus-galli*) eastern North America total evidence approach using RAPD-DNA and DNA sequences. *Can. J. Bot*., 78: 1505–1513

Rutledge, J., R.E. Talbert and C.H. Sneller, 2000. RAPD analysis of genetic variation among propanil-resistant and susceptible *Echinochloa crus-galli* populations in Arkansas. *Weed Sci*., 48: 669–674

Santaella, J.P.R., F. Bastida, A.R. Franco and R.D. Prado, 2006. Morphological and molecular characterization of different *Echinochloa* spp. and *Oryza sativa* populations. *J. Agric. Food Chem*., 54: 1166–1172

Stankiewicz, M., G. Gadamski and S.W. Gawronski, 2001. Genetic variation and phylogenetic relationships of triazine-resistant and triazine-susceptible biotypes of *Solanum nigrum*: analysis using RAPD markers. *Weed Res.*, 41: 287–300

Sterling, T.M., D.C. Thompson and L.B. Abbott, 2004. Implications of invasive plan variation for weed management. *Weed Technol*., 18: 1319–1324

Tasrif, A., A.S. Juraimi, J. Kadir, S.S. Sastroutomo and S. Napis, 2004. Genetic diversity of *Echinhloa crus-galli var. crus-galli* (L.) Beauv. (Barnyardgrass: *Poaceae*) ecotypes in Malaysia and Indonesia as revealed by RAPD markers. *Asian J. Plant Sci*., 32: 231–238

Tayyar, R.I., J.H.T. Nguyen and J.S. Holt, 2003. Genetic and morphological analysis of two novel nutsedge biotypes from California. *Weed Sci*., 5: 731–739

Tsuji, R., A.J. Fischer, M. Yoshino, A. Roel, J.E. Hill and Y. Yamasue, 2003. Herbicide-resistant late watergrass (*Echinochloa phyllopogon*): Similarity in morphological and amplified fragment length polymorphism traits. *Weed Sci*., 51: 740–747

Vellend, M., 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. *Amer. Nat*., 166: 199–215

Vigueira, C.C., K.M. Olsen and A.L. Caicedo, 2013. The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. *Heredity (Edinb)*, 110: 303–311

Vilatersana, R., T. Garnatje, A. Susanna and N. Garcia-Jacas, 2005. Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification. *Bot. J. Linn. Soc*., 147: 375–383

Whitney, B., B.D. Forest, K. Al-Khatib and A.J. Fischer, 2017. Predicting yield losses in rice mixed-weed species infestations in California. *Weed Sci*., 65: 61–72

Yabuno, T., 1996. Taxonomy and genealogy of *Echinochloa* plants. *In: Natural history of Echinochloa Plants*, pp: 16–28. Yabuno, T. and H. Yamaguchi (eds.). DowElanco (Japan), Tokyo, Japan

Yamaguchi, H., S. Umemoto and Y. Masanaga, 1996. Studies on barnyard grasses, especially on non-shattering form of *Echinochloa oryzicola* Vasing., in Yungui plateau, China. *Weed Res. Jpn*., 41: 111

**(Received 29 September 2017; Accepted 24 November 2017)**