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# Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) resistance to acetolactate synthase-inhibiting and other herbicides in rice in Turkey

*Koray Kacan*[1], *Nihat Tursun*[2], *Hayat Ullah*[3], *Avishek Datta*[3]*

[1]*Department of Plant and Animal Production, Ortaca Vocational School, Mugla Sitki Kocman University, Mugla, Turkey*
[2]*Department of Plant Protection, Faculty of Agriculture, Malatya Turgut Ozal University, Malatya, Turkey*
[3]*Department of Food, Agriculture and Bioresources, School of Environment, Resources and Development, Asian Institute of Technology, Klong Luang, Pathum Thani, Thailand*
**Corresponding author: datta@ait.ac.th; avishek.ait@gmail.com*

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**Abstract:** Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) is one of the most yield-limiting weeds in rice in Turkey. Barnyardgrass resistance to common herbicides has been reported worldwide; however, such information is largely lacking in the country. The objective of this study was to determine the resistance spectrum of different barnyardgrass populations to the most commonly-used herbicides in rice in Turkey. The susceptibility of 40 barnyardgrass populations was evaluated. The samples were collected from fields with intensive rice cultivation in Balıkesir and Çanakkale provinces. Seeds were picked from barnyardgrass plants suspected to be herbicide-resistant because of their survival in the rice fields after herbicides application. A total of 38 populations were resistant to penoxsulam, and the resistance index of these populations ranged from 2 to 39. A total of 24 out of the 38 barnyardgrass populations showed a $GR_{50}$ (herbicide dose causing a 50% reduction in plant dry matter) value higher than the recommended penoxsulam dose (20.2 g a.i./ha) in rice. Among these 24 barnyardgrass populations, 25, 29.2 and 45.8% populations exhibited high, moderate and low level of penoxsulam resistance, respectively. From the penoxsulam-resistant populations (38), the response of 14 populations (low to high resistance to penoxsulam) to six commonly-used herbicides for barnyardgrass control in rice was evaluated. The selected 14 populations showed resistance to almost all herbicides tested, with the lowest average resistance being determined against profoxydim and the highest average resistance against molinate herbicide. Resistance levels against six commonly-used herbicides in rice ranged from 2 to 34.

**Keywords:** chemical control; herbicide resistance; *Oryza sativa* L.; enzyme inhibitor; weed management

Rice (*Oryza sativa* L.) is one of the most widely-grown cereal crops in the world with approximately 782 million tonnes of annual production (FAO 2018). In Turkey, the average rice yield is 7 824 kg/ha, while the world average is 4 678 kg/ha (FAO 2018). The total annual rice production in Turkey is about 940 000 tonnes, which is not sufficient to meet the domestic demand (FAO 2018). Monoculture paddy rice production is common in Turkey's Marmara region, which is the most important rice-producing area. Weed infestation is among the major constraints for sustainable rice production in the country as weed competes with rice for nutrients and other important resources and acts as host for insect pests and diseases. Yield losses in rice caused by uncontrolled weeds have been reported between 15% and 42% in Turkey depending on rice cultivar, crop establishment method, type of weed species and weed densities (Mennan et al. 2012). Herbicides are commonly used for controlling weeds in crop production system. However, repeated and extensive use of the same (one) herbicide or different herbicides with the same mode of action over time has led to the development of herbicide resistance worldwide since the 1970's (Holt 1992, Avcı and Uygur 2008, Knezevic et al. 2009, Burgos et al. 2013, Datta and Knezevic 2013, Datta et al. 2017).

Herbicide resistance has been reported in 70 countries and 92 crops (Heap 2020). Herbicide-resistant weed species have rapidly increased over the last few decades and approximately five new cases per year have been recorded between 1990 and 2015 on average (Kniss 2018). Globally, 164 cases (species × country × site of action) of herbicide-resistant weed populations and 35 cases (country × site of action) of herbicide-resistant barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) populations have been reported in rice (Heap 2020). Resistance to acetolactate synthase (ALS) enzyme inhibitors, acetyl coenzyme A carboxylase (ACCase) enzyme inhibitors, photosystem II inhibitors, synthetic auxins, long chain fatty acid inhibitors, lipid inhibitors, carotenoid biosynthesis inhibitors and cellulose inhibitors has been found in weeds including barnyardgrass (Heap 2020). It was reported that monocotyledon weeds are generally resistant to ACCase and ALS inhibitor herbicides in rice across various countries (Heap 2020).

Barnyardgrass is an annual grass weed, which reproduces through seed and can grow up to 150 cm in height (Bagavathiannan et al. 2012). It is a widely distributed weed species and is recognised as the world's most serious weed of rice affecting up to 36 crops in 61 countries (Riar et al. 2012, Chen et al. 2016). It can proliferate in temperate, tropical and subtropical regions, and a single barnyardgrass plant is able to produce up to 39 000 seeds (Bagavathiannan et al. 2012, Miller et al. 2018). Barnyardgrass causes economic damage to fruits, vegetables and other crops, and it has become a seriously problematic weed in Turkish rice fields and elsewhere over the last two decades (Isık et al. 2000, Kaya Altop and Mennan 2011, Mennan et al. 2012, Kraehmer et al. 2016, Peterson et al. 2018). Because of its wide ecological tolerance, high adaptability, rapid germination, abundant seed production and strong competitive ability, economic losses from its proliferation continue to mount (Lopez-Martinez et al. 1999, Mennan et al. 2012, Heap 2014, Bajwa et al. 2015). This noxious weed can cause yield losses between 21% and 79% in rice depending on the cropping system and management (Ottis and Talbert 2007, Wilson et al. 2014). The most effective method of controlling barnyardgrass is the use of synthetic herbicides, but resistant biotypes have evolved due to repeated and excessive use of the same (one) herbicide or different herbicides with the same mode of action over time, leading to reduced herbicide effectiveness and serious productivity losses (Datta et al. 2017, Song et al. 2017, Fang et al. 2019). Current chemical weed control methods have thus become insufficient (Chauhan and Abugho 2013, Kaya Altop et al. 2014, Guo et al. 2017).

Barnyardgrass resistance to common herbicides with different modes of action was first detected in Canadian maize (*Zea mays* L.) fields in 1990 (Stephenson et al. 1990, Heap 2018). However, the resistance of barnyardgrass to the most commonly-used herbicides in rice is largely lacking in Turkey. Many Turkish farmers primarily depend on ALS-inhibiting herbicides (azimsulfuron, bispyribac-sodium, penoxsulam, imazamox) for weed control in rice. The other widely-used herbicide group in rice is ACCase enzyme inhibitors (cyhalofop-butyl, profoxydim, fenoxaprop-P-ethyl). In addition, growers also use two more herbicides belonging either to lipid biosynthesis inhibitor (molinate) or pigment inhibitor (clomazone, aclonifen, amitrol) chemical families in rice. More detailed information on the resistance of barnyardgrass to the most commonly-used herbicides in rice in Turkey is thus urgently needed. This information would help farmers better understand effective suppression of this weed and optimise their crop yields. Therefore, the objective of this study was to determine the resistance spectrum of different barnyardgrass populations to the most commonly-used herbicides in rice in the provinces of Çanakkale and Balıkesir in the Marmara region, which is Turkey's most important rice-producing area.

## MATERIAL AND METHODS

**Seed collection.** Panicles of 39 barnyardgrass populations suspected of being resistant against ALS- and ACCase-inhibiting herbicides as well as one susceptible population were collected from various rice fields in Balıkesir (40°06'N, 27°47'E) and Çanakkale (40°16'N, 27°17'E) provinces of the Marmara region between August and September 2014 (Table 1). Panicles were collected from rice fields where inconsistent control of barnyardgrass had been repeatedly reported by growers with the application of ALS- and ACCase-inhibiting herbicides. Barnyardgrass panicles with suspected susceptibility were collected from fields and roadside areas where no herbicide was previously applied. Sampling sites were randomly selected within each field based on the presence of barnyardgrass during the time of

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Table 1. Barnyardgrass populations listed by global positioning system coordinates from which they were collected in 2014

| Number | Population | Latitude (°N) | Longitude (°E) |
|---|---|---|---|
| 1 | CAK4 | 40.413 | 27.302 |
| 2 | CAC4 | 40.211 | 27.184 |
| 3 | BAG3 | 40.074 | 27.391 |
| 4 | BAT5 | 40.073 | 27.392 |
| 5 | BAG4 | 40.093 | 27.395 |
| 6 | BAT1 | 40.083 | 27.391 |
| 7 | BAM4 | 40.063 | 27.562 |
| 8 | BAT2 | 40.081 | 27.385 |
| 9 | BAM1 | 40.060 | 27.560 |
| 10 | BAS1 | 40.173 | 27.164 |
| 11 | CAB3 | 40.154 | 27.123 |
| 12 | CAT6 | 40.044 | 27.373 |
| 13 | BAM6 | 40.053 | 27.564 |
| 14 | CAC3 | 40.063 | 27.551 |
| 15 | BAG2 | 40.105 | 27.401 |
| 16 | CAB6 | 40.162 | 27.155 |
| 17 | CAK1 | 40.200 | 27.185 |
| 18 | CAC1 | 40.190 | 27.152 |
| 19 | BAM7 | 40.062 | 27.551 |
| 20 | BAG5 | 40.043 | 27.363 |
| 21 | CAC5 | 40.194 | 27.154 |
| 22 | BAT3 | 40.075 | 27.390 |
| 23 | BAB4 | 40.156 | 27.128 |
| 24 | BAG1 | 40.092 | 27.411 |
| 25 | BAM5 | 40.035 | 27.540 |
| 26 | BAT4 | 40.103 | 27.400 |
| 27 | CAC6 | 40.174 | 27.140 |
| 28 | CAC7 | 40.172 | 27.142 |
| 29 | CAB1 | 40.182 | 27.161 |
| 30 | BAM8 | 40.015 | 27.510 |
| 31 | CAC2 | 40.175 | 27.139 |
| 32 | CAK3 | 40.203 | 27.171 |
| 33 | CAK6 | 40.204 | 27.172 |
| 34 | CAB2 | 40.055 | 27.581 |
| 35 | BAM2 | 40.014 | 27.501 |
| 36 | CAK2 | 40.204 | 27.173 |
| 37 | BAM3 | 40.035 | 27.556 |
| 38 | BAB7 | 40.062 | 27.583 |
| 39 | CAK5 | 40.208 | 27.178 |
| 40 | CAB5* | 40.073 | 27.573 |

*indicates susceptible population

collection. The number of panicles collected was dependent on the density of barnyardgrass present within each field. A handheld global positioning system was utilised to record the coordinates for each sampling site during the time of panicle collection. Panicles were threshed after collection, seeds were cleaned, air dried and separately stored in paper bags in a refrigerator at 4 °C until further use.

**Greenhouse experiments.** The susceptibility of barnyardgrass populations to both ALS-inhibitor group involving three herbicides (azimsulfuron, bispyribac-sodium and penoxsulam) and ACCase-inhibitor group involving two herbicides (cyhalofop-butyl and profoxydim) was evaluated. In addition, barnyardgrass resistance to lipid biosynthesis inhibitor group involving one herbicide (molinate) and pigment inhibitor group involving one herbicide (clomazone) was tested (Table 2).

Seeds from each group (resistant and susceptible populations) were randomly selected and 10 seeds per group were planted in plastic pots (25 cm height and 15 cm wide). The pots were filled with a 2:1:1 mixture (*w*/*w*) of soil, peat and sand (Mahmood et al. 2016). Pots were arranged in a completely randomised design with five replications. After emergence, seedlings were thinned to five plants per pot. The experiment was maintained in a greenhouse under natural sunlight. During the vegetation period, the average daily minimum temperature was 20 ± 2 °C and the maximum average temperature was 35 ± 2 °C. All pots were irrigated by an automatic tap water irrigation system whenever necessary during the growing period.

Pre-emergence herbicides (molinate and clomazone) were applied five days before sowing of barnyardgrass and water was applied at the second day after planting following manufacturer directions. Post-emergence herbicides (azimsulfuron, bispyribac-sodium, penoxsulam, cyhalofop-butyl and profoxydim) were applied at the 2–4 leaf stage of barnyardgrass. Herbicides were applied using a backpack sprayer equipped with a F110 flat-fan nozzle, which contains four nozzles with a 2 m working width, and it was set at 304 kPa pressure. Herbicide doses used in this study were determined by mixing in a solution totalling 300 L of water/ha. The amount of water was calibrated as at a field application, and homogeneous distribution of the same amount of herbicide was ensured in the experiment. No adjuvant was mixed with any herbicide.

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Table 2. Description of the tested herbicides and used application doses in Experiments 1 and 2

| Herbicide | Site of action | Manufacturer | Formulation | Tested application doses (g a.i./ha) |
|---|---|---|---|---|
| Azimsulfuron | ALS or AHAS inhibitor | Dupont | 50% WDG | 0, 7.5, 15, 30 (recommended dose), 60, 120 and 240 |
| Bispyribac-sodium | ALS or AHAS inhibitor | Bayer | 420 g/L SC | 0, 5.25, 10.5, 21 (recommended dose), 42, 84 and 168 |
| Penoxsulam | ALS or AHAS inhibitor | Dow AgroSciences | 25.2 g/L OD | 0, 5.05, 10.1, 20.2 (recommended dose), 40.4, 80.8 and 161.6 |
| Cyhalofop-butyl | ACCase inhibitor | Agrobest | 200 g/L EC | 0, 75, 150, 300 (recommended dose), 600, 1 200 and 2 400 |
| Profoxydim | ACCase inhibitor | BASF | 75 g/L EC | 0, 28.125, 56.25, 112.5 (recommended dose), 225, 450 and 900 |
| Molinate | lipid biosynthesis inhibitor | Koruma | 750 g/L EC | 0, 900, 1 800, 3 600 (recommended dose), 7 200, 14 400 and 28 800 |
| Clomazone | pigment inhibitor | Entosav | 480 g/L EC | 0, 180, 360, 720 (recommended dose), 1 440, 2 880 and 5 760 |

ALS – acetolactate synthase; AHAS – acetohydroxyacid synthase; ACCase – acetyl coenzyme A carboxylase; WDG – water dispersible granules; SC – suspension concentrate; OD – oil dispersion; EC – emulsifiable concentrate; a.i. – active ingredient

**Experiment 1: Penoxsulam resistance of 40 barnyardgrass populations.** The first greenhouse experiment was conducted between January and April 2015 at the Plant Protection Department Laboratory of the Mugla Sitki Kocman University located in Mugla, Turkey. In this experiment, resistance spectrum of 40 barnyardgrass populations to penoxsulam was determined. Application doses of penoxsulam are presented in Table 2.

**Experiment 2: Resistance of 15 barnyardgrass populations to different herbicides.** The second greenhouse experiment was conducted between June and September 2015 at the same location where the first experiment was carried out. In this experiment, 15 barnyardgrass populations (14 penoxsulam-resistant populations and 1 penoxsulam-susceptible population) were selected from the first greenhouse experiment (Table 3). Penoxsulam-resistant barnyardgrass populations (14) were selected among the 38 populations that exhibited low to high resistance to penoxsulam based on their resistance index (RI) values (between 2 and 39) (Table 3), and those populations that produced seeds from the survived plants in Experiment 1. Penoxsulam-susceptible barnyardgrass population (1) was selected between two populations that exhibited no resistance to penoxsulam based on their RI values (< 2). These populations were treated to determine their resistance to six commonly-used herbicides (azimsulfuron, bispyribac-sodium, cyhalofop-butyl, profoxydim, molinate and clomazone) in rice. These herbicides were applied at 0, 1/4, 1/2, 1, 2, 4 and 8 fold of their recommended dose (Table 2).

Plants in both experiments were harvested at 35 and 28 days after pre-emergence and post-emergence herbicide application, respectively. All five plants in each pot were harvested and the plant samples were oven-dried at 70 °C to a constant weight to determine their dry matter (DM), which was later converted to "per plant" basis. Plant DM was expressed as a percentage of untreated plants. For both experiments, $GR_{50}$ (herbicide dose causing a 50% reduction in plant dry matter) values were calculated for each barnyardgrass population under a particular herbicide application based on the analyses of the dose-response curves. The $GR_{50}$ value is defined as the effective herbicide dose that caused a 50% DM reduction relative to untreated plants. Whole-plant DM was assessed for both experiments.

**Data analysis.** There were five replications of each treatment and each experiment was repeated two times. DM reduction (%) data were subjected to a nonlinear regression analysis over herbicide dose using the three-parameter log-logistic model where the lower limit (*C* term) was fixed at zero (Knezevic et al. 2007, Ulloa et al. 2011):

$$Y = D/\{1 + \exp[B (\log X - \log E)]\}$$

where: $Y$ – response (e.g., percent DM reduction); $D$ – upper limit; $B$ – slope of the line at the inflection point (also known as the rate of change); $E$ – dose resulting in a 50% response between the upper and lower limit (also known as inflection point, $GR_{50}$).

Table 3. $GR_{50}$ (herbicide dose causing a 50% reduction in plant dry matter) values (g a.i./ha) and resistance index of 40 barnyardgrass populations against penoxsulam based on whole-plant dry matter (Experiment 1)

| Population | Regression parameter (± standard error) | | | RI |
|---|---|---|---|---|
| | $GR_{50}$ | *t*-value | *P*-value | |
| CAC3 | 280.2 ± 3.7 | 18.76 | 0.00 | 39 |
| BAT5 | 211.7 ± 20.7 | 10.6 | 0.55 | 29 |
| BAT2 | 128.9 ± 13.5 | 19.8 | 0.00 | 18 |
| BAT3 | 117.8 ± 3.4 | 23.12 | 0.00 | 16 |
| BAG1 | 106.9 ± 3.6 | 21.88 | 0.00 | 15 |
| CAK4 | 76.2 ± 8.1 | 10.90 | 0.30 | 11 |
| BAT1 | 66.8 ± 12.5 | 10.95 | 0.35 | 9 |
| BAM5 | 60.2 ± 0.8 | 13.95 | 0.00 | 8 |
| BAG4 | 58.5 ± 5.6 | 20.42 | 0.02 | 8 |
| BAM4 | 55.5 ± 14.3 | 20.02 | 0.05 | 8 |
| CAC7 | 49.1 ± 1.2 | 4.46 | 0.00 | 7 |
| BAT6 | 45.5 ± 4.2 | 21.05 | 0.00 | 6 |
| CAB4 | 40.8 ± 6.4 | 3.28 | 0.00 | 6 |
| BAT4 | 39.1 ± 2.7 | 12.91 | 0.00 | 5 |
| CAK6 | 37.8 ± 0.07 | 10.7 | 0.00 | 5 |
| CAB3 | 36.8 ± 4.2 | 10.95 | 0.35 | 5 |
| BAM2 | 29.8 ± 5.1 | 10.84 | 0.00 | 4 |
| BAM3 | 28.9 ± 1.7 | 5.3 | 0.00 | 4 |
| CAC4 | 27.6 ± 3.1 | 2.55 | 0.02 | 4 |
| BAS1 | 26.0 ± 2.2 | 2.36 | 0.03 | 4 |
| BAB1 | 24.4 ± 5.3 | 1.87 | 0.00 | 3 |
| BAM6 | 22.9 ± 1.5 | 1.57 | 0.13 | 3 |
| BAM7 | 21.2 ± 0.7 | 10.59 | 0.12 | 3 |
| BAM8 | 20.4 ± 4.1 | 4.98 | 0.00 | 3 |
| CAK5 | 20.1 ± 3.7 | 8.7 | 0.00 | 3 |
| CAB2 | 19.2 ± 2.1 | 4.5 | 0.00 | 3 |
| CAK3 | 18.7 ± 0.9 | 1.9 | 0.02 | 3 |
| BAG2 | 18.5 ± 3.4 | 10.54 | 0.60 | 3 |
| CAC1 | 18.3 ± 1.2 | 1.57 | 0.13 | 3 |
| BAM1 | 17.8 ± 2.2 | 7.37 | 0.00 | 2 |
| CAC5 | 17.7 ± 1.3 | 20.02 | 0.00 | 2 |
| CAK1 | 17.5 ± 3.5 | 9.80 | 0.00 | 2 |
| CAC2 | 17.2 ± 2.5 | 1.8 | 0.08 | 2 |
| CAB7 | 16.3 ± 2.1 | 8.3 | 0.00 | 2 |
| CAK2 | 15.1 ± 3.1 | 2.1 | 0.04 | 2 |
| BAG5 | 14.7 ± 1.57 | 9.34 | 0.00 | 2 |
| CAB6 | 12.5 ± 1.3 | 10.98 | 0.06 | 2 |
| CAC6 | 11.7 ± 1.8 | 0.65 | 0.50 | 2 |
| CAB5 (susceptible) | 7.2 ± 1.4 | 3.94 | 0.00 | 1 |
| BAG3 | 6.4 ± 1.5 | 8.98 | 0.00 | 1 |

RI – resistance index; a.i. – active ingredient

$GR_{50}$ values were used to compare DM reduction of each barnyardgrass population under a particular herbicide (Matzenbacher et al. 2015, Chen et al. 2016). Analyses of the dose-response curves were performed using the R software (R version 2.15.3, R Development Core Team 2013) utilising the drc (dose-response curves) statistical add-on package (Knezevic et al. 2007, Knezevic and Datta 2015). A lack-of-fit test at the 5% level was not significant for any of the dose-response curves indicating that the log-logistic model was appropriate for the data analyses (Ulloa et al. 2011, Leskovsek et al. 2012, Datta et al. 2013).

For each herbicide, the susceptibility of each barnyardgrass population was determined by calculating resistance index as described by Beckie and Tardif (2012), Yang et al. (2013) and Chen et al. (2016). It was calculated by dividing the $GR_{50}$ value of each suspected resistant population by the $GR_{50}$ value of one suspected susceptible sample (in the present study, population CAB5). Using the same methods as outlined by Beckie and Tardif (2012), the herbicide susceptibility was classified into five groups: no resistance (RI < 2); low resistance (RI = 2–5); moderate resistance (RI = 6–10); high resistance (RI = 11–100) and very high resistance (RI > 100).

## RESULTS

**Resistance to penoxsulam.** In the first greenhouse experiment, 38 populations out of the total amount (40) were found to be resistant to penoxsulam and the RI values ranged from 2 to 39 (Table 3). Therefore, to achieve a 50% reduction in DM, it was necessary to use doses that were 2–39 times higher for the resistant populations than for the susceptible one. Barnyardgrass populations that were resistant to penoxsulam were less affected by this herbicide compared with barnyardgrass populations that were susceptible.

A total of 24 out of 38 barnyardgrass populations collected from rice fields showed a $GR_{50}$ value higher than the recommended penoxsulam dose (20.2 g a.i. (active ingredient)/ha) (Table 3). The RI values of these 24 barnyardgrass populations ranged from 3 to 39. Among these 24 barnyardgrass populations, 6 populations exhibited high resistance (RI = 11–39), 7 populations showed moderate resistance (RI = 6–9) and 11 populations had low resistance (RI = 3–5) to penoxsulam.

**Resistance of selected barnyardgrass populations to other herbicides.** Population CAB5 was highly susceptible to most of the tested herbicides, with a $GR_{50}$ value < 50% of the recommended doses (Table 4). From the 14 tested populations, nine had $GR_{50}$ values ranging from 1.1–3.9 times the recommended dose of azimsulfuron. For clomazone, only two populations (BAT1 and CAB6) had $GR_{50}$ values higher than the recommended dose. Overall, fewer populations required a higher than the recommended dose of pigment inhibitor (clomazone: 2 populations) and lipid biosynthesis inhibitor (molinate: 4 populations) to result in a 50% reduction in DM. In contrast, more populations required a higher than the recommended dose of ACCase inhibitor (cyhalofop-butyl: 6 populations, profoxydim: 7 populations) and ALS-inhibitor (azimsulfuron: 9 populations, bispyribac-sodium: 10 populations) to result in a 50% DM reduction.

Resistance index was obtained by dividing $GR_{50}$ values of all the resistant populations by the $GR_{50}$ value of the susceptible one (CAB5) under a particular herbicide (Table 4). For 14 penoxsulam-resistant barnyardgrass populations that were tested to azimsulfuron, the RI values ranged from 1 to 20 (Table 5). All populations treated with cyhalofop-butyl had RI values between 3 and 24. Overall, all populations treated with profoxydim had RI values between 1 and 5, which were substantially lower than for any other herbicide. Among 14 resistant populations, the following ones showed high resistance (RI = 11 to 100): 5 populations to azimsulfuron (RI = 11 to 20), 4 populations to bispyribac-sodium (RI = 13 to 21), 6 populations to cyhalofop-butyl (RI = 12 to 24), 5 populations to molinate (RI = 12 to 34) and 6 populations to clomazone (RI = 14 to 21). A total of 4 populations to azimsulfuron (RI = 6 to 10), 5 populations to bispyribac-sodium (RI = 7 to 9), 4 populations to cyhalofop-butyl (RI = 6 to 9), 2 populations to molinate (RI = 8 to 10) and 3 populations to clomazone (RI = 6 to 10) exhibited moderate resistance (RI = 6 to 10). Population CAB3 had the highest resistance to molinate (RI = 34) followed by population BAT2 with the RI value of 29 (Table 5).

## DISCUSSION

Like in most parts of the world, farmers in Turkey also use herbicides for controlling weeds in rice. Barnyardgrass is among the most troublesome weeds of rice in the world including Turkey and it has developed resistance to herbicides (Norsworthy et al. 1998, Isık et al. 2000, Hoagland et al. 2004, Mennan et al. 2012, Heap 2018), which has seriously constrained the

Table 4. The $GR_{50}$ (herbicide dose causing a 50% reduction in plant dry matter) (± standard error) values (g a.i./ha) and the recommended doses of six commonly-used herbicides in rice in Turkey for 14 penoxsulam-resistant populations and 1 penoxsulam-susceptible population (Experiment 2)

| Herbicide | CAC3 | BAT5 | BAT2 | BAT3 | CAK4 | BAT1 | BAG4 | CAB3 | CAC4 | BAS1 | BAM1 | CAK1 | CAB6 | CAB5* | BAM4 | Recommended dose (g a.i./ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Azimsulfuron | 15.5<br>± 4.1 | 116.8<br>± 11.5 | 42.2<br>± 6.9 | 62.4<br>± 5.3 | 18.2<br>± 1.3 | 62.3<br>± 6.7 | 58.7<br>± 8.1 | 40.2<br>± 3.3 | 34.1<br>± 4.1 | 3.9<br>± 0.4 | 22.2<br>± 4.1 | 90.5<br>± 1.6 | 88.6<br>± 12.1 | 5.9<br>± 2.3 | 13.9<br>± 0.7 | 30 |
| Bispyribac-sodium | 17.6<br>± 6.2 | 20.7<br>± 4.3 | 55.4<br>± 8.4 | 23.5<br>± 6.1 | 10.3<br>± 0.5 | 29.2<br>± 7.4 | 41.3<br>± 4.3 | 41.4<br>± 4.4 | 28.9<br>± 5.4 | 94.2<br>± 5.6 | 61.2<br>± 6.7 | 56.0<br>± 8.9 | 7.3<br>± 0.5 | 4.4<br>± 0.1 | 38.3<br>± 8.7 | 21 |
| Cyhalofop-butyl | 94.1<br>± 8.4 | 134.2<br>± 49.8 | 333.8<br>± 11.2 | 110.7<br>± 14.6 | 172.1<br>± 18.2 | 264.5<br>± 37.8 | 89.7<br>± 11.5 | 406.3<br>± 1.2 | 402.2<br>± 11.2 | 480.0<br>± 13.8 | 684.7<br>± 10.4 | 183.6<br>± 22.6 | 680.2<br>± 34.3 | 28.4<br>± 0.3 | 182.6<br>± 5.1 | 300 |
| Profoxydim | 84.5<br>± 2.1 | 116.8<br>± 14.5 | 54.2<br>± 12.9 | 117.8<br>± 3.4 | 157.8<br>± 27.4 | 58.8<br>± 7.1 | 139.2<br>± 5.8 | 109.9<br>± 2.3 | 77.8<br>± 38.5 | 266.9<br>± 12.2 | 333.0<br>± 0.1 | 120.5<br>± 1.6 | 88.0<br>± 0.2 | 64.2<br>± 2.3 | 64.3<br>± 15.3 | 112.5 |
| Molinate | 1 500.7<br>± 9.8 | 350.5<br>± 11.7 | 5 508.9<br>± 8.6 | 570.5<br>± 16.7 | 608.2<br>± 27.4 | 2 206.5<br>± 5.7 | 205.4<br>± 3.5 | 6 300.6<br>± 6.5 | 1 900.8<br>± 17.6 | 150.1<br>± 0.3 | 620.8<br>± 9.3 | 402.6<br>± 11.3 | 4 222.6<br>± 34.2 | 187.3<br>± 5.1 | 4 700.2<br>± 14.7 | 3 600 |
| Clomazone | 260.4<br>± 5.9 | 618.9<br>± 35.2 | 400.1<br>± 11.5 | 207.6<br>± 12.1 | 240.5<br>± 12.7 | 768.8<br>± 34.1 | 21.3<br>± 4.7 | 38.7<br>± 3.5 | 581.6<br>± 22.9 | 594.3<br>± 6.6 | 168.4<br>± 3.8 | 540.6<br>± 6.2 | 821.1<br>± 3.1 | 39.6<br>± 0.2 | 164.7<br>± 2.5 | 720 |

*indicates susceptible population; a.i. – active ingredient

Table 5. Resistance index values of 14 penoxsulam-resistant barnyardgrass populations against six commonly-used herbicides in rice in Turkey (Experiment 2)

| Herbicide | CAC3 | BAT5 | BAT2 | BAT3 | CAK4 | BAT1 | BAG4 | BAM4 | CAB3 | CAC4 | BAS1 | BAM1 | CAK1 | CAB6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Azimsulfuron | 2.6 | 19.8 | 7.2 | 10.6 | 3.1 | 10.6 | 9.9 | 2.4 | 6.8 | 5.8 | 0.7 | 3.8 | 15.3 | 15.0 |
| Bispyribac-sodium | 4.0 | 4.7 | 12.6 | 5.3 | 2.3 | 6.6 | 9.4 | 8.7 | 9.4 | 6.6 | 21.4 | 13.9 | 12.7 | 1.7 |
| Cyhalofop-butyl | 3.3 | 4.7 | 11.8 | 3.9 | 6.1 | 9.3 | 3.2 | 6.4 | 14.3 | 14.2 | 16.9 | 24.1 | 6.5 | 23.9 |
| Profoxydim | 1.3 | 1.8 | 0.8 | 1.8 | 2.5 | 0.9 | 2.2 | 1.0 | 1.7 | 1.2 | 4.2 | 5.2 | 1.9 | 1.4 |
| Molinate | 8.0 | 1.9 | 29.4 | 3.1 | 3.2 | 11.8 | 1.1 | 25.1 | 33.6 | 10.1 | 0.8 | 3.3 | 2.1 | 22.5 |
| Clomazone | 6.6 | 15.6 | 10.1 | 5.2 | 6.1 | 19.4 | 0.5 | 4.2 | 1.0 | 14.7 | 15.0 | 4.3 | 13.7 | 20.7 |

productivity of rice (Mennan et al. 2012, Peterson et al. 2018). In Turkey, rice grain yield loss due to the presence of barnyardgrass has been reported between 6% and 30% depending on weed density, rice cultivar, location and growing season (Mennan et al. 2012). Herbicide resistance makes weed control further difficult.

In the present study, 38 populations were found to be resistant (low to high) to penoxsulam, out of which 6 populations exhibited high resistance (RI = 11 to 39) to this herbicide (Table 3). Other populations showed low to moderate resistance. In addition, 14 populations with low to high resistance to penoxsulam also exhibited resistance to six other commonly-used herbicides in rice in Turkey. The present results are in line with the previous findings where barnyardgrass populations were found resistant to a variety of herbicides including clomazone, penoxsulam, bispyribac-sodium and cyhalofop-butyl (Yang et al. 2013, Norsworthy et al. 2014, Iwakami et al. 2015, Chen et al. 2016), as well as other ACCase and ALS inhibitors in rice (Beckie and Tardif 2012, Heap 2018). In this study, population BAG4 showed low to moderate resistance to all post-emergence herbicides (ALS and ACCase inhibitors), but exhibited susceptibility to two pre-emergence herbicides (molinate and clomazone) confirming the effectiveness of pre-emergence herbicides (Table 5). Similarly, BAT3 and BAM1 populations also exhibited susceptibility to molinate and clomazone. CAB3 and BAS1 populations were susceptible to only one of the pre-emergence herbicides; all the other populations showed low to moderate resistance to two pre-emergence herbicides.

Overall, this study confirmed that barnyardgrass populations collected from rice fields in Balıkesir and Çanakkale provinces of the Marmara region in Turkey have developed resistance to penoxsulam, where a total of 24 out of 38 populations showed a $GR_{50}$ value higher than the recommended penoxsulam dose of 20.2 g a.i./ha. In addition, the selected 14 out of the 38 barnyardgrass populations also exhibited varied levels of resistance (RI = 2 to 34) to six commonly-used herbicides in rice. These results are consistent with other studies showing that penoxsulam-resistant barnyardgrass populations were 2–53 times more resistant to other frequently-used herbicides in rice compared with penoxsulam-susceptible populations (Chen et al. 2016). However, in the present study, 14 penoxsulam-resistant populations were relatively more susceptible to profoxydim than to other herbicides (Table 5). This relative susceptibility of penoxsulam-resistant populations to one of the ACCase inhibitor herbicides (profoxydim) could be due to population differences in morphology and genetic backgrounds as well as changes in chemical weed management practice (Kaya Altop and Mennan 2011). Turkish farmers are heavily dependent on ALS-inhibiting herbicides (azimsulfuron, bispyribac-sodium, penoxsulam) for weed control in rice, which could lead to the development of herbicide-resistant weed populations. Better control of penoxsulam-resistant populations with ACCase inhibitor herbicide (profoxydim) might be the result of using a new herbicide with different mode of action than ALS-inhibiting herbicide (penoxsulam).

Fourteen barnyardgrass populations selected from the 38 penoxsulam-resistant populations showed resistance to other ALS and ACCase inhibitor herbicides at different levels (RI = 2–34). BAG4 population showed susceptibility to clomazone and molinate-effective herbicides, whereas BAS1 population exhibited susceptibility to azimsulfuron and molinate. In addition, each population, except BAT5, BAT3, CAK4, BAM1 and CAK1, exhibited susceptibility to at least one active herbicide. The penoxsulam-resistant barnyardgrass populations (14) showed different levels of susceptibility to ACCase inhibitor herbicides cyhalofop-butyl and profoxydim. On the other hand, these populations exhibited almost similar level of resistance (the same total RI value) to other ALS inhibitor herbicides (azimsulfuron and bispyribac-sodium) with at least 4 populations showing high resistance (RI = 11 to 21) against each herbicide. The $GR_{50}$ values of the penoxsulam-resistant populations were also higher than those of the susceptible population for six herbicides, which could be linked with the differences in their genetic backgrounds. Genetic variations between herbicide-resistant and herbicide-susceptible populations dictate the formation of populations with different phenological characteristics, thereby enhancing ecological compatibility by increasing adaptive abilities (Tursun 2012). Genetic diversity among weed species is strongly influenced by climatic and geographical conditions (Burgos et al. 2013). Species and strains with high genetic diversity have a better adaptive ability to changing environmental conditions in time and space (Kaya Altop and Mennan 2011). Genetic diversity occurring in weeds triggers the development of a higher resistance of those species to certain herbicides, thereby making control of these weeds more difficult. Therefore, comprehensive understanding of germination biology, plant phenology and genetic backgrounds of susceptible and resistant barnyardgrass is important in devising an effective integrated weed management program against this noxious weed. A judicious selection of herbicides, herbicide rotations, appropriate doses and targeted applications have been recommended as good management practices against the evolution of herbicide resistance (Powels and Yu 2010, Bajwa et al. 2015).

In conclusion, the present study confirmed that barnyardgrass populations have evolved resistance to penoxsulam in rice in Balıkesir and Çanakkale provinces of the Marmara region in Turkey. Moreover, barnyardgrass populations with resistance to penoxsulam herbicide have also developed multiple resistance to different commonly-used herbicides in rice at different levels. Penoxsulam-resistant barnyardgrass populations exhibited the lowest average resistance against profoxydim and the highest average resistance against molinate herbicide. It is important to maintain and/or increase herbicide diversity to delay selection of herbicide resistance in cropping systems. Application of sequential or tank-mix herbicides with different modes of action can improve the control and may delay or avoid the evolution of herbicide-resistant weeds. Use of soil-applied herbicide after crop planting could also be an effective control strategy to minimise the impacts of herbicide-resistant weeds. Diversification of crop and weed management practices emphasizing on non-chemical weed control tactics are important tools for proactive management of herbicide-resistant weeds. Irrigation water, soil tillage tools and other contamination routes must be eliminated to prevent seed dispersal from rice fields infested with resistant barnyardgrass to other fields. In addition, proper management methods should be applied to minimise the resistant weed seeds reserve in the soil.

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