Crop Protection 65 (2014) 202–206

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Short communication

Detecting ALS and ACCase herbicide tolerant accession of *Echinochloa oryzoides* (Ard.) Fritsch. in rice (*Oryza sativa* L.) fields

Emine Kaya Altop [a], Husrev Mennan [a], Jens Carl Streibig [b,*], Unal Budak [a], Christian Ritz [c]

[a] Ondokuz Mayıs University, Agriculture Faculty, Department of Plant Protection, 55139 Samsun, Turkey
[b] Department of Plant and Environmental Sciences, The University of Copenhagen, DK 2630 Taastrup, Denmark
[c] Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 25 June 2014
Accepted 20 July 2014
Available online

Keywords:
ALS and ACCase inhibitors
Dose response
Sensitivity
Rice

ABSTRACT

Inconsistent control of *Echinochloa oryzoides* has been reported repeatedly by farmers in the major rice growing area of Turkey. Greenhouse studies confirmed the existence of cross and multiple herbicide tolerance of *E. oryzoides* accessions including acetolactate synthase (penoxsulam, bispyribac-sodium) and acetyl CoA carboxylase (cyhalofob-butyl) inhibiting herbicides. Comparison of 95% lower confidence intervals of ED_{90} derived from log-logistic dose–response curves, and twice the recommended field rates of the herbicides showed some, but not distinct separation of susceptible and tolerant accessions. We used a novel method to separate heterogeneous data without *a priori* knowledge of grouping into more than one group. On the basis of the distribution of ED_{90} it was possible to identify two distinct groups of the 172 accessions tested, 78% were not controlled by ALS inhibitors (penoxsulam, and bispyribac-sodium) at recommended field rates; and 38% were not controlled by the ACCase Inhibitor (cyhalofob-butyl) at twice the field rates. The effective response level of ED_{90} resulted in 64 and 14 tolerant accessions to ALS and ACCase, respectively. Fourteen accessions showed multiple resistances to ALS and ACCase Inhibitors.

Some of the accessions were strongly tolerant to both herbicide modes of action and had 100% survival even at 6 times the recommended rates. Most of these tolerant accessions were from Marmara region, predominantly in Edirne and Balıkesir, which are the regions without any crop rotation.

1. Introduction

Echinochloa is an economically important genus with several species being considered as noxious weeds in agriculture, especially in rice paddies (Holm et al., 1977). *Echinochloa oryzoides* (Ard.) Fritsch. is listed among the most noxious weeds in rice paddies throughout the world (Damalas et al., 2008; Işık et al., 2000; Tabacchi et al., 2006). The broad ecological tolerance such as the ability to mimic rice, rapid germination, growth and abundant seed production makes it a successful weed.

Rice is an important staple crop in Turkey, because of its high domestic consumption. Rice growing is concentrated in the middle Black Sea and Trachea region, with small areas of rice growing in south-eastern Anatolia. Farmers have changed their seeding method from transplanting to wet seeding because of rising labor costs and also better control of *Echinochloa crus-galli* (L.) P. Beauv. and other grasses. In this system, fields are kept water saturated for 7–10 days after seeding to facilitate root growth and anchorage of the rice seedlings. The continuous cropping of rice in this system has inevitably resulted in the proliferation of highly competitive weed species strongly adapted to the aquatic environment. Thus, *E. crus-galli* has been replaced by *E. oryzoides* in many fields as *E. oryzoides* is only partially controlled by continuous flooding and it often results in dense weed infestation (Hill et al., 2001; Phuong et al., 2005).

Yield losses caused by uncontrolled weeds may range from 15 to 42% depending on weed species, weed densities, rice cultivar and seeding method (Mennan et al., 2012a,b). Many farmers depend on herbicides for weed control in the water seeding system. Since the early 1990s acetolactate synthase inhibitors have been used widely in rice fields (Saari et al., 1994). The other widely used herbicide group is Acetyl coenzyme A carboxylase (ACCase) inhibitors that selectively control grass species (Delye et al., 2002).

Herbicides used to control *Echinochloa* spp. have been extensively applied to rice for over 30 years and now farmers complain of

* Corresponding author. The University of Copenhagen, Faculty of Life Sciences, Dept. Agriculture and Ecology (Crop Science), DK 2630 Taastrup, Denmark.
E-mail addresses: hmennan@omu.edu.tr (H. Mennan), jcs@life.ku.dk (J.C. Streibig).

http://dx.doi.org/10.1016/j.cropro.2014.07.011
0261-2194/

unacceptable control of *E. oryzoides*. Continuous use of herbicides with the same mechanisms of action leads to the evolution of herbicide-resistant weed population (Holt, 1993). Currently, there are 303 resistant biotypes within 211 species (115 dicotyledonous and 82 monocotyledonous) worldwide (Heap, 2012). Herbicide-resistant *E. oryzoides* was reported in the USA more than ten years ago (Albert et al., 2000; DeWitt et al., 1999; Fischer et al., 2000).

In previous studies, repeated use of molinate, propanil, thiobencarb, fenoxaprop and bispyribac-sodium in rice has led to the evolution of resistant *Echinochloa* spp. biotypes (Baltazar and Smith, 1994; Caseley et al., 1996; Hoagland et al., 2004; Norsworthy et al., 1998; Valverde et al., 2000). Resistant biotypes of *Echinochloa crus-galli* and *Echinochloa colona* (L.) Link. have been reported in the USA, Greece, Italy, Portugal Sri Lanka, and Thailand (Heap, 2009). Multiple herbicide resistance is another problem for ALS and ACCase inhibitors and has been reported in accession of *Echinochloa phyllopogon* (stapf) Koss. and *E. oryzoides* involving four different herbicides of three chemical families with different modes of action (Fischer et al., 2000).

The objectives of this study were to evaluate (1) how to control *E. oryzoides* accessions that have become increasingly difficult to control with commonly used penoxsulam, bispyribac-sodium and cyhalofob-butyl, (2) to confirm the existence of herbicide tolerant *E. oryzoides* accessions associated with direct-seeded rice in Turkey, and (3) to determine cross and multiple resistance involving two or more of these herbicides.

2. Material and methods

2.1. Seed source

Seeds of *E. oryzoides* were collected in different rice growing regions of Turkey (Table 1). In 2009; 20, 39, 3, 29, 53, 8, 6, 9 and 5 different *E. oryzoides* seed samples were collected from nine provinces. 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. Approximately 500 g *E. oryzoides* seeds were collected from each field and seeds were cleaned and stored at room temperature until experiments were initiated.

Table 1
The list of materials used in study.

Locality	Accession No	Number of accession
Samsun, Alaçam	SAM-E-1, SAM-E-8	2
Samsun, Bafra	SAM-E-11, SAM-E-14~18, SAM-E-25, SAM-E-35~42	15
Samsun, Terme	SAM-E-67, SAM-E-69, SAM-E-78, SAM-E-81~84	7
Balıkesir, Gönen	BAL-E-4, BAL-E-10, BAL-E-16~20, BAL-E-24~27, BAL-E-30, BAL-E-31, BAL-E-33, BAL-E-38	15
Balıkesir, Manyas	BAL-E-41, BAL-E-43, BAL-E-44, BAL-E-47~50, BAL-E-55, BAL-E-66, BAL-E-69, BAL-E-75, BAL-E-80, BAL-E-86, BAL-E-90	14
Bursa, Merkez	BUR-E-4, BUR-E-10, BUR-E-17	3
Çorum, Kargı	COR-E-2, COR-E-5, COR-E-9, COR-E-12, COR-E-14~16, COR-E-25	8
Çorum, Osmancık	COR-E-27~31, COR-E-34, COR-E-36 COR-E-38, COR-E-39 COR-E-41~45, COR-E-47, COR-E-52	16
Çorum, Bayat	COR-E-55, COR-E-58, COR-E-63	3
Çorum, Dodurga	COR-E-65, COR-E-67, COR-E-71	3
Edirne, İpsala	EDİ-E-2, EDİ-E-4, EDİ-E-7, EDİ-E-8~11, EDİ-E-14, EDİ-E-16, EDİ-E-19~22, EDİ-E-69, EDİ-E-74~78, EDİ-E-89~95, EDİ-E-101, EDİ-E-105~111, EDİ-E-114, EDİ-E-128~132	42
Edirne, Meriç	EDİ-E-25, EDİ-E-28	2
Edirne, Uzunköprü	EDİ-E-39, EDİ-E-40, EDİ-E-45, EDİ-E-47	4
Edirne, Merkez	EDİ-E-31, EDİ-E-35, EDİ-E-37, EDİ-E-38	4
Edirne, Havsa	EDİ-E-50, EDİ-E-52, EDİ-E-55, EDİ-E-56	4
Edirne, Keşan	EDİ-E-139, EDİ-E-144, EDİ-E-150, EDİ-E-152	4
Kastamonu, Tosya	KAS-E-5, KAS-E-7, KAS-E-8, KAS-E-10, KAS-E-13	5
Kastamonu, Hanönü	KAS-E-2, KAS-E-4	2
Kırklareli, Babaeski	KIR-E-2, KIR-E-4, KIR-E-7	3
Kırklareli, Pehlivanköy	KIR-E-10, KIR-E-11	2
Sinop, Boyabat	SIN-E-8, SIN-E-10, SIN-E-17, SIN-E-20, SIN-E-21	5
Sinop, Durağan	SIN-E-28, SIN-E-31	2
Sinop, Saraydüzü	SIN-E-4, SIN-E-5	2
Tekirdağ, Hayrabolu	TEK-E-2, TEK-E-4, TEK-E-5	3
Tekirdağ, Malkara	TEK-E-10, TEK-E-11	2
Total		172

2.2. Dose–response experiments

In order to rule out some of the most susceptible accessions, we did a single-dose assay experiment proposed by Moss (Moss et al., 1999) to determine possible tolerance among the 172 accessions. If the efficacy of herbicides were less than 80% these accessions were included in the subsequent dose–response experiments.

The putative tolerant accessions, based on the single-dose-assay results, were seeded into cell trays (each cell 25 ml) containing commercial potting mix. Plants were grown at average daily temperatures ranging from 24 to 30 °C and at a 16 h day length. Natural light, was supplemented with artificial light if needed. When germinated, seedlings were transplanted into 7 L square plastic pots (18.5 × 18.5 cm) filled with rice paddy field soil. Each pot was fertilized before transplanting and after first tillering with ammonium sulfate (21%N, 24%S) at rates equivalent to 400 kg ha^{-1} in two split doses. After seedling establishment, each pot contained 4 equidistantly spaced uniform plants and herbicides were applied at the 3–4 true leaf stages. Penoxsulam (Cherokee™ 25.2 g ai kg^{-1}) was applied at rates equivalent to 0, 5.04, 10.08, 20.16, 40.32, 80.64, 161.28 and 322.56 g ha^{-1} to 128 *E. oryzoides* accessions., bispyribac-sodium (Nominee™ 420 g ai kg^{-1}) was applied at 0, 5.25, 10.50, 21, 42, 84, 168, and 336 g ha^{-1} equivalent to 135 *E. oryzoides* accessions and finally cyhalofob-butyl (Clincher™ 200 g ai kg^{-1}) was applied at equivalent 0, 37.5, 75, 150, 300, 600, 1200, and 2400 g ha^{-1} to 66 *E. oryzoides* accessions.

Dose ranges were generated according to registered field rates for post-emergence application of these herbicides. These rates correspond to 0, 0.25 0.5, 1, 2 4, 8, and 16 times the recommended field rate of the products in Turkey. Dose–response experiments for each herbicide were in a completely randomized design with four replications per treatment. All experiments were independently made twice. Twenty-one days after treatment (DAT) the above-ground shoots were harvested and dry weights determined.

2.3. Data analysis

A preliminary analysis of the dose–response curves within accessions showed that the data were best described with a three-parameter log-logistic curve (Ritz, 2010; Ritz and Streibig, 2005).

$$y = \frac{D}{1 + \exp(b(\log(x) - \log(ED_{50})))}$$

y is biomass plant^{-1}, D is the upper value of y, b is proportional to the slope of the curve around ED_{50}, which is the dose required to

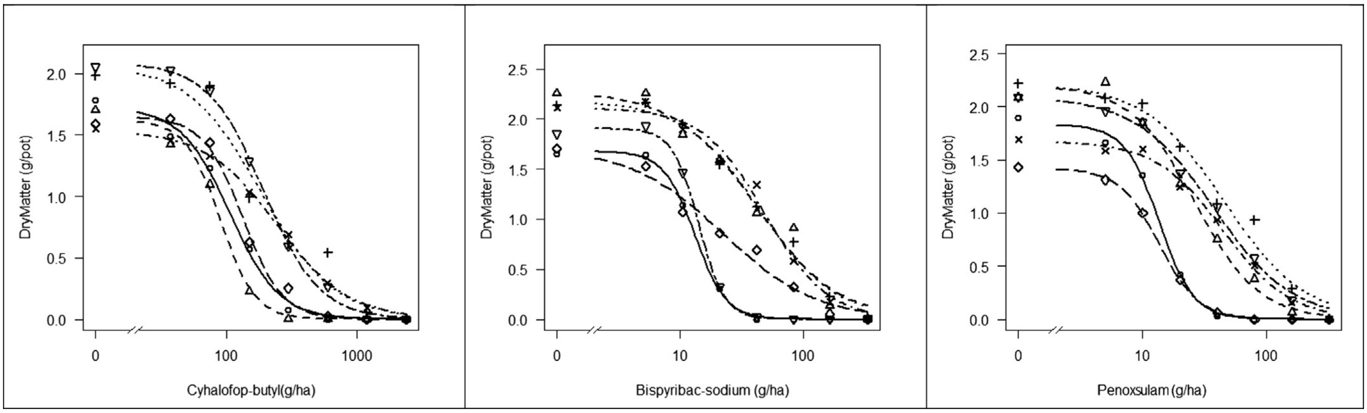

Fig. 1. The effect of cyhalofob-buty, bipyribac-sodium and penoxsulam on tolerant, the curves displaced to the right, and susceptible accession of *E. oryzoides*.

halve the biomass relative to *D*. Analysis of the dose–response curves were done using **R** (version 2.15.2) with the add-on package *drc* (version 2.03.0) (Ritz and Streibig, 2005). The model was fitted simultaneously to all curves within a herbicide and experimental treatment. The ED_{90} response level and its 95% confidence intervals were derived from the model and graphical analysis of residuals was used to assess regression fits (Ritz and Streibig, 2005). On the basis of two independent experimental runs, we did a weighted mixed ANOVA model where the 95% lower confidence limit of ED_{90} was the response, the herbicide was the fixed effect and the experimental runs were random effects.

The 95% lower confidence limit of the ED_{90} response levels were input into a finite mixture model that can be used to model bimodal distributions that often result from heterogeneous samples, such as the mixture of two normal distributions with different mean and/or standard deviations (Grün and Leisch, 2007). As the distribution of the ED_{90} parameters was approximately log normally distributed, calculations were done on log(ED_{90}) values (Morse and Bickle, 1967).

3. Results

All herbicide treatments at their recommended rate provided different levels of *E. oryzoides* control at 21 DAT when compared to the non-treated control. Some dose–response curves, shown in Fig. 1, illustrated large differences among susceptibility of accessions within herbicides. The dose ranges of the herbicides were adequate to describe the curves, ranging from virtually no effect at low to large effect at high doses. The upper limits of herbicides differed among accessions lying between 1.5 and 2 g ha^{-1}. The shapes of the response curves did not vary much except for some few curves with low relative slopes (Fig. 1).

The dose–response curves were all generated in the greenhouse and consequently, the recommended rate used in the field may not be directly translated into the greenhouse situation. In order to give a conservative estimate of the relation between the effect and recommended rate, we derived the ED_{90} levels with associated 95% confidence intervals (Ritz, 2010; Ritz and Streibig, 2005) and compared the lower 95% confidence level to twice the recommended rate in the field (Fig. 2).

Although there is some indication of differential susceptibility for cyhalofob-butyl, the limits are not very clear for bispyribac-sodium and penoxsulam (Fig. 2). For cyhalofob-butyl, 42 out of 66 accessions had a lower ED_{90} confidence limit higher than twice the recommend rate. For bispyribac-sodium 67 out of 134 were tolerant and, for penoxsulam, 73 accessions out of 129 were tolerant. However, we do not have any additional evidence of the mechanism of putative tolerance/resistance, so an ambiguously defined grouping on the basis of genetics is not possible.

Fig. 3 shows the results of using the finite mixture model (Grün and Leisch, 2007) and in all three instances there were two distinct groups significantly different from each other. The relative potencies with associated 95% confidence intervals within herbicides were 3.9 (3.4–4–5), 3.9 (3.4–4.3) and 4.2 (3.7–4.8),

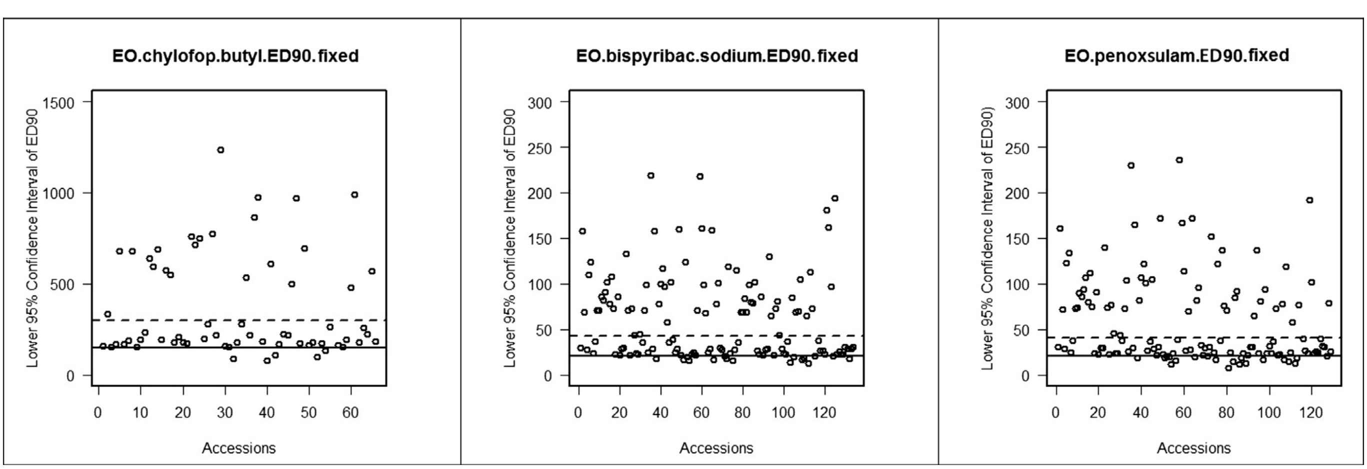

Fig. 2. The distribution of the lower 95% confidence limit of ED_{90} for the various accessions. The solid line is the recommended rate and the broken line is twice recommended rate.

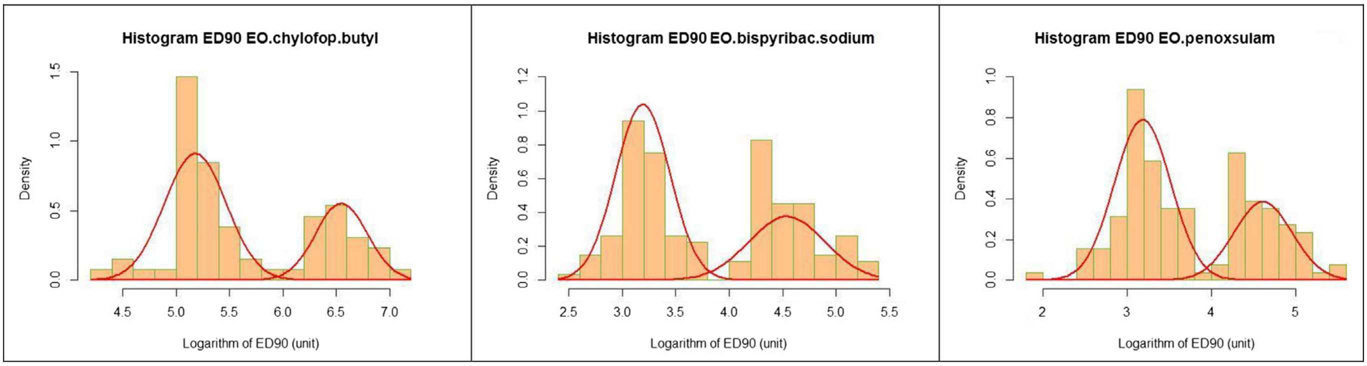

Fig. 3. Separation of biotypes based upon log(ED_{90}) The curves are the normal distributions for the various biotypes.

respectively. Fig. 3 shows that the boundaries between classifications are not clear-cut; there are individual accessions that overlap the two groups.

Most of the tolerant accessions were from the Marmara region, predominantly from Edirne and Balıkesir with continuous rice growing and without any crop rotation. Relative potency of bispyribac-sodium was almost the same as that of cyhalofob-butyl. Some accessions had 100% survival at 6 times the recommended application rate (Fig. 2) and were located in Edirne and Balıkesir. This commonly observed pattern showed the very high risk of relying upon continuous rice cropping with its inevitable shift in weed flora to more tolerant species and the development of herbicide resistance.

4. Discussion

In a field study where material was collected from non-sprayed fields and fields perennially sprayed with ALS inhibitors and various mixture of ALS inhibitors and auxin herbicides, the relative potency between the accessions were close to 2.0. At this level farmers already had noticed problems with weed tolerance (Mennan et al., 2012a,b).

Relative potencies of around 4.0 were rather large and, as expected; farmers already had discovered a reduced herbicide effect several years before the relative potency had reached 4.0. However, inconsistent control of *E. oryzoides* in rice fields has been reported repeatedly by farmers in the Marmara and Karadeniz regions. Many of them were solving their weed problems by using higher doses, which exacerbated the development of herbicide resistance in the future. However, in the greenhouse we usually see a clearer difference between putative tolerant and susceptible biotypes.

One of the novel issues presented in this paper is the use of the finite mixture model that can separate accessions into groups where the grouping initially is not clear cut. Consequently, this method could be a versatile tool to identify the initial development of herbicide tolerant biotypes of weeds and the first indication of whether or not more elaborate molecular and biochemical research is needed to find the mode/site of tolerance/resistance.

Acknowledgments

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

References

Albert, J.F., Comfort, M.A., David, E.B., James, E.H., 2000. Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. Weed Sci. 48, 225–230.
Baltazar, A.M., Smith Jr., R.J., 1994. Propanil-resistant barnyardgrass (*Echinochloa crus-galli*) control in rice (*Oryza sativa*). Weed Technol. 8, 576–581.
Caseley, J.C., Leah, J.M., Riches, C.R., Valverde, B.E., 1996. Combating propanil resistance in (*Echinochloa colona*) with synergists that inhibit acylamidase and oxygenases. In: Proceedings of the Second International Weed Control Congress, Slagelse, Denmark, pp. 455–460.
Damalas, C.A., Dhima, K.V., Eleftherohorinos, I.G., 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.
Dèlye, C., Matèjicek, A., Gasquez, J., 2002. PCR-based detection of resistance to acetyl-CoAcarboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds.) and ryegrass (*Lolium rigidum* Gaud). Pest Manag. Sci. 58, 474–478.
DeWitt, T.C., Vickery, C., Heier, J., 1999. Control of herbicide resistant watergrass in Northern California rice with Regiment™ herbicide. Proc. Calif. Weed Sci. Soc. 51, 182–183.
Fischer, A.J., Bayer, D.E., Carriere, M.D., Ateh, C.M., Yim, K.O., 2000. Mechanisms of resistance to bispyribac-sodium in an *Echinochloa phyllopogon*. Pestic. Biochem. Physiol. 68, 156–165.
Grün, B., Leisch, F., 2007. FlexMix: an R package for finite mixture modelling. R. News 7, 8–13.
Heap, I.M., 2009. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.com (accessed 03.10.09.).
Heap, I.M., 2012. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.com (accessed 11.12.12.).
Hill, J.E., Mortimer, A.M., Namuco, O.S., Janiya, J.D., 2001. Water and weed management in direct-seeded rice: are we headed in the right direction? In: Peng, S., Hardy, B. (Eds.), Rice Research for Food Security and Poverty Alleviation. International Rice Research Institute, Philippines, pp. 491–510.
Hoagland, R.E., Norsworthy, J.K., Carey, F., Talbert, R.E., 2004. Metabolically based resistance to the herbicide propanil in Echinochloa species. Weed Sci. 52, 475–486.
Holm, L., Plucknett, D., Pancho, J., Herberger, J., 1977. The World's Worst Weeds: Distribution and Biology. University of Hawaii Press, Honolulu, p. 609.
Holt, J.S., 1993. Mechanisms and agronomic aspects of herbicide resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 203–209.
Işık, D., Mennan, H., Ecevit, O., 2000. Determination of wild species in rice fields in Samsun province. J. Agric. Fac. O.M.Ü 15, 99–104.
Mennan, H., Streibig, J.C., Ngouajio, M., Kaya, E., 2012a. Tolerance of Two *Bifora radians* Bieb accassion to ALS inhibitors in winter wheat. Pest Manag. Sci. 68, 116–122.
Mennan, H., Ngouajio, M., Sahin, M., Isık, D., Kaya-Altop, E., 2012b. Competitiveness of rice (*Oryza sativa* L.) cultivars against *Echinochloa crus-galli* (L.) Beauv. in water-seeded production systems. Crop Prot. 41, 1–9.
Morse, P.M., Bickle, A., 1967. The combination of estimates from similar experiments, allowing for inter-experiment variation. Am. Stat. Assoc. 62, 241–250.
Moss, S.R., Clarke, J.H., Blair, A.M., Culley, T.N., Read, M.A., Ryan, P.J., Turner, M., 1999. The occurrence of herbicide-resistant grass-weeds in the United Kingdom and a new system for designating resistance in screening assays. In: Proceedings of the Brighton Crop Protection Conference on Weeds, U.K, pp. 179–184.
Norsworthy, J.K., Talbert, R.E., Hoagland, R.E., 1998. Chlorophyll fluorescence for rapid detection and confirmation of propanil-resistant barnyardgrass (*Echinochloa crus-galli*). Weed Sci. 46, 163–169.
Phuong, L.T., Denich, M., Vlek, P.L.G., Balasubramanian, V., 2005. Suppressing weeds in direct-seeded lowland rice: effects of methods and rates of seeding. J. Agron. Crop Sci. 191, 185–194.
Ritz, C., Streibig, J.C., 2005. Bioassay analysis using R. J. Stat. Softw. 12, 1–22.
Ritz, C., 2010. Towards a unified approach to dose-response modeling in ecotoxicology. Environ. Toxicol. Chem. 27, 220–229.
Saari, L.L., Cotterman, J.C., Thill, D.C., 1994. Resistance to acetolactate synthase inhibiting herbicides. In: Powles, S.B., Holtum, J.A.M. (Eds.), In Herbicide

Resistance in Plants: Biology and Biochemistry. Lewis Publishers, Boca Raton, FL, pp. 83–139.
Tabacchi, M., Mantegazza, R., Spada, A., Ferrero, A., 2006. Morphological traits and molecular markers for classification of Echinochloa species from Italian rice fields. Weed Sci. 54, 1086–1093.
Valverde, B.E., Riches, C.R., Caseley, J.C., 2000. Prevention and Management of Herbicide Resistant Weeds in Rice: Experience from Central America with *Echinochloa Colona*. Cámara de Insumos Agropecuarious, Costa Rica, p. 123.