J. Sher-e-Bangla Agric. Univ., 13(1&2): 1-9, January & July 2022 ISSN 1997-6038

MORPHOLOGICAL AND PHENOLOGICAL VARIABILITY OF *Echinochloa* ACCESSIONS AND THEIR HERBICIDE SENSITIVITY

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ABSTRACT

Echinochloa species are the most problematic grass weeds in rice fields worldwide. The species of this genus have high intra- and interspecific variability, with many ecotypes. Based on this, the study was conducted to characterize the morphology and phenology of Bangladesh *Echinochloa* accessions and evaluate their herbicide sensitivity. The results showed a distinct variability of the growth characteristics among Echinochloa ecotypes. The four *Echinochloa* accessions, designated *Echinochloa crus-galli* ecotypes A, B, C, and D, were discovered in the various rice-growing regions of Bangladesh. The same ecotypes were treated with pre-and post-emergence herbicides in pot culture and the puddle and dry sowing conditions to determine the herbicide sensitivity. Among the pre-emergence herbicides, all except butachlor in dry sowing conditions and pyrazosulfuron ethyl in both puddle and dry sowing conditions were effective against ecotypes of *Echinochloa* detected. Bispyribac sodium and metamifop were effective in both the four-leaf and eight-leaf stages of *E. crus-galli* ecotype D. In contrast, fenoxaprop–p–ethyl was effective at the four-leaf stage. *E. crus-galli* ecotype B was effectively controlled by quizalofop-p-ethyl when sprayed at the eight-leaf stage and fenoxaprop–p–ethyl at the four-leaf stage. *E. crus-galli* ecotype C was less susceptible to all the herbicides. However, quizalofop-p-ethyl and fenoxaprop-p-ethyl, although effective at the four-leaf stage in reducing the weed persistence index (WPI). This information helps make weed management decisions and is informative in understanding the speciation and adaptation of weedy *Echinochloa* species.

Keywords: *Echinochloa* accessions, morphology, pre-emergence herbicides, post-emergence herbicides, weed persistence index

INTRODUCTION

Knowledge on the basic biology of weeds is essential for developing sustainable weed management systems. *Echinochloa* is a genus belonging to Poaceae of about 50 weed species with a worldwide dispersion. It has been reported to be a serious weed in 36 crops (Holm *et al.,* 1991), particularly in rice, where its similar habit and appearance make it difficult to distinguish when young. *Echinochloa* species are self-pollinating, but gene flow by wind-mediated outcrossing can occur (Bagavathiannan *et al.,* 2012). Correctly identifying *Echinochloa* species is challenging because its morphological characteristics tend to intergrade. The widely distributed *E. crus-galli* is mainly known for its high phenotypic plasticity (Maun and Barrett, 1986; Masum *et al.*, 2016). As a result, synonyms were misclassified and used incorrectly at the species and intraspecies levels. (Michael, 2003). Numerous species of Echinochloa exhibit weed-like characteristics, including prolific seed generation, protracted emergence durations due to dormancy, photoperiod insensitivity, and rapid vegetative growth. *Echinochloa* is a C_4 plant that utilizes carbon dioxide more effectively than C_3 plants like rice and wheat. This characteristic, and various weedy traits, have made *Echinochloa* a great competitor in crop production systems in both flooded (e.g., rice) and non-flooded (e.g., soybean, maize, various others) fields (Bagavathiannan *et al.,* 2011).Consequently, proper identification of (sub) species is a prerequisite for optimization of weed control comprising an appropriate choice of herbicide and dose tailored to the weeds present. Therefore, a better knowledge of the distribution of variability within the genus *Echinochloa* would help in its taxonomic classification and in designing a rational strategy for herbicide testing.

Biological invasion severely threatens agro-biodiversity, ecological stability, and agricultural sustainability. Recently, herbicide resistance has become a conundrum that is haunting ecosystem

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stability, weed management, and sustainable crop production (Heap, 1999). Therefore, herbicide resistance in weeds necessitates the continued monitoring of herbicide resistance evolution in weed species (Asaduzzaman *et al*., 2022). Nowadays, *E. crus-galli* is included among the most dangerous herbicide-resistant weeds in the world (Beckie and Reboud, 2009). It shows morphologic variability and differences in response to herbicides (Claerhout *et al.,* 2015). It has evolved both target-site and non-target-site herbicide resistance, due to which a quest for alternative herbicides is also in danger (Norsworthy *et al*., 2014). Besides good management practices to reduce resistance evolution, the investigation into biological and ecological mechanisms is much needed. Various biotypes of *E. crus-galli* are now resistant to multiple herbicides (Wilson *et al.,* 2014; Masum *et al.,* 2019). Moreover, the continuous use of the same herbicides with a similar mode of action has evolved resistance in *E. crus-galli.* Undoubtedly, herbicide resistance in *E. crus-galli* is a severe threat to sustainable crop production in a large area globally.

Despite being one of the world's most significant warm-season annual grass weeds, *E. crus-galli,* relatively few researchers have examined the population plasticity of its growth and development and responses to herbicides. These studies are infrequent in Bangladesh. Therefore, these investigations aimed to obtain specific information on the growth and development of *E. crus-galli*, creating an emerging concern towards herbicide resistance. This knowledge may help develop more effective control methods for this weedy grass.

MATERIALS AND METHODS

Seed source

Existing *Echinochloa* spp. of Sher-e-Bangla Agricultural University (SAU) farm and farmer's field in Bangladesh were used as test species. A field survey was conducted in rice fields of SAU farm and different locations in Bangladesh to identify different *Echinochloa* spp.

Growing of plants

Echinochloa accessions were sown in trays and grown under a plastic rain shelter. The soil was shielded from weed growth by geotextile membranes. After 18 days, plants at the four-leaf stage were planted outside in pots in a completely randomized block design with three replications. The experimental unit (pot) consisted of five plants of a given accession.

Manual labor was used to eradicate the remaining other weeds. All plants were screened for qualitative and quantitative parameters regarding vegetative and generative traits.

Morphological and phenological observation

Growth traits such as plant type, plant height, number of tillers, flag leaf length and width, panicle length, spikelet weight, fresh weight of 100-seeds, and panicle awn were assessed at the maturity stages. Plant type (prostrateness, Fig. 1) and panicle awn were considered as qualitative characters visually rated on a scale from none (0), moderate (1) to severe (2) (Park *et al*., 1995; Yamasue, 1997). In addition, phonological events such as emergence date, heading time, and growth duration were also evaluated.

Whole-plant herbicide resistance assay

A pot culture study was conducted from March to June 2021 under a plastic rain shelter at SAU's Agronomy field laboratory. Plastic pots (50 cm x 40 cm) were used for the experiment, and 3/4th of the pots were filled with soil collected from the field. The soil was silty clay loam (sand 26%, silt 45%, and clay 29%) with pH 5.8, C 0.45%, N 0.12%, HPO_4^{2-} 0.40 mg g^{-1} soil, and K^+ 0.70 mg g^{-1} soil. Each pot was sown with 25 seeds of *Echinochloa spp*. After seed germination, five seedlings were maintained per pot. The seeds were pretreated with 1M ethanol under darkness for three days to improve germination - a modification of the procedure suggested by Masum *et al.* (2016). A completely randomized design was adopted for experiments, and separate experiments were conducted for the different *Echinochloa* types with three replications for each treatment. The ecotypes of *Echinochloa* were treated with pre-emergence herbicide with a dose of 0.75 kg a.i. ha^{-1} of pretilachlor, 1.25 kg a.i. ha^{-1} of pyrazosulfuron ethyl, 1.5 kg a.i. ha^{-1} of pendimethalin, (0.18+ 0.40) kg a.i. ha^{-1} of (bensulfuron methyl + pretilachlor), (0.04 + 0.14) kg a.i. ha^{-1} of (bensulfuron methyl + acetachlor), 1.25 kg a.i. ha^{-1} of butachlor, 0.10 kg a.i. ha^{-1} of oxadiazon, (0.0015 + 0.06) kg a.i. ha^{-1} of (pyrazosulfuron-ethyl + pretilachlor), along with untreated control. All the herbicides were applied in both puddle sowing and dry sowing conditions. Post-emergence herbicides were applied at four-leaf and eight-leaf stages of *Echinochloa* spp. and treated with 0.12 kg a.i. ha^{-1} of quizalofop-p-ethyl, 0.024 kg a.i. ha^{-1} of carfentrazone- ethyl, 0.025 kg a.i. ha^{-1} of bispyribac sodium, 0.06 kg a.i. ha^{-1} of fenoxaprop–p–ethyl, 0.015 kg a.i. ha^{-1} of ethoxysulfuron, 0. 125 kg a.i. ha^{-1} of metamifop and untreated control. The quantity of herbicide was calculated based on the recommendation and the pot's diameter. Six ml of the solution was then carefully sprayed in each pot using a hand sprayer. The efficacy of pre-emergence herbicides was studied under both dry sowing and puddle sowing conditions. In dry sowing conditions, *Echinochloa* seeds were sown in pots filled with soil and irrigated lightly without flooding, simulating rainfall. Herbicides were sprayed on the day after sowing. In puddle sowing conditions, soil in the pots was puddled by hand, and excess water was removed after settling the clay. Twenty-five seeds were sown on the top layer and, after one day, again flooded to a depth of three centimeters. Water was drained after one day, and herbicides were sprayed on the third day after sowing. Flooding was again done after 24 hours and continued for the duration of the study.

Weed control efficiency (WCE) was calculated based on the number of surviving weed seedlings in each pot (Mani *et al.,* 1973) by using the following equation-

WCE = x-y/x × 100, where x = Number of *Echinochloa* seedlings in unweeded control and y = Number of *Echinochloa* seedlings in the treated plot.

The efficacy of each herbicide was determined on the basis of seedling survival percentage and weed persistence index (WPI). WPI was estimated using the following formula of Mishra and Misra (1987).

WPI= (p/q) x (b/a), where p = Dry weight of weeds in the treated plot, q = Dry weight of weeds in the control plot, a = Weed count in the treated plot, and b = Weed count in the control plot.

WPI indicates the degree of resistance of the plant that survived the herbicide and is expressed in terms of dry matter production. The data on seedling survival percentage and weed persistence index were transformed by angular transformation to normalize their distribution.

The statistical software 'STATIX 10' was used to analyze the data, and the means were compared based on the least significant difference (LSD) at a 0.05 level of significance.

RESULTS AND DISCUSSION

Identification of *Echinochloa crus-galli* accessions

Different types of *E. cruss-galli* were collected from different locations based on their prostrateness (Fig. 1), having awn, and seed size. The growth form of individual *E. cruss-galli* plants in the study population ranged from almost prostrate to upright.

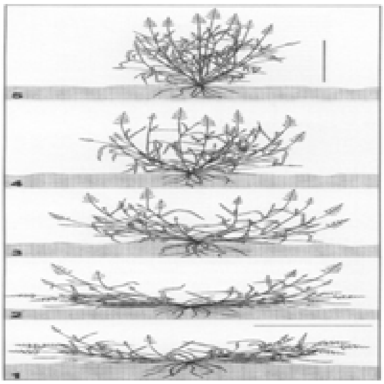

Fig. 1. The collected ecotypes of *Echinochloa crus-galli* were selected as of the growth patterns displayed by Norris, 1996

Within the population, variation in prostrateness has been observed for *E. crus-galli* accession. According to Fig. 1, awn and seed size, *E. crus-galli* accessions are classified into four types (Fig. 2).

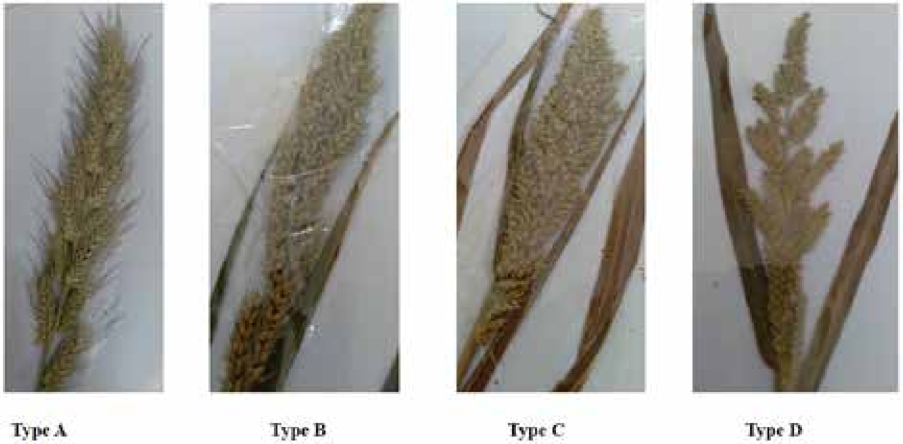

Fig. 2. Spikes of different ecotypes designated Type A, Type B, Type C, and Type D of *Echinochloa crus-galli* are detected in Bangladesh.

Variability in growth characteristics among *Echinochloa* ecotypes

Variability in growth characteristics among *Echinochloa* ecotypes is presented in Table 1. Results of the growth characteristics among *Echinochloa* ecotypes collected from different rice-growing areas of Bangladesh showed distinct variability. The seedling emergence period varies significantly among species, showing a wide range of days to emergence (Table 1). The range of emergence was from 3-7 days, and heading time was from 41 to 59 days. When the growth duration ranged from 86 days to 98 days. The diversity in emergence behavior across *Echinochloa* populations presents a big problem for weed management. These species can emerge across a wide window of time, allowing many to escape weed management tactics (Scursoni *et al*., 2007). In general, growth characteristics variability among *Echinochloa* ecotypes might be affected by geographic origin. This observation was in line with the work of Johnson *et al*. (1989). They reported that the ecotype of some Sweetvetch (*Hedysarum boreale* Nutt.) differed in their taxonomic distance values, and the difference is not always closely related to specific characteristics of the collection sites. Widespread species tend to have more genetic variability than close relations with narrow distribution (Maki, 1999). Damalas *et al*. (2008) studied the morphological variation among 12 populations of *Echinochloa* species in the same environment in Greece.

Plant height differed across species. Type D was the shortest species compared to other species and produced significantly lower tillers than other species, but *Echinochloa* were equal producers of tillers. The high tillering capability allows *Echinochloa* ecotypes to produce many panicles (and seeds), contributing to its abundance. Competition drastically reduces the number of tillers in rice fields-generally less than ten productive tillers plant^{-1} (Zhang *et al*., 2021). However, with gaps in crop rows and field edges, *Echinochloa* species can quickly reach their tillering potential and maximum seed production. *Echinochloa* in this study produced fewer tillers, with high variability in tillering than *Echinochloa* in Greece (115-131 tillers) (Damalas *et al*., 2008).

E. crus-galli type C had the longest flag leaf among the three species, significantly longer than other ecotypes. Big flag leaves provide a competitive advantage because of the light interception's large surface area and higher photosynthesis capacity. A big plant such as *E. crus-galli* type C needs to fix a large amount of carbon to develop a large plant canopy and biomass. Also, big flag leaves afford a higher capability to shade adjacent plants and gain a competitive advantage (Weiner and Thomas, 1986). Panicle lengths were significantly different among species. *E. crus-galli* type C had the longest panicles, followed by *E. crus-galli* type B, and *E. crus-galli* type D. *E. crus-galli* type A had the shortest panicles at 12.83 cm (Table 1). The estimated total seed output plant^{-1} differed significantly between species after accounting for the total number of panicles. *E. crus-galli* type C produced the highest amount of seed (533 seeds panicle^{-1}), and *E. crus-galli* type A produced the lowest (240 seeds panicle^{-1}). However, the *E. crus-galli* type A and B were found awns. The awn length depended on its habitat's relative humidity (Maun and Barett, 1986). Thus, the awn length of *E. crus-galli* type A (21 mm) varied significantly, while *E. crus-galli* type B had 10 mm awns (Table 1). With such a considerable variation in this trait, one can only associate the most obvious generalities to a species, such as being predominantly awnless, predominantly long-awned, or having long awns only on apical spikelets in a panicle.

The spikelet characteristics (spikelet length and width) of the three species were significantly different. *E. crus-galli* type C had the longest (4.10 mm) and widest (2.13 mm) spikelets. There was a wide range of spikelet sizes among *Echinochloa* accessions in many plant studies (Gould *et al*., 1972; Michael, 2003; Tabacchi *et al*., 2006). As expected, greater root-to-foliage ratios were found for *E. crus-galli* type C plants.

Table 1. Growth characteristics of collected *Echinochloa crus-galli* accessions

E. crus-galli accessions	Emer-gence (days)	Average length of tiller (cm)	Tillers plant^{-1} (Nos.)	Heading Time (Days)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Spikelet length (mm)	Spikelet width (mm)	Seeds panicle^{-1}	Length of awn (mm)	Growth duration (Days)	Root foliage ratio
Type A	7 a	82.33 c	50.33 a	59.33 a	17.17 bc	1.13	12.83 b	2.63 b	1.40 ab	240.00 c	21 a	95.67 a	0.14 bc
Type B	3 b	130.33 a	44.00 a	41.67 b	23.40 ab	1.33	17.70 ab	3.53 a	1.70 ab	353.33 b	10 b	86.00 b	0.18 b
Type C	7 a	108.67 b	44.67 a	52.67 b	27.67 a	1.27	20.23 a	4.10 a	2.13 a	533.33 a	-	98.00 a	0.23 a
Type D	5 b	37.33 d	29.00 b	47.00 c	13.30 c	1.23	14.57 ab	2.50 b	1.37 b	205.00 c	-	93.33 a	0.11 c
SE(±)	0.75	6.761	4.055	1.73	2.94	0.13	2.88	0.31	0.19	43.58	0.57	2.29	0.01
CV%	4.23	15.59	9.35	3.99	17.67	13.56	6.66	1.72	1.45	19.53	1.33	5.30	12.06

In a column means having similar letter(s) are statistically similar, and those having dissimilar letter(s) differ significantly at a 0.05level of probability.

Herbicides susceptibility

In pots treated with pretilachlor, pendimethalin, bensulfuron methyl + pretilachlor, bensulfuron methyl + acetachlor, oxadiazon, and pyrazosulfuron-ethyl + pretilachlor, there was no seed germination under either puddle sowing or dry sowing conditions. These pre-emergence herbicides were thus 100% efficient in controlling all four types of *Echinochloa*. However, seedling emergence was observed in pots treated with butachlor in dry sown condition, whereas in puddle condition, no seedling was found. In the case of pyrazosulfuron-ethyl, seedling emergence was observed in three types (A, B, and C) of *Echinochloa,* whereas there was no germination of types D. So, butachlor could be applied in stagnant water, and pyrazosulfuron-ethyl is recommended to be used 6-9 days after sowing, which could be the reason for their poor performance in the present study where they were applied in dry condition and 3-6 days earlier, respectively. Plants grown in dry sown conditions were more susceptible to pyrazosulfuron-ethyl than those in wet sowing conditions. The susceptibility to pyrazosulfuron-ethyl was in the order *E. crus-galli* type D > *E. crus-galli* type C > *E. crus-galli* type A > *E. crus-galli* type B (Table 2). Post-emergence herbicides had varying effects on the different types of *Echinochloa*. Although herbicides that were known to be highly effective against the weed were evaluated, they were less effective in pot culture studies. The data on WPI are presented in Table 3.

Application of bispyribac sodium at the eight-leaf stage was highly effective against *E. crus-galli* type A as there was no seedling survival. Bispyribac sodium has also been reported to be effective in rice nurseries and the main field against *E. crusgalli* (Duary and Mukherjee, 2013). At the four-leaf stage, the survival seedlings' persistence was low (0.04). The survival percentage of the weed was less when quizalofop-p-ethylc, carfentrazone-ethyl, fenoxaprop-p-ethyl, and metamifop were applied at the four-leaf stage. At the eight-leaf stage, these herbicides performed poorly, resulting in high dry matter

Table 2. Efficacy of butachlor and pyrazosulfuron-ethyl on *Echinochloa* spp.

Ecotype of *Echinochloa*	Treatments	Dry sowing conditions		Puddle sowing conditions	
		Emergence (%)	WCE (%)	Emergence (%)	WCE (%)
Type A	butachlor	18.67	61.86	-	-
	Control	50.67		-	
	pyrazosulfuron ethyl	24.00	63.55	26.67	55.51
	Control	54.66		62.67	
Type B	butachlor	8.00	87.29	-	-
	Control	61.33		-	
	pyrazosulfuron ethyl	22.67	71.99	21.33	66.75
	Control	60.00		66.67	
Type C	butachlor	12.00	79.80	-	-
	Control	60.00		-	
	pyrazosulfuron ethyl	20.00	66.87	28.00	48.73
	Control	58.67		54.67	
Type D	butachlor	30.67	46.95	-	-
	Control	60.00		-	
	pyrazosulfuron ethyl	0.00	78.611	42.67	24.49
	Control	58.67		57.33	

Table 3. Effect of post-emergence herbicides on Weed Persistence Index (WPI) of *Echinochloa spp.*

Treatments	*Dose (kg a.i. ha^{-1})*	*Echinochloa type A*		*Echinochloa type B*		*Echinochloa type C*		*Echinochloa type D*	
		Four leaf stage	**Eight leaf stage**	**Four leaf stage**	**Eight leaf stage**	**Four leaf stage**	**Eight leaf stage**	**Four leaf stage**	**Eight leaf stage**
Quizalofop-p-ethylc	0.12	0.05	0.27	0.11	0.00	0.44	0.67	0.00	0.21
Carfentrazone-ethyl	0.024	0.06	0.86	0.41	0.65	0.69	0.92	0.81	0.80
Bispyribac sodium	0.025	0.04	0.00	0.37	0.34	0.41	0.54	0.00	0.42
Fenoxaprop–p–ethyl	0.06	0.02	0.64	0.08	0.27	0.19	0.53	0.09	0.94
Ethoxysulfuron	0.015	0.09	0.86	0.18	0.92	0.54	0.65	0.60	0.67
Metamifop	0. 125	0.18	0.45	0.00	0.15	0.48	0.46	0.00	0.17

production by the surviving seedlings. At the eight-leaf stage, persistence (0.86) was higher when carfentrazone-ethyl was applied. Though an earlier application produced better results, the effect was seen to be inadequate, pointing to a likely need for either a higher dose of application or a still earlier application. Sharma *et al*. (2004) recommended a dose of 0.09 kg ha^{-1} to control *Echinochloa* in the nursery. It was suggested that a higher amount is required for suppressing *Echinochloa* in an advanced stage. The time of application also has to be precise, as a slight variation in the age of the weed seedlings can render a herbicide ineffective. The application of metamifop killed off *E. crus-galli* type B at the four-leaf stage or quizalofop-p-ethylc at the eight-leaf stage.

Contrary to its effect on *E. crus-galli* type A and *E. crus-galli* type D, bispyribac sodium was seen to be ineffective against *E. crus-galli* type B at both leaf stages of application. Earlier application of bispyribac sodium was impractical in suppressing most of the seedlings, while at the later stage, the persistence of the surviving seedlings was high. Fenoxaprop-p-ethyl was a suitable herbicide for *E. crus-galli* type B at the four-leaf stage, as the seedling persistence was only 0.08. A similar result was reported by Singh *et al.* (2004). Chauhan and Abugho (2012) observed less effective control of *E. crus-*

galli when fenoxaprop + ethoxy sulfuron (150 + 18 g ha^{-1}) was sprayed at the eight-leaf stage. In contrast, the weed control efficiency at the four-leaf stage was 68%. In the present experiment, the surviving seedlings showed high persistence at the eight-leaf stage.

Differences in anatomical, physiological, and biochemical traits may help make weeds best adapted to applied herbicides. *E. crus-galli* type C was significantly different from the other species of *Echinochloa* studied in its response to herbicides. Bispyribac sodium was less effective against this species at the four-leaf and eight-leaf stages, and the surviving seedlings were highly persistent. Azmi (2002) observed that certain weed species might establish when a particular herbicide is continuously used due to inherent properties or sublethal concentrations, leading to resistance. This may lead to changes in the weed spectrum and composition and distribution.

For *Echinochloa* type D, the application of quizalofop-p-ethylc and fenoxaprop-p-ethyl at the four-leaf stage was less effective. Fenoxaprop-p-ethyl performed better at the eight-leaf stage of the weed, suggesting that application at this stage would be more effective. Bispyribac sodium and metamifop were effective at both stages of the weed, indicating their superiority in controlling *Echinochloa* type A. Chauhan and Abugho (2012) reported that post-emergence application of bispyribac sodium at four-leaf stage reduced the biomass of *Echinochloa* up to 95%. Walia *et al.* (2008) noted that bispyribac sodium (0.4 kg ha^{-1}) could significantly reduce *Echinochloa*'s biomass accumulation when applied 30 days after sowing. There was no seedling survival when quizalofop-p-ethylc, bispyribac sodium, and metamifop were used at the four-leaf stage. Ethoxysulfuron was the least effective among the herbicides applied as the seedling survival was 100%, and the surviving plants were highly persistent.

Detailed knowledge of how individual weeds grow and reproduce may improve the accuracy of predicting population dynamics and crop losses due to competition and may point to ways to improve control strategies. This comprehensive study of a single *Echinochloa* population emphasizes the extreme variability within a population. The findings imply that *Echinochloa crus-galli* ecotypes have developed and adapted in Bangladesh. Morphological variations among *E. crus-galli* ecotypes in Bangladesh are not very high. The ecotype variations will affect the successful management of *Echinochloa crus-galli* using a chemical or potential biocontrol agent. However, molecular traits analysis would be helpful to characterize the level of genetic variability among *E. crus-galli* ecotypes. Suppose new strains (biotypes) of the *Echinochloa* grass migrates from one to another regions and cross-pollinate. In that case, the genetic diversity may increase, and the control of *E. crus-galli* in rice production may become more complex.

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