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Morphophysiological diversity and its association with herbicide resistance in *Echinochloa* ecotypes

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Research Article

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Abstract

The genus *Echinochloa* constitutes some of the most prominent weed species found in rice (*Oryza sativa* L.) production worldwide. The taxonomy of *Echinochloa* is complex due to its morphological variations. The morphophysiological diversity and taxonomic characteristics of *Echinochloa* ecotypes infesting rice fields in Texas are unknown. A total of 54 *Echinochloa* ecotypes collected during late-season field surveys in 2015 and 2016 were characterized in a common garden in 2017. Plants were characterized for 14 morphophysiological traits, including stem angle; stem color; plant height; leaf color; leaf texture; flag leaf length, width, and angle; days to flowering; panicle length; plant biomass; seed shattering; seed yield; and seed dormancy. Principal component analysis indicated that 4 (plant height, flag leaf length, seed shattering, and seed germination) of the 14 phenological traits characterized here had significantly contributed to the overall morphological diversity of *Echinochloa* spp. Results showed wide interpopulation diversity for the measured traits among the *E. colona* ecotypes, as well as diverse intrapopulation variability in all three *Echinochloa* species studied, including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], junglerice [*Echinochloa colona* (L.) Link], and rough barnyardgrass [*Echinochloa muricata* (P. Beauv.) Fernald]. Taxonomical classification revealed that the collection consisted of three *Echinochloa* species, with *E. colona* being the most dominant (96%), followed by *E. crus-galli* (2%), and *E. muricata* (2%). Correlation analysis of morphophysiological traits and resistance status to commonly used preemergence (clomazone, quinclorac) and postemergence herbicides (propanil, quinclorac, imazethapyr, and fenoxaprop-ethyl) failed to show any significant association. Findings from this study provided novel insights into the morphophysiological characteristics of *Echinochloa* ecotypes in rice production in Texas. The morphological diversity currently present in *Echinochloa* ecotypes could contribute to their adaptation to selection pressure imposed by different management tools, emphasizing the need for a diversified management approach to effectively control this weed species.

Introduction

The genus *Echinochloa* contains the most troublesome weeds of rice (*Oryza sativa* L.) production systems worldwide. It comprises about 50 species, most of which are found in the tropics (Michael 1983). *Echinochloa* spp. are highly adaptive and competitive due to traits such as high fecundity, seed dormancy, and genetic diversity (Lopez-Martinez et al. 1999; Maun and Barrett 1986). Barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and junglerice [*Echinochloa colona* (L.) Link] are the two most problematic *Echinochloa* species infesting rice fields in the southern United States (Bryson and Reddy 2012). *Echinochloa crus-galli* is an annual grass weed that mimics rice, especially at the seedling stage. However, by the time it can be easily differentiated from rice, crop yield loss may have already occurred (Holm et al. 1977). It is an erect-growing plant, reaching up to 2 m tall. The leaves are linear to lanceolate, extending up to 40-cm long and 0.5- to 1.5-cm wide (Chin 2001). *Echinochloa colona*, a species like *E. crus-galli* in appearance, is also an annual grass weed, but has a prostrate growth habit, with plants growing up to 0.6-m tall (Hruševar et al. 2015). In general, *E. colona* has lanceolate leaves with green or purple stems that are often branched at the base (Zimdahl et al. 1989). Rough barnyardgrass [*Echinochloa muricata* (P. Beauv.) Fernald], another species found in U.S. rice production, often grows erect (0.8- to 1.6-m tall), with stem nodes sometimes having sparse hairs (Gould et al. 1972). *Echinochloa colona* and *E. crus-galli* can flower throughout the year, whereas *E. muricata* usually flowers during midsummer to early fall (Chauhan and Johnson 2009; Tahir 2016; Vengris et al. 1966).

The taxonomy of the genus *Echinochloa* is very complex due to continuous morphological variation exhibited by the taxa and the formation of several intergrading polymorphic complexes, which are difficult to classify (Barrett and Wilson 1981). In many cases, *Echinochloa* spp. lack conspicuous identification characteristics, which can lead to misidentification (Costea and Tardif 2002; Michael 1983). Although *Echinochloa* species show a high degree of autogamy, occasional outcrossing is sufficient to facilitate gene exchange among populations (Maun and Barrett 1986). In rice production in Texas, *E. crus-galli* has long been thought to be the most prominent species of *Echinochloa* (popularly known as "barnyard" among growers and crop consultants in the region). However, visits to Texas rice fields by the authors have suggested the distribution of more *E. colona* populations than what was previously perceived.

In U.S. rice production, different *Echinochloa* spp. were reported to have evolved resistance to various herbicides (Heap 2021), and multiple herbicide resistance is also common (Rouse et al. 2018). Herbicide resistance is often associated with certain plant morphological characteristics. For example, in a detailed survey of *Echinochloa* in rice fields in northern Greece, Damalas et al. (2006) found significant morphological differences among the populations, which were consistently linked with the level of sensitivity to different rice herbicides used in the region. Tsuji et al. (2003) compared the morphology of 15 herbicide-resistant and 8 susceptible late watergrass [*Echinochloa phyllopogon* (Stapf) Kosso-Pol.] populations from rice fields in Sacramento Valley, CA, and found that the resistant populations were shorter in height, with shorter and narrower flag leaves, thinner culms, and smaller spikelets compared with the susceptible plants. In that study, the morphological similarity among the resistant populations was also greater than that of the susceptible populations. Maity et al. (2021) reported positive correlation between herbicide resistance and 100-seed weight as well as seed dormancy in Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot].

Though *Echinochloa* spp. are important weeds in rice production in Texas, knowledge of the morphophysiological diversity and species composition of different ecotypes is limited. Moreover, multiple resistance (different combinations) was confirmed for propanil, quinclorac, imazethapyr, and fenoxaprop in several *Echinochloa* ecotypes collected in Texas (Liu 2018), but whether certain morphophysiological characteristics are associated with altered sensitivity to herbicides in these ecotypes is unknown. The objectives of this research were to (1) characterize different *Echinochloa* ecotypes collected from rice fields in Texas for different morphophysiological traits and (2) determine potential association between plant morphophysiological traits and herbicide-resistance status in the ecotypes studied.

Materials and Methods

Plant Materials

Echinochloa samples were collected across the rice-growing region in Texas, known as the "Texas Rice Belt" (Figure 1), during late July/early August in 2015 and 2016. Surveys followed a semi-stratified methodology, and the survey sites were randomly selected on a Google® map using the ITN Converter software (v. 1.88; Benichou Software, http://www.benichou-software.com) without any prior knowledge of the distribution of *Echinochloa* spp. in specific fields.

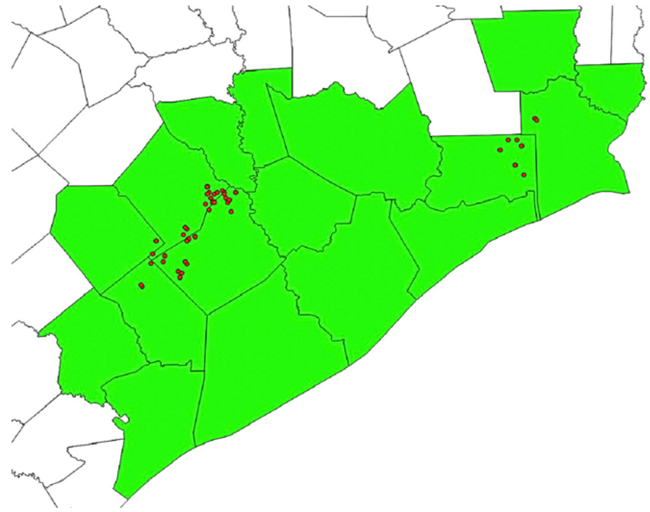

Figure 1. Collection sites (dots) of different *Echinochloa* ecotypes used in the experiment. Field surveys were conducted in all rice-growing counties highlighted in green; however, seeds were available in harvestable quantities only in select fields.

More details of the survey methodology can be found in Liu et al. (2020). A total of 232 fields were surveyed, but samples were collected only in fields where the *Echinochloa* escapes were present. In each survey field, approximately 20 mature panicles were randomly collected and pooled into a single composite sample. A total of 54 samples were collected, representing 54 *Echinochloa* ecotypes. Samples were subsequently dried in a hot-air oven at 50 C for 48 h, hand threshed, cleaned, and stored in ziplock bags before use in the experiment. Sensitivity of the collected *Echinochloa* ecotypes to five different herbicides (clomazone, propanil, quinclorac, imazethapyr, and fenoxaprop) commonly used in Texas rice production was evaluated in a parallel study (Liu 2018).

Morphological Characterization

Seedlings of each ecotype were raised at the Norman Borlaug Center for Southern Crop Improvement Greenhouse Research Facility at Texas A&M University, College Station, during March 2017. Seeds were germinated in petri dishes (9-cm diameter) and transplanted to individual pots (10-cm diameter, 9-cm height) at the 1-leaf seedling growth stage. When the seedlings were about 15- to 25-cm tall, they were transplanted in a common garden established at the David Wintermann Rice Research and Extension Center located near Eagle Lake, TX. The soil type at the study location was Hockley silt loam (fine, smectitic, hyperthermic Typic Albaqualfs; 61% sand, 31% silt, and 8% clay), with 0.75% organic matter content and 6.1 pH.

The climate at this location is characterized by mild winters and hot and humid summers, with an average annual rainfall of 114 cm. The experiment included all the 54 *Echinochloa* ecotypes established in rows (7 plants per row), arranged in a randomized complete block design with four replications (i.e., 4 rows of 7 plants each per ecotype). The distance between plants within a row was 1 m, and the distance between rows was 1.2 m. The experimental area was maintained weed-free throughout the study period by a combination of hand weeding and glyphosate application between rows using a sponge roller at approximately biweekly intervals. The plots were surface irrigated as needed. Rice (variety: 'Clearfield™

111') was planted at 78.4 kg ha^{-1} all around the plots to provide a safe border. In each row, 3 plants (out of the 7 plants) were randomly selected (avoiding borders) and tagged for detailed morphophysiological characterization.

A total of 14 traits were recorded from each tagged plant: stem angle; stem color; leaf color; leaf texture; flag leaf length, width, and angle; days to flowering; panicle length; plant height at maturity; plant biomass; seed shattering; seed yield; and seed dormancy. Stem angle (growth habit) was determined visually relative to the horizontal plane (1 = prostrate/open canopy; 2 = intermediate; 3 = erect/closed canopy). Flag leaf angle was determined visually relative to the main culm (1 = leaf angle ≤45°; 2 = leaf angle >45°). The length and width (measured at the widest point of the leaf) of flag leaves were measured from five random tillers plant^{-1}. Leaf texture was assessed by rubbing fingers along the surface of three random flag leaves in each plant (1 = smooth; 2 = intermediate; 3 = rough). Plant height was measured from the base of the plant to the tip of the panicle on the main stem. Plants were monitored weekly for initiation of flowering. The length of five randomly selected panicles (fully developed) was measured for each plant. To collect any shattered seed, the panicles of each plant were enclosed in Delnet® bags (Delstar Technologies, Middletown, DE, USA) before seed maturity. Plants were harvested at maturity by clipping the entire plant from the base. Samples were dried and weighed for plant biomass determination. Seeds were threshed and weighed for estimation of seed yield. Taxonomical classification of each *Echinochloa* ecotype was carried out based on Carretero (1981).

Evaluation of Seed Dormancy

Seed samples were stored at room temperature (25 C) after harvest. Seed germination tests were carried out for all *Echinochloa* ecotypes at 210 d after harvest. Twenty-five seeds of each ecotype were placed on a moistened filter paper in a petri dish (9-cm diameter) and incubated at 30 C with a light intensity of 90 μmol m^{-2} s^{-1}. The test included two replications and two experimental runs for each ecotype. Petri dishes were arranged in a completely randomized design. Seedlings were counted and removed every 4 d, up to 12 d. The petri dishes were re-randomized after each evaluation timing. Viability of nongerminated seeds was determined using the tetrazolium test (Overaa 1984). Seed were immersed in 2, 3, 5-triphenyltetrazolium chloride (1%) staining solution and incubated at 30 C for 24 h before being examined. Seeds with pink or red embryos were considered alive and dormant.

Data Analysis

Statistical analyses were performed using JMP® Pro (v. 13.1, SAS Institute, Cary, NC, USA). A principal component analysis (PCA) was conducted using JMP® Pro based on the 14 morphophysiological variables measured in this study to determine the most important traits that contribute to grouping of ecotypes based on eigenvalues. From the PCA output, four variables were chosen as principal traits for K-means clustering to group the *Echinochloa* ecotypes. The number of clusters was determined based on the fit statistic with the largest cubic clustering criterion value, using JMP® Pro. The resistance status of each ecotype for the herbicides clomazone, propanil, quinclorac, imazethapyr, and fenoxaprop (screening methodology and results are described in Liu [2018]) was used to calculate the percentage of ecotypes resistant to one or more herbicide sites of action (SOAs) within each hierarchical cluster. A multiple resistance (MR) status number was assigned to each ecotype representing the total SOAs to which each ecotype showed resistance (MR = 0, susceptible ecotype; MR = 1, resistant to one SOA; etc.). A Pearson correlation analysis between the herbicide-resistance status and the 14 morphophysiological traits measured was performed in JMP® Pro.

Results and Discussion

Species Composition

Based on the taxonomic characteristics of the plants, 52 of the total 54 ecotypes studied were identified as *E. colona* and 1 each as *E. crus-galli* and *E. muricata* (Table 1; Figure 2). Results show that *E. colona* is the most prevalent *Echinochloa* species infesting rice fields in Texas. Similar findings have been reported by Bryson and Reddy (2012) in a study involving 240 *Echinochloa* accessions collected from six midsouthern U.S. states (Alabama, Arkansas, Kentucky, Louisiana, Mississippi, and Tennessee), where *E. colona* was the most commonly found species throughout the region. Likewise, in eastern Arkansas, 73 of the 94 accessions of *Echinochloa* collected from rice and soybean [*Glycine max* (L.) Merr.] fields were identified as *E. colona* (Tahir 2016). The rest of the samples included *E. crus-galli*, *E. muricata*, and coast cockspurgrass [*Echinochloa walteri* (Pursh) A. Heller]. In the present survey, *E. walteri* was not observed in rice fields in Texas. Tahir (2016) indicated that high fecundity and low seed dormancy of *E. colona* have likely contributed to its widespread occurrence in the region.

Phenotypic Differentiation within and among Echinochloa Ecotypes

About half (48%) of the *E. colona* ecotypes showed prostrate or intermediate growth habit with short plants (average height: 48 cm), whereas *E. crus-galli* (127 cm) and *E. muricata* (137 cm) exhibited erect growth habit (Table 2; Figure 2). Our results corroborate the observations of Tahir (2016) on *Echinochloa* ecotypes characterized in Arkansas with respect to species characteristics. Prostrate growth habit allows a plant to have maximum ground coverage with open canopy, whereas an erect growth habit with closed canopy helps avoid shading from nearby plants. Rice breeders intentionally select for an erect plant growth characteristic to improve production efficiency (Monsi et al. 1973). The erect growth habit of *E. crus-galli* is one of the examples of crop mimicry in weed species (Barrett 1983). Moreover, plants tend to exhibit erect growth habit when they grow in a dense population (van Hinsberg and van Tienderen 1997). Observations from our field survey showed that *E. colona* plants were like rice plants in height, though *E. crus-galli* and *E. muricata* were much taller than the rice canopy.

The majority (73%) of *E. colona* ecotypes had green color leaves, while the rest (27%) had a mixture of plants with purple or green color leaves within the same ecotype (Table 2). The *E. crus-galli* and *E. muricata* ecotypes characterized here also had a mixture of plants with either purple or green color leaves within each ecotype (Table 2). It is likely that ecotypes showing a mixture of phenotypic traits might have resulted from inter/intraspecific hybridization (Burgos et al. 2014; Singh et al. 2017). It is often observed in the field that the leaf blades of *E. colona* have purple marks; however, this is not a reliable key for species identification (Bryson and Reddy 2012). Tahir (2016) indicated that *E. muricata* was the only species that has green leaves with purple margins, which may help distinguish this species from *E. crus-galli*. This trait was not

Table 1. Details of the *Echinochloa* ecotypes collected in rice production fields in Texas.

Ecotype	Latitude °N	Longitude °W	Species
ECH-1	29.35	96.61	*E. colona*
ECH-2	29.35	96.61	*E. colona*
ECH-3	29.23	96.64	*E. colona*
ECH-4	29.11	96.69	*E. colona*
ECH-5	29.12	96.70	*E. colona*
ECH-6	29.28	96.63	*E. colona*
ECH-7	29.27	96.56	*E. colona*
ECH-8	29.24	96.57	*E. colona*
ECH-9	29.19	96.48	*E. colona*
ECH-10	29.18	96.46	*E. colona*
ECH-11	29.16	96.47	*E. colona*
ECH-12	29.16	96.47	*E. colona*
ECH-13	29.16	96.47	*E. colona*
ECH-14	29.23	96.43	*E. colona*
ECH-15	29.37	96.38	*E. colona*
ECH-16	29.36	96.42	*E. colona*
ECH-17	29.35	96.43	*E. colona*
ECH-18	29.35	96.43	*E. colona*
ECH-19	29.38	96.45	*E. colona*
ECH-20	29.41	96.43	*E. colona*
ECH-21	29.57	96.29	*E. colona*
ECH-22	29.51	96.30	*E. crus-galli*
ECH-23	29.51	96.30	*E. colona*
ECH-24	29.55	96.28	*E. colona*
ECH-25	29.60	96.30	*E. colona*
ECH-26	29.81	94.57	*E. colona*
ECH-27	29.83	94.44	*E. colona*
ECH-28	29.97	94.36	*E. colona*
ECH-29	29.96	94.35	*E. colona*
ECH-30	29.63	96.31	*E. colona*
ECH-31	29.63	96.31	*E. colona*
ECH-32	29.63	96.31	*E. colona*
ECH-33	29.63	96.31	*E. muricata*
ECH-34	29.59	96.27	*E. colona*
ECH-35	29.54	96.32	*E. colona*
ECH-36	29.24	96.44	*E. colona*
ECH-37	29.59	96.31	*E. colona*
ECH-38	29.60	96.25	*E. colona*
ECH-39	29.61	96.22	*E. colona*
ECH-40	29.60	96.21	*E. colona*
ECH-41	29.59	96.21	*E. colona*
ECH-42	29.57	96.20	*E. colona*
ECH-43	29.55	96.19	*E. colona*
ECH-44	29.56	96.18	*E. colona*
ECH-45	29.60	96.14	*E. colona*
ECH-46	29.60	96.14	*E. colona*
ECH-47	30.61	96.35	*E. colona*
ECH-48	29.50	96.17	*E. colona*
ECH-49	29.55	96.27	*E. colona*
ECH-50	29.42	96.44	*E. colona*
ECH-51	29.68	94.43	*E. colona*
ECH-52	29.73	94.48	*E. colona*
ECH-53	29.86	94.47	*E. colona*
ECH-54	29.86	94.52	*E. colona*
ECH-50	29.42	96.44	*E. colona*
ECH-51	29.68	94.43	*E. colona*
ECH-52	29.73	94.48	*E. colona*
ECH-53	29.86	94.47	*E. colona*
ECH-54	29.86	94.52	*E. colona*
ECH-50	29.42	96.44	*E. colona*
ECH-51	29.68	94.43	*E. colona*
ECH-52	29.73	94.48	*E. colona*
ECH-53	29.86	94.47	*E. colona*
ECH-54	29.86	94.52	*E. colona*
ECH-51	29.68	94.43	*E. colona*
ECH-52	29.73	94.48	*E. colona*
ECH-53	29.86	94.47	*E. colona*
ECH-54	29.86	94.52	*E. colona*

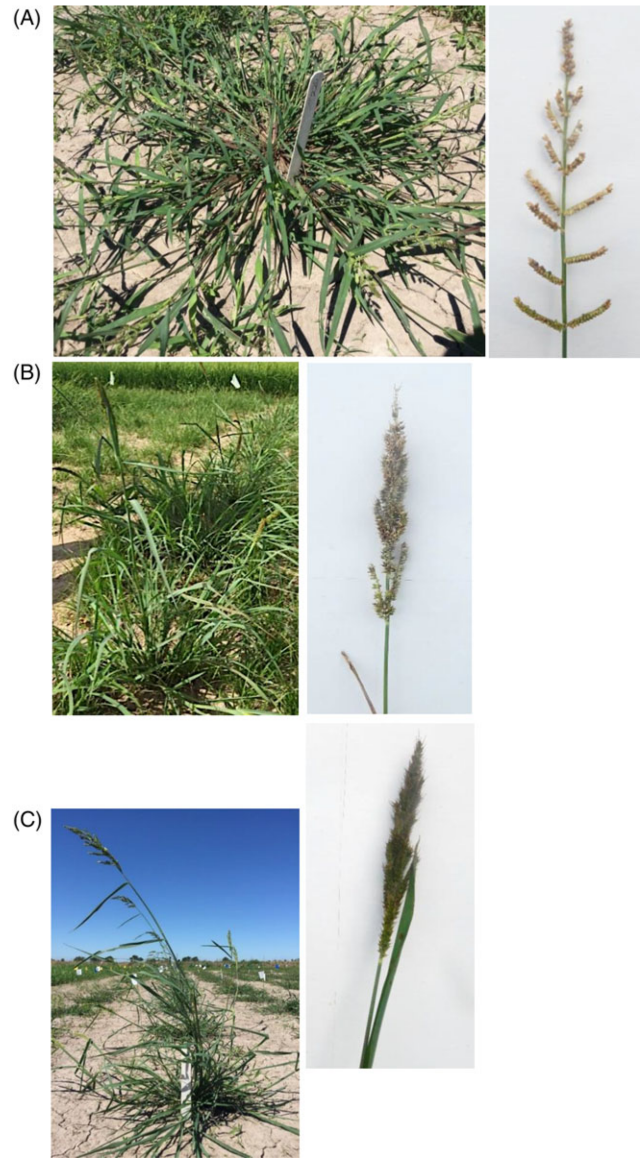

Figure 2. Photos of three *Echinochloa* ecotypes: (A) *E. colona*; (B) *E. crus-galli*; (C) *E. muricata.*

observed in the *E. muricata* ecotype studied here. With respect to stem color, 92% of the *E. colona* ecotypes had purple stems, and the rest had a mixture of plants with purple or green stems. *Echinochloa muricata* had green-based stems, whereas *E. crus-galli* had plants with both green- or purple-based stems. Tabacchi et al. (2006) reported in an Italian rice system that basal stem color was an important morphological trait for distinguishing different *Echinochloa* accessions. The ecological advantage of stem color, however, is not clear.

Nearly 87% of the *E. colona* ecotypes had smooth (hairless) leaf texture, and the rest had a mixture of individuals with both smooth and rough-textured leaves; *E. crus-galli* and *E. muricata* had smooth leaves. This finding is largely consistent with the report of Holm et al. (1977) on *Echinochloa* spp. collected from the Mississippi delta region of Arkansas, where *E. colona*, *E. crus-galli*, and *E. muricata* all had hairless leaf blades. They did indicate that *E. muricata* may have some long hairs at the base of the leaf blades,

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Table 2. Morphophysiological characteristics of different *Echinochloa* spp. collected in rice production fields in Texas.

Species[a]	*N*	Plant height	Flag leaf length	Flag leaf width	Panicle length	Seed shattering	Germination	Days to flowering initiation[b]			
		cm		mm		%		28	35	21–28	28–35
E. colona	52	48	11	8	99	20	83	2	7	1	42
E. crus-galli	1	127	17	12	172	30	85	0	0	0	1
E. muricata	1	137	18	12	173	43	86	0	0	0	1

		Panicle color[c]			Stem color[c]			Canopy structure		
Species	*N*	P	G	P & G	P	G	P & G	Prostrate	Erect	Intermediate
E. colona	52	17	2	33	48	0	4	25	0	27
E. crus-galli	1	0	1	0	0	0	1	0	1	0
E. muricata	1	0	1	0	0	1	0	0	1	0

		Leaf texture[d]			Flag leaf angle			Leaf color[c]		
Species	*N*	S	R	S & R	0°–45°	46°–90°	Variable: 0°–90°	P	G	P & G
E. colona	52	45	0	7	3	1	48	0	38	14
E. crus-galli	1	1	0	0	0	1	0	0	0	1
E. muricata	1	1	0	0	0	1	0	0	0	1

[a]Species identified based on taxonomic classification.
[b]Measured as days after transplanting; the range indicates intra-ecotype variability in initiation of first flowering.
[c]Color: P, all plants within the ecotype exhibiting purple color; G, all plants within the ecotype exhibiting green color; P & G, plants within a given ecotype with both plants of purple color and plants of green color.
[d]Leaf texture: S, smooth; R, rough; S & R, both smooth and rough.

but this characteristic was not observed in the current study. Leaf hairs may directly influence the retention and uptake of herbicides (Himel et al. 1990). For instance, Sterling and Jochem (1999) found that the white locoweed (*Oxytropis sericea* Nutt.), which has fine hairs on the leaf surface, exhibited high uptake of the herbicide metsulfuron.

The average flag leaf length of *E. colona* was 11 cm (range: 5.3 to 17 cm), considerably shorter than that of *E. crus-galli* (17 cm) and *E. muricata* (18 cm) (Table 2). Likewise, *E. colona* had narrower (8-mm wide) flag leaves than *E. crus-galli* (12-mm wide) or *E. muricata* (12-mm wide). The surface area of a flag leaf is associated with photosynthetic capacity (Damalas et al. 2008; Evans and Rawson 1970). High flag leaf surface area allows the plant to intercept more sunlight for photosynthesis and assimilate carbon. The longer and wider flag leaves of *E. crus-galli* and *E. muricata* could produce sufficient energy to support the taller plant stature, compared with *E. colona*. With respect to flag leaf angle, erect angles (<45°) in grasses allow for more light penetration to the lower parts of the plant canopy and improve overall light interception by the plant (Mantilla-Perez and Salas Fernandez 2017). In fact, self-shading rather than leaf angle per se is important for light interception and carbon gain (Falster and Westoby 2003). In the current study, the majority (48 out of 52, 92%) of *E. colona* ecotypes exhibited high within-ecotype variation (0° to 90°) for flag leaf angle. Considering that *E. colona* largely mimics rice crop height, the within-ecotype variability for leaf angle might allow it to maximize light and carbon capture depending on crop density. In contrast, *E. crus-galli* and *E. muricata* had flag leaves with much wider angles (46° to 90°). Given that these two species grew taller than the rice canopy, the broader flag leaves and wider flag leaf angles may have provided better light interception, while shading the rice crop underneath (Weiner and Thomas 1986).

The majority (95%) of *E. colona* began flowering within 28 to 35 d after transplanting, and flowering continued for several weeks. Both *E. crus-galli* and *E. muricata* also initiated flowering within the same time frame as the majority of *E. colona*. Reports indicate that *E. colona* and *E. crus-galli* can flower throughout the year (Chauhan and Johnson 2009; Vengris et al. 1966), whereas *E. muricata* usually flowers during midsummer to early fall (Tahir 2016). A long flowering window increases opportunities for outcrossing among different *Echinochloa* biotypes and species. Even though members of *Echinochloa* are autogamous and predominantly self-pollinating, occasional outcrossing could still happen (Maun and Barrett 1986). Pollen-mediated gene flow (PMGF) was detected among *E. crus-galli* plants at the maximum tested distance of 50 m (Bagavathiannan and Norsworthy 2014). An extended flowering window has implications for the spread of herbicide resistance through PMGF across fields.

With respect to panicle length, *E. colona* had the shortest (9.9 cm) panicles compared with *E. crus-galli* (17.2 cm) and *E. muricata* (17.3 cm). It was reported that the inflorescence length and seed production are positively correlated in *E. crus-galli* (Norris 1992). Two different panicle colors are usually observed in *Echinochloa*: purple and green. The majority (63%) of *E. colona* had a mix of both purple- and green-colored panicles within the ecotype (Table 2), whereas the remaining 33% or 4% of the ecotypes had purple- or green-colored panicles, respectively. Both *E. crus-galli* and *E. muricata* had green-colored panicles. Upon maturity, the caryopsis of the green panicles typically turned brown, while the purple-colored panicles turned dark brown.

Seed shattering is an important weedy trait that contributes to species' persistence in the field. Results showed that *E. crus-galli* and *E. muricata* had greater seed-shattering abilities with 30% and 43% at plant maturity, respectively, compared with *E. colona* (20%). When tested at 7 mo after harvest, the vast majority of the ecotypes had already overcome dormancy, exhibiting 83%, 85%, and 86% germination for *E. colona*, *E. crus-galli*, and *E. muricata*, respectively. Honěk and Martinková (1996) reported that freshly harvested seeds of *Echinochloa* spp. typically exhibit dormancy, the length of which can vary from 3 to 7 mo. Seed dormancy is an important bet-hedging mechanism that facilitates temporal dispersal of a species, allowing for persistence under unpredictable environmental conditions (Baskin and Baskin 2004). Unfortunately, seed dormancy of the freshly harvested seed could not be tested in the present study due to practical circumstances. Nevertheless, tetrazolium test results suggest that the vast majority of the seed might germinate in the following season

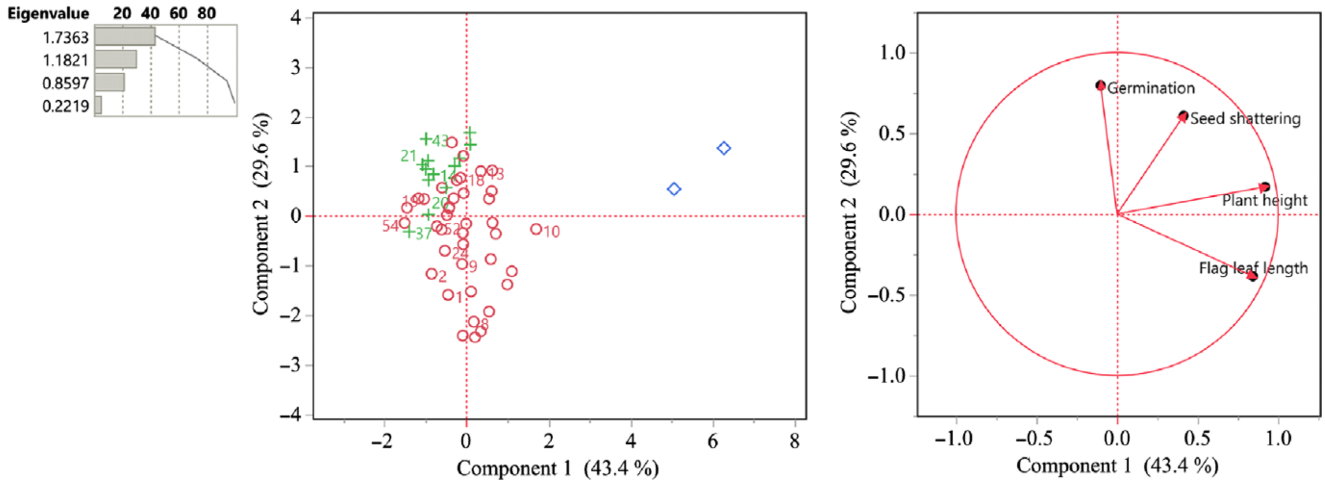

Figure 3. Principal component analysis showing the traits (plant height, flag leaf length, seed shattering, and seed germination) that significantly contributed to the overall morphological diversity of *Echinochloa* ecotypes investigated in this study.

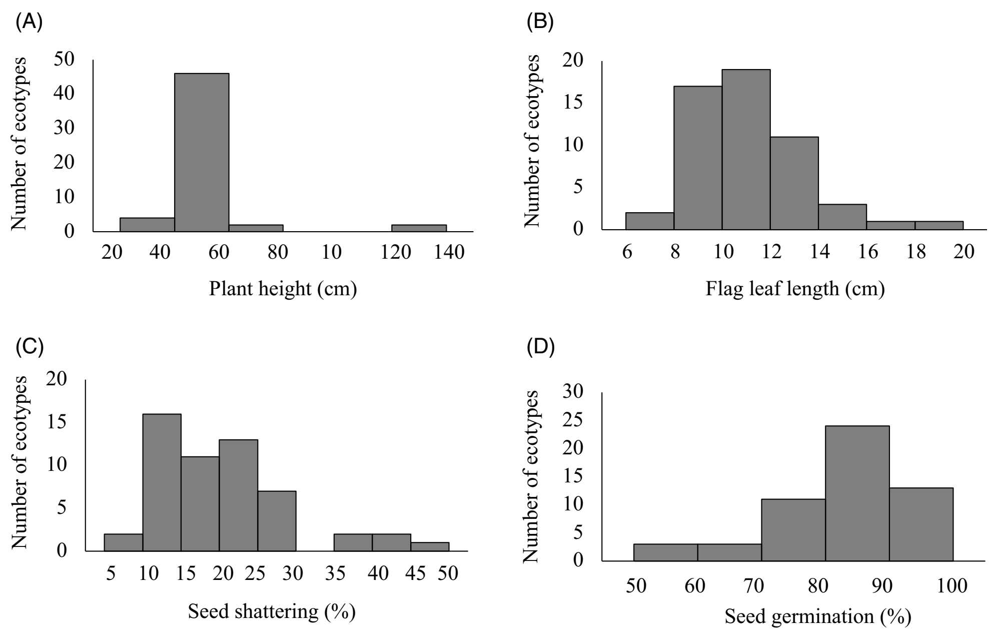

Figure 4. Histogram for the four important traits identified by the principal component analysis among the *Echinochloa* ecotypes collected from Texas rice fields: (A) plant height, (B) flag leaf length, (C) seed shattering, and (D) seed germination.

should germination requirements be fulfilled. Animal grazing during the rotational year typically practiced in the region might be beneficial for the management of *Echinochloa* spp.

Clustering of Ecotypes Based on Four Significant Phenotypic Traits

The PCA (Figure 3) indicated that 4 of the 14 phenological traits characterized in the study— plant height, flag leaf length, seed shattering, and seed germination—contributed significantly to the overall morphological diversity of *Echinochloa* spp. The frequency distribution of the four traits that significantly contributed to *Echinochloa* ecotype diversity is displayed in Figure 4. Based on these four traits, K-means cluster analysis grouped the 54 *Echinochloa* ecotypes into five clusters (Table 3; Figure 5).

Cluster 1 comprised 28% of the characterized ecotypes, with an average plant height of 51 cm, slightly taller than the plants in the Cluster 2 (47 cm), and an average flag leaf length of 12 cm. Seed-shattering potential was the lowest (14%) in this cluster, and seed germination was 86%. Seed shattering is an important weedy trait that typically favors dispersal and persistence of a weed population through seedbank replenishment (Holm et al. 1977). Low weed seed shattering ability of certain ecotypes can be exploited for harvest weed seed control strategies, which rely on the ability of weeds to retain seeds at the time of harvest for seed collection and destruction (Walsh et al. 2018). Cluster 2, which represented the highest number of ecotypes (43%), was characterized by the highest germination capacity (88%). It also had purple- or green-colored panicles.

Cluster 3 consisted of 7% of the total ecotypes, having the shortest (9 cm) flag leaf length and the greatest (40%) seed

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Table 3. K-means cluster analysis of different *Echinochloa* ecotypes based on the four most discriminating traits.[a]

Cluster	*N*	Plant height	Flag leaf length	Flag leaf width	Panicle length	Seed shattering	Germination	Days to flowering initiation[b]			
		——cm——		——mm——		——%——		28	35	21–28	28–35
1	15	51	12	8	101	14	86	0	3	1	11
2	23	47	10	8	97	21	88	1	1	0	21
3	4	43	9	7	93	40	82	0	1	0	3
4	10	48	13	8	101	18	66	1	2	0	7
5	2	132	18	12	172	36	86	0	0	0	2

		Panicle color[c]			Stem color[c]			Canopy structure		
Cluster	*N*	P	G	P & G	P	G	P & G	Prostrate	Erect	Intermediate
1	15	4	0	11	14	0	1	7	0	8
2	23	7	2	14	22	0	1	11	0	12
3	4	0	0	4	3	0	1	1	0	3
4	10	6	0	4	9	0	1	6	0	4
5	2	0	2	0	0	1	1	0	2	0

		Leaf texture[d]			Leaf angle			Leaf color[c]		
Cluster	*N*	S	R	S & R	0°–45°	46°–90°	Variable: 0°–90°	P	G	P & G
1	15	13	0	2	0	0	15	0	11	1
2	23	21	0	2	0	0	22	0	17	1
3	4	4	0	0	0	1	3	0	3	1
4	10	7	0	3	1	0	9	0	7	1
5	2	2	0	0	1	0	0	0	0	1

[a]Clusters based on plant height, flag leaf length, seed shattering, and seed germination.
[b]Measured as days after transplanting; the range indicates intra-ecotype variability in initiation of first flowering.
[c]Color: P, all plants within the ecotype exhibiting purple color; G, all plants within the ecotype exhibiting green color; P & G, plants within a given ecotype with both plants of purple color and plants of green color.
[d]Leaf texture: S, smooth; R, rough; S & R, smooth and rough.

shattering. The plants in this cluster were also the shortest (43 cm) compared with other groups. Cluster 4 consisted of 18% of the ecotypes characterized. This cluster had the lowest germination rate (66%). This is notable, because plants in other clusters showed very high germination. Low germination could be attributed to seed dormancy, which is regulated by genetic and environmental factors (Bewley 1997; Chauhan and Johnson 2009). As discussed earlier, seed dormancy allows for species' persistence (Baskin and Baskin 2004).

Cluster 5 had two ecotypes (4% of the total ecotypes) that were phenotypically very distinct from others. A taxonomic identification revealed that these two ecotypes were of different species: *E. crus-galli* and *E. muricata*. Plants were the tallest (132 cm) with the largest flag leaf (18-cm length, 1.2-cm width) and panicle length (17.2 cm). Tall plants can suppress the surrounding vegetation through shading and thereby can be more competitive for resources (Weiner and Thomas 1986). This cluster had the second highest seed shattering (36%) and germination capacity (86%).

Correlation Analysis of Morphophysiological Traits and Their Association with Herbicide Resistance

Correlation analysis (Figure 6) performed on 14 morphophysiological traits revealed that plant height was highly correlated with flag leaf width (r = 0.79), panicle length (r = 0.92), stem color (r = 0.91), and canopy structure (r = 0.83). In general, *E. crus-galli* and *E. muricata* in the current study were taller and exhibited wider flag leaves and longer panicles compared with *E. colona*. Similar findings were reported by Tahir (2016) in Arkansas, where the widest flag leaves and longest panicles were observed in *E. crus-galli* and *E. muricata*, rather than in *E. colona*. Stem color was highly correlated with canopy structure (0.81), which represents the plant growth habit. Specifically, *Echinochloa* ecotypes with purple stem color tend to have open geometry with shorter stature. Conversely, ecotypes with greener stems were taller, with a closed canopy. An open canopy allows for better light penetration, though open canopy with decumbent or prostrate growth habit may pose disadvantages for hybridization. In this respect, *E. crus-galli* and *E. muricata* may have greater chances of hybridization in a rice field, owing to taller stature and larger inflorescences (OLA and MAFF 2002).

No significant association was observed between phenotypic traits and herbicide-resistance status (Figure 6). The multiple herbicide resistance profile of *Echinochloa* within each cluster is displayed in Figure 5. In Cluster 1 (total 15 ecotypes), 26% were susceptible to all four herbicides, whereas 37%, 11%, 21%, and 5% of the ecotypes were resistant to one, two, three, and four herbicide SOAs, respectively. In Cluster 2 (total 23 ecotypes), 24%, 38%, and 14% of the ecotypes were resistant to one, two, and three herbicide SOAs, respectively, whereas the rest (24%) were susceptible. None of the ecotypes in this cluster were resistant to all four tested herbicides.

Cluster 3 consisted of three ecotypes, of which two were resistant to one SOA, and one ecotype was resistant to three SOAs. In Cluster 4, which comprised nine ecotypes, 22% were susceptible, whereas 42% and 11% were resistant to two and three SOAs, respectively. All four clusters were composed of *E. colona* ecotypes only, and frequency of resistance to one or more SOAs was independent of cluster groupings. Similar results have been reported by Rouse et al. (2018) in a study conducted in Arkansas, where 40% of the *E. colona* ecotypes were found resistant to only one SOA and 33% were resistant to two or more herbicides. Cluster 5 consisted of two ecotypes, one of them was susceptible (*E. crus-galli*) and the other one (*E. muricata*) was resistant to one SOA. In contrast to the current findings, *E. crus-galli* in Arkansas exhibited higher frequency of three-way resistance (three

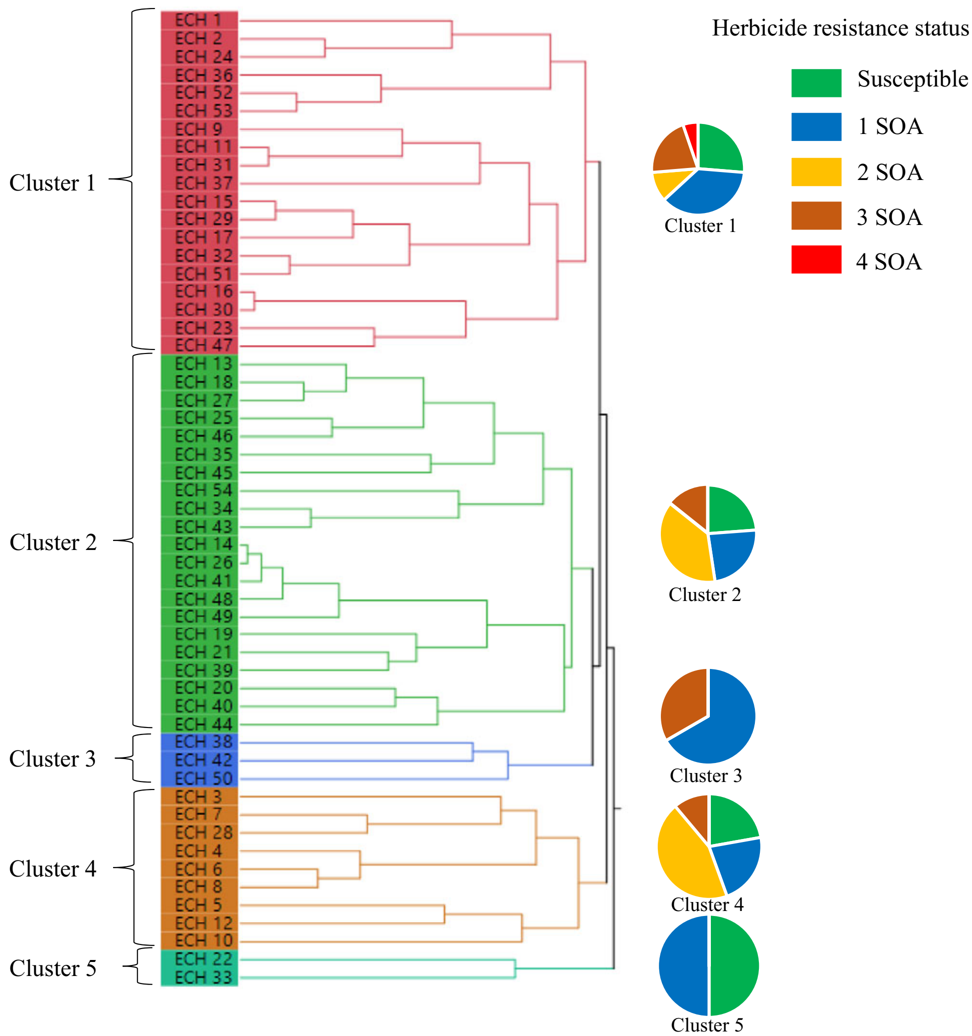

Figure 5. Hierarchical cluster analysis for the 54 *Echinochloa* ecotypes, along with the herbicide-resistance status for the ecotypes grouped within each cluster shown in pie charts. Green indicates proportion of susceptible ecotypes within the cluster, whereas other colors indicate resistance to one or more herbicide sites of action (SOAs).

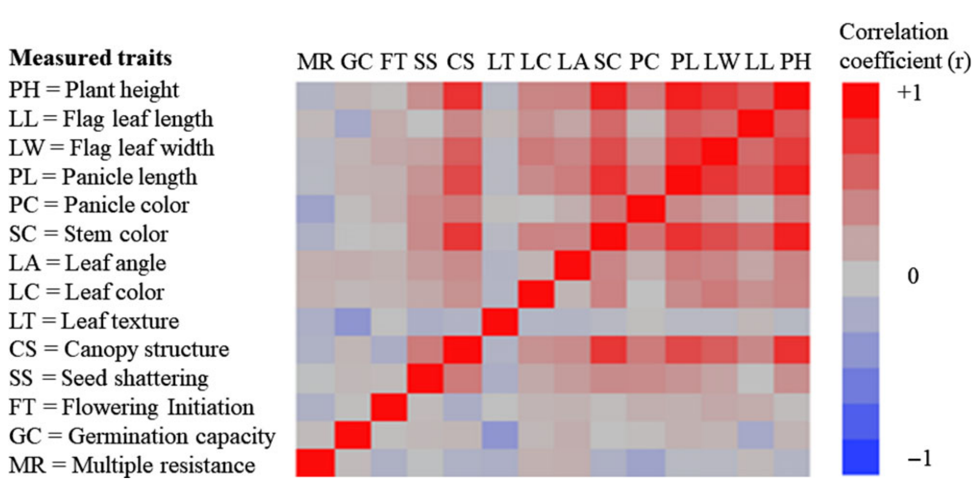

Figure 6. Correlation analysis between herbicide-resistance status and 13 morphophysiological traits measured in 54 *Echinochloa* ecotypes collected in rice production fields in Texas.

SOAs) compared with other species (Rouse et al. 2018). However, due to low sample size of *E. crus-galli* and *E. muricata* available in the current study, resistance frequencies could not be compared across the different *Echinochloa* spp.

This common garden study revealed the occurrence of three *Echinochloa* species in Texas rice production, namely *E. colona*, *E. crus-galli*, and *E. muricata*, among which *E. colona* is the predominant species. Even though *Echinochloa* spp. are commonly referred to as "barnyardgrass," it is vital that the research and extension community is cognizant of the inaccuracy of attribution. The 14 morphophysiological traits investigated in this study were found to vary greatly within and among the ecotypes. Certain morphological traits, notably plant height and flag leaf size, could help with *Echinochloa* species classification. However, association between herbicide resistance and weed morphophysiological traits was not evident. Overall, the presence of high diversity for different morphological traits among different *Echinochloa* ecotypes characterized in this study suggests their ability for adaptation to different selection agents. The findings support the fact that *Echinochloa* spp. show high risk for the evolution of herbicide resistance and emphasize the need for proactive diversified management to tackle this species.

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