Morphophysiological diversity and its association with herbicide resistance in Echinochloa ecotypes

Rui Liu1, Vijay Singh2, Seth Abugho3, Hao-Sheng Lin4, Xin-Gen Zhou5 and Muthukumar Bagavathiannan5

1Graduate Research Assistant, Texas A&M University, College Station, TX, USA; current: Kansas State University Agricultural Research Station, Hays, KS, USA; 2Assistant Research Scientist, Texas A&M University, College Station, TX, USA; current: Virginia Tech Eastern Shore Agricultural Research and Extension Center, Painter, VA, USA; 3Graduate Research Assistant, Texas A&M University, College Station, TX, USA; 4Undergraduate Researcher, Texas A&M University, College Station, TX, USA; 5Associate Professor, AgriLife Research and Extension Center, Beaumont, TX, USA and 6Associate Professor, Texas A&M University, College Station, TX, USA

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 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 (Hrushev 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].

Although *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. 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 Wintemmmen 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™'
Echinochloa crus-galli had long smooth flag leaves (Table 2). 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
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. *Echinocloa 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 *Echinocloa* 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.

### 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        | 96.16         | *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*   |

![Figure 2. Photos of three Echinochloa ecotypes: (A) E. colona; (B) E. crus-galli; (C) E. muricata.](https://doi.org/10.1017/wsc.2021.64)
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 *E. muricata* 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 et al. 2017). In fact, self-shading rather than leaf angle per se is important for light interception and carbon gain (Falster et al. 2017).

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

| Speciesa | N | Plant height | Flag leaf length | Flag leaf width | Panicle length | Seed shattering | Germination | Days to flowering initiationb |
|----------|---|--------------|-----------------|----------------|---------------|----------------|-------------|-----------------------------|
| *E. colona* | 52 | 48 | 11 | 8 | 99 | 20 | 83 | 28–35 |
| *E. crus-galli* | 1 | 127 | 17 | 12 | 172 | 30 | 85 | 21–28 |
| *E. muricata* | 1 | 137 | 18 | 12 | 173 | 43 | 86 | 28–35 |

| Species | N | Panicle length | Stem color | Canopy structure |
|---------|---|----------------|-------------|-----------------|
| *E. colona* | 52 | 17 | 33 | 0 | 25 |
| *E. crus-galli* | 1 | 1 | 0 | 48 | 0 |
| *E. muricata* | 1 | 0 | 0 | 10 | 0 |

| Species | N | Leaf texture | Flag leaf angle | Leaf color |
|---------|---|-------------|----------------|-----------|
| *E. colona* | 52 | 45 | 7 | 3 | 1 |
| *E. crus-galli* | 1 | 1 | 0 | 1 |
| *E. muricata* | 1 | 1 | 0 | 1 |

| Speciesa | N | Panicle color | Stem color | Canopy structure |
|----------|---|---------------|-------------|-----------------|
| *E. colona* | 52 | P | G | P & G | 28–35 |
| *E. crus-galli* | 1 | P | G | P & G | 21–28 |
| *E. muricata* | 1 | P | G | P & G | 28–35 |

*Species identified based on taxonomic classification.

1Measured as days after transplanting; the range indicates intra-ecotype variability in initiation of first flowering.

2Within-ecotype variability in initiation of first flowering.

3Seed 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.
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...
plants of green color. Wider flag leaves and longer panicles compared with E. muricata and E. crus-galli (Baskin 2004). Earlier, seed dormancy allows for species factors (Bewley 1997; Chauhan and Johnson 2009). As discussed seed dormancy, which is regulated by genetic and environmental very high germination. Low germination could be attributed to rate (66%). This is notable, because plants in other clusters showed ecotypes characterized. This cluster had the lowest germination compared with other groups. Cluster 4 consisted of 18% of the 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

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

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

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

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 |
|---------|---|--------------|-----------------|-----------------|----------------|----------------|-------------|--------------------------|
|         |   | (cm)         | (mm)           |                 |                |                |             | 28–35 | 21–28 | 28–35 |
| 1       | 15 | 51           | 8              | 101             | 14             | 86             | 0           | 3 | 1 | 11 |
| 2       | 23 | 47           | 8              | 97              | 21             | 88             | 1           | 1 | 0 | 21 |
| 3       | 4  | 43           | 7              | 93              | 40             | 82             | 0           | 1 | 0 | 3  |
| 4       | 10 | 48           | 8              | 101             | 18             | 66             | 1           | 2 | 0 | 7  |
| 5       | 2  | 132          | 12             | 172             | 36             | 86             | 0           | 0 | 0 | 2  |

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 findings support the fact that herbicide resistance and weed morphophysiological traits were 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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