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Research Article

Interspecific and intraspecific differences in seed germination response to different temperatures of three *Echinochloa* rice weeds: A case study with 327 populations

Yang Chen¹, Aatiqa Masoom², Zeyue Huang³, Jiahao Xue⁴, Guoqi Chen⁵

¹Graduate student, Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College (Research Institute of Rice Industrial Engineering Technology) of Yangzhou University, Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, China. ² Graduate student, Agricultural College, Yangzhou University, Yangzhou, China. ³ Graduate student, Agricultural College, Yangzhou University, Yangzhou, China. ⁴ Graduate student, Agricultural College, Yangzhou University, Yangzhou, China. ⁵ Associate Professor, Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College (Research Institute of Rice Industrial Engineering Technology) of Yangzhou University, Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, China.

Author for correspondence: Guoqi Chen; Email: chenguoqi@yzu.edu.cn, The first two authors contributed equally to this work.

Abstract

Echinochloa crus-galli var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Petermann (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG) are all serious rice (*Oryza sativa* L.) weeds, which are usually treated as a single species in weed management practices. To determine interspecific and intraspecific differences in seed germination responding to different temperatures among the three *Echinochloa* weeds, we conducted field surveys and collected 66 EC, 141 ECM, and 120 EG populations from rice fields of East China in 2022; and tested their seed germination under 28/15C (day/night), 30/20C, and 35/25C regimes, simulating temperatures of rice planting periods for double-cropping early rice, single-cropping rice, and double-cropping late rice, respectively. In EC, ECM, and EG, seed percentage germination (cumulative percent of germinated seed) and germination index (sum of the ratio of germinated seeds to the corresponding days) increased with increasing temperatures. At 28/15C, the average percentage germination of EC populations (67.5%) was significantly ($P < 0.05$) higher than ECM (46.4%) and EG (43.7%); GD_{50} (duration for 50% total germination) for EC populations (5.2 d) was significantly shorter than ECM (5.9 d) and EG (5.8 d). At 35/25C, the percentage germination of EC (90.7%), ECM (80.5%), and EG (80.3%) were all significantly the highest among the three temperature treatments, respectively, and the GD_{50} values for EC (2.5 d), ECM (2.6 d), and EG (2.7 d) were all significantly the lowest. At 30/20C and 35/25C, average germination percentage of populations collected from transplanted rice fields were significantly higher than that of populations collected from direct-seeded rice fields. Moreover, among EG populations, the longitudes and latitudes of collection locations were significantly correlated with seed percentage germination and germination indices. According to the interspecific differences and intraspecific variations of *Echinochloa* species, weed management strategies should also be customized according to the species and population characteristics in seed germination.

Keywords: *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), *Echinochloa glabrescens* Munro ex Hook. f. (EG), seed germination

Introduction

The genus *Echinochloa* includes numerous problematic rice (*Oryza sativa* L.) weeds, such as *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC) (Holm et al. 1979), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook f. (EG) (Chen et al. 2019) in rice planting areas in Asia. EC, ECM, and EG are very similar in morphology, and are usually treated as a single species in weed management practices. Spikelets of EC are 3-4 mm in length, and lower lemma extends into an awn to 3 cm. Spikelets of ECM are about 3 mm in length, awnless, or with awns shorter than 5 mm, while inflorescences of ECM are usually branched. The lower lemma of EG is convex, coriaceous, and shining; while the lower lemma of EC and ECM are flat and herbaceous (Chen et al. 2019; Chen and Phillips 2006). Our previous studies had found that the harmfulness of ECM and EG is not lower than that of EC and even higher in some regions. Zhang et al. (2014) found that 6 plants·m⁻² of EC and ECM resulted in 10.8% -25.3% and 19.2% -39.7% reduction in rice yield, respectively. Opena et al. (2014) found that EG may cause a yield loss of 7% - 87%.

EC, ECM, and EG are propagated by seed (Chen et al. 2022; Li 1998). Therefore, knowledge on seed germination of these weed species is of key importance for their integrated management (Chen et al. 2023b; Masin et al. 2014; Rezvani et al. 2021). Temperature is the primary factor influencing seed germination. EC did not germinate below 10C, and its percentage germination increased with temperature increasing from 10C to 24C (Loddo et al. 2018). The optimal germination temperature for EC was around 25-30C (Kovach et al. 2010). EC buried in the autumn had a higher average percentage germination than seeds buried in the spring, and the optimal germination temperature for EC decreased with increasing seed age (Martinkova and Honek 2013; Martinkova et al. 2006). In the case of alternating light and dark, the percentage germination of EG populations at 25/15C, 30/20C, and 35/25C were similar (Opena et al. 2014). The average percentage germination of ECM was 54.0 % at 25C, and 0 % at 17C.

To date, research on *Echinochloa* species has been mainly on EC, and most studies on the seed biology of *Echinochloa* species based on one to two populations. Yoshioka et al. (1998) confirmed soil CO₂ was responsible for causing intermittent flushes of germination by testing 50 EC populations. The differences in biological and ecological traits among these three

species remain undefined. Considering the widespread distribution of the three *Echinochloa* species and the seriousness of their damage in rice fields, a comparative study of the variations between EC, ECM, and EG is necessary. It is the basis for differentiated and efficient integrated management.

In 2022, we collected 66, 141, and 120 populations of EC, ECM, and EG, respectively, from rice fields in East China. We compared the germination characteristics of these populations under different temperature regimes. It had been reported that there were significant intraspecific differences in the initial germination temperature of EC (Royo-Esnal et al. 2022). Nevertheless, the existence of intraspecific differences in ECM and EG had yet to be determined. With all 327 populations collected from rice fields in East China, the aims of this study were to 1) reveal intraspecific differences in seed germination of EC, ECM, and EG at different temperatures simulating different rice planting periods; 2) compare seed germination of EC, ECM, and EG at different temperatures.

Materials and Methods

Sampling and Investigation

In October 2022, we conducted field surveys on the occurrence of EC, ECM, and EG in rice fields of East China (Table S1), and collected seeds from each rice field surveyed. A total of 250 independent rice fields (sites) occurring EC, ECM, or EG were randomly surveyed, with an interval of >5 km for adjacent sites (Figure 1, and Table S1). Each site surveyed a rice field covering an area of about 0.1 – 0.2 ha. The identification of *Echinochloa* species was based on our previous work (Chen et al. 2019). A total of 327 *Echinochloa* populations were collected, including 66 EC, 141 ECM, and 120 EG populations. Panicles with mature seeds were randomly collected from more than 100 individuals of each population with a pollen bag (100 mesh, 30 cm by 45 cm), and mature seeds were collected by hand. The relative dominance of *Echinochloa* species in rice fields was scored 0.1, 0.5, 1, 2, 3, 4, or 5 by visual inspection according to Qiang (2005) and our previous field surveys (Chen et al. 2013). Seeds were air-dried and stored in our lab at room temperature fluctuating from 15C to 25C. From March to June 2023, the 1000-seed weight of each population was determined by weighing five replicates of 100 mature seeds.

Experimental Design

To determine the adaptation of the three *Echinochloa* species to temperatures of the beginning of rice planting seasons in East China, three temperature regimes were set responding to three different rice cropping patterns in East China: single-cropping rice, double-cropping early rice, and double-cropping late rice. Single-cropping rice is mainly sown or transplanted around June 20, double-cropping early rice is usually transplanted around May 20, and double-cropping late rice is often transplanted around July 20. Therefore, seeds were incubated at alternating temperatures of 28/15C (day/night), 30/20C, and 35/25C with a 12/12h (light/dark) photoperiod to simulate the temperature of double-cropping early rice, single-cropping rice, and double-cropping late rice, respectively. From April to June 2023, about seven months after collecting, seed germination for each population under the above three temperature regimes was determined in incubators (Changzhou Haibo Instrument Equipment Co., Ltd., HBZ-400B) with three replications. Fifty seeds were placed into a 9-cm diameter Petri dish with two pieces of filter paper (Hangzhou Fuyang Beimu Pulp & Paper Co., Ltd.), which contained 6 ml distilled water. The germinated seeds with a visible radicle were counted and removed (Rehman et al. 2011). Percentage germination of each Petri dish was determined daily for 21 d. From May to June 2024, 20% of 327 *Echinochloa* populations were selected randomly for germination repeatedly.

Statistical Analysis

The percentage germination of each dish was determined by the percentage of germinated seeds out of the number of sown seeds (50). Germination index for each dish was determined by equation 1 (Schmer et al. 2012).

$$GI = \sum (GT / DT) \quad (\text{Eq. 1})$$

where GT is the number of seed germination per day; and DT is the germination day corresponding to GT . Coefficient of variations (CV s) for variables were determined using equation 2 (Munthali et al. 2012).

$$CV = \sigma / \mu \times 100\% \quad (\text{Eq. 2})$$

where μ is the sample mean, and σ is the sample standard deviation.

A three-parameter logistic function was fitted to test days and accumulated temperature required for germination (equation 3), using the “drc” add-on package in R 3.1.3 (Ritz et al. 2015):

$$Y = a / [1 + (x / e)^b] \quad (\text{Eq. 3})$$

where Y denoted the total germination (%) at day x of the germination or accumulated temperature x after sowing; a was the upper limit; b indicates the slope; e was the days required for 50% of total germination (GD_{50}) or the accumulated temperature required for 50% of total germination (TD_{50}). Accordingly, the days required for 90% of total germination (GD_{90}) and the accumulated temperature required for 90% of total germination (TD_{90}) were determined.

To determine differences in germination indices among three temperature regimes, data were subjected to analysis of variance in SPSS software (version 26.0, IBM, Armonk, NY, USA), using the one-way analysis of variance (ANOVA) procedure. Data were checked for normality and constant variance prior to analysis. Treatment means were separated using LSD test at $P = 0.05$. The general linear model (GLM) in SPSS was used to test data of environmental factors and germination indices; *Echinochloa* species, planting methods, and collection cities were set as dependent variable; GD_{50} , GD_{90} , and percentage germination were set as fixed factors. Correlations among the latitudes and longitudes of seed collection sites and germination indices, and 1000-seed weight and relative dominance, percentage germination, and GD_{50} were determined with SPSS using correlate analysis. The independent sample T-test method in SPSS software was used to compare the differences in percentage germinations of *Echinochloa* species collected from direct seeding rice fields and transplanting rice fields at different temperature regimes. The data presented means \pm SEs.

Results and Discussion

Distribution and 1000-seed Weight

Among the 250 collection sites (Figure 1), two *Echinochloa* species co-occurred in 68 collection sites, and three *Echinochloa* species co-occurred in five collection sites. ECM was the most frequent species (43.0%), followed by EG (36.6%) and EC (20.4%). Average values of relative dominance among seed collecting rice fields were 2.5, 2.5, and 2.7 (Figure S1), for

EC, ECM, and EG populations, respectively, which did not show significant differences. *Echinochloa* species differed in their average 1000-seed weights. EC were significantly the heaviest (average 1000-seed weight of 2.92 g), followed by EG and ECM with 1000-seed weights of 2.40 g and 2.26 g (Figure 2), respectively. Interestingly, thousand-seed weight of the 327 *Echinochloa* populations showed significant and positive correlations with relative dominance, percentage germination and germination index (Figures 3 and 4).

Interspecific Differences

At 28/15C, the average percentage germination of EC (67.5%) was significantly higher ($P < 0.05$) than that of ECM (46.4%) and EG (43.7%) (Figure 5), and the coefficients of variations (CVs) were 35.9, 43.2, and 44.2, respectively (Table S2). The average percentage germinations of the three species at 30/20C were like those of the same species at 28/15C (Table S3). Among the 141 EG populations, the latitude of population collection sites significantly and positively correlated with the percentage germination at 28/15C and 30/20C (Table 1); the longitudes of population collection sites significantly and negatively correlated the percentage germination at 30/20C. When temperature regime increased to 35/25C, the average percentage germinations of EC, ECM, and EG were 90.7%, 80.5%, and 80.3%, and the coefficients of variation of EC, ECM, and EG decreased to 11.2, 17.5, and 17.8, respectively (Tables 1 and S2).

The average germination indices of EC, ECM, and EG were 5.6, 3.8, and 3.6 at 28/15C, respectively (Figure 6), and the coefficients of variation were 41.0, 55.7, and 52.2, respectively. At 35/25C, the average germination indices of EC, ECM, and EG were also significantly highest among different treated temperatures, with 13.7, 9.8, and 9.7, respectively. The percentage germination and germination index of EC was higher than that of the other two species regardless of temperature regimes.

The percentage germination and germination process of EC were significantly higher and faster than those of ECM and EG at the same temperature treatments, which might be related with seed mass. Zhou et al. (2021) found that the smaller common ragweed (*Ambrosia artemisiifolia* L.) seeds germinated faster with a study of 26 populations. Here, the 1000-seed of EC was heavier than ECM and EG by 29.2% and 21.7%, respectively (Figure 2).

Meanwhile, species of *Echinochloa* and the city where the population was collected had significant influences on the GD₅₀ and GD₉₀ values and percentage germination (Table 2). Moreover, percentage germination at high temperatures was significantly influenced by the planting method (direct seeding or transplanting) at the site where the seed was collected. Specifically, at 30/20C, average germination percentage of overall populations collected from transplanted rice fields (62.9±2.6%) were significantly higher than that of overall populations collected from direct-seeded rice fields (52.5±2.1%), as well as the comparison at 35/25C (85.9±1.7% VS 76.9±1.5%) (Table S4). Chemical control time window against *Echinochloa* species in rice fields is before planting or sowing to the rice bolting stage, which is about 10 days shorter in transplanting rice fields than in direct seeding rice fields (Li et al. 2015; Sun et al. 2014). Thus, *Echinochloa* species populations escaping chemical control applications lean to have a longer growth period in transplanting rice fields than those in direct seeding rice fields. This longer development time often resulted in higher quality seeds (Chen et al. 2017), which could lead to a significantly high percentage germination of *Echinochloa* seeds collected from transplanting rice fields.

Compared to the average GD₅₀ and GD₉₀ value of ECM and EG, those of EC were significantly highest among three temperature regimes (Figures 7 and 8). The GD₅₀ values of *Echinochloa* species showed significantly and positively correlated with the latitude of population collection sites and negatively correlated with the longitudes of population collection at 35/25C, respectively. Considering the different climate zones in the world, accumulated temperature was more universal (Hou et al. 2014). At 28/15C, the average accumulated temperature of EC, ECM, and EG to 50% total germination were 111.0C, 127.3C, and 124.7C, respectively (Table 3). At 35/25C, the average accumulated temperature of EC, ECM, and EG to 50% total germination were 74.3C, 76.4C, and 80.2C, respectively. Six indices of ECM and EG seed germination showed no significant differences at the same temperature treatments, excluding the GD₉₀ and TD₉₀ at 30/20C and 35/25C (Table S5).

Interspecific differences were obvious among the three *Echinochloa* species and seed germination could affect by the latitudes and longitudes of the population collection sites (Zhou et al. 2021). Cheng et al. (2022) found that smooth cordgrass (*Spartina alterniflora* Loisel.) seeds germinated earlier at higher latitudes. In our results, germination indices of EG

were also impacted by the latitudes and longitudes of collection sites (Table 1). EG seeds in the northwest region had a lighter 1000-seed weight (Table S6), the percentage germination lent to be higher, and the germination process lent to be slower. Furthermore, the city where the population was collected significantly affected the seed percentage germination and germination process of tested *Echinochloa* species populations (Table 2). This effect may be related to the influence of local rice cultivation, field management, climate, and other environmental factors (Li et al., 2009). Among these factors, previous crops were the most critical for weed impact, followed by tillage intensity and environmental parameters (Hanzlik and Gerowitt 2011). Additionally, geographical location and agricultural practices could affect the genetic variation of EC (Altop and Mennan 2011).

Intraspecific Differences

Percentage germination and germination index of three *Echinochoa* species increased with temperature increments, which was significant at 35/25C. At 28/15C, the average GD₅₀ and GD₉₀ values of EC (5.2 and 7.4), ECM (5.9 and 10.5), and EG (5.8 and 10.0) were significant highest, followed by treatments at 30/20C with EC (4.6 and 6.1), ECM (5.1 and 7.4), and EG (5.2 and 8.2). At 35/25C, the GD₅₀ and GD₉₀ value of EC (2.5 and 3.4), ECM (2.6 and 3.8), and EG (2.7 and 4.0) were all significantly the lowest among different temperatures. The GD₅₀ and GD₉₀ values of EC were the lowest at the same temperature conditions, and the GD₉₀s of ECM and EG had significant differences at 35/25C (Tables S3 and S4).

Seed germination of the 66 EC populations at different temperatures simulating different rice planting periods varied considerably, and intraspecific differences decreased significantly with increasing temperature (Tables S2 and S3). Aritz Royo-Esnal et al. (2022) found that one EC population collected in Norway lent to emerge earlier than the other population collected in Italy. Intraspecific variations in seeds percentage germination and GD₅₀ were also found in a study with 25 EC populations (Martinkova and Honek 1997). The 66 EC populations study showed the CVs of 35.9 and 24.3 in percentage germination and GD₅₀ values at 28/15C, while the CVs of both indices decreased dramatically at 35/25C. Serra et al. (2018) found EC populations exhibited varying degrees of adaptability to environmental conditions during the seed germination stage. Intraspecific variations included population-level variations,

between-individual variations, and within-individual variations, which affected by the maternal plant (Albert et al. 2011). Our study was at population-level, these intraspecific variations would affect by genotypic compositions of populations and a temporal variability in the environment. Intraspecific variations in the germination process and the percentage germinations of EC were notable at low temperatures, and such variations were narrowed with increasing temperatures. Increasing temperatures increased the percentage germination and accelerated the germination process (Bastiani et al. 2015; Derakhshan et al. 2018). Marambe et al. (2002) found percentage germinations of EC at 34/31C were 27-29% higher compared to those at 28/24C with two populations. In this study, the average percentage germinations of 66 EC populations at 35/25C was 90.7%, which was significantly higher than those at 28/15C and 30/20C, respectively; the GD_{50} of 66 EC populations at 35/25C was 2.5 d, which was significantly lower than those at 28/15C and 30/20C. The *CV* of the average percentage germinations among 66 EC populations decreased from 40.7 to 11.2, with treated temperature increased from 30/20C to 35/25C, as well as germination index, GD_{50} , and TD_{50} .

Seed germination of the 141 ECM and the 120 EG populations also showed notable intraspecific variations, which also decreased significantly with the increasing temperature. When the temperature increased to 35/25C, the *CVs* of GD_{50} among 141 ECM and 120 EG populations significantly decreased to 16.9 and 18.1, respectively, as well as percentage germination, germination index, and TD_{50} . The accumulated temperature could serve as a reference for the growth and development of wheat, which required an accumulation of 70 – 80C to produce each leaf on the main stem (Li et al. 2009). Whereas, the accumulated temperatures required for EC, ECM, and EG seeds germination at high temperatures were significantly lower than that at low temperatures. Combined with repetition of the second year (Table S7), seed germination of three *Echinochloa* species response to temperature had significant intraspecific variations.

Management Strategies

In managing rice weeds, different species of *Echinochloa* species are usually treated as a single species (Guo et al. 2017). Nevertheless, our results suggested interspecific differences among EC, ECM, and EG, as well as intraspecific variations among populations of the same

species. Hence, management strategies against EC, ECM, and EG should also be customizable according to the target populations. Especially EC co-occurred with any two other *Echinochloa* species in rice field. The significant correlation between 1000-seed weight, percentage germination and relative dominance of *Echinochloa* weed populations warned farmers to pay more attention to the rice fields with serious *Echinochloa* weed damage and formulate reasonable and effective control measures.

At the temperatures simulating the planting period of double-cropping early rice (28/15C) and single-cropping rice (30/20C), the percentage germinations of three *Echinochloa* species ranged greatly, with a long duration. For long germination periods of three *Echinochloa* species, one-time pre-emergence chemical control is not sufficient to control later-emerging seeds. For double-cropping late rice (35/25C), a majority of the 327 *Echinochloa* population tested showed more than 90% within 3 – 4 d. Therefore, pre-emergence chemical control at 4 d after rice seeding or transplanting could be highly effective (Chen et al. 2023a), as well as stale seedbed strategies (Chen et al. 2022). When two or three *Echinoachloa* species occurred in the same field at low temperature (28/15C and 30/25C), species identification was crucial. At low temperatures, ECM and EG can be treated with the same weed management in view of the similar germination durations of ECM and EG. However, germination duration of EC was shorter than the other two species, making pre-emergency control should be applied in 5-6 d when EC occurred in rice field. Moreover, the longer germination periods of ECM and EG suggested they were more likely to avoid the pre-emergence herbicide treatment in rice fields partially. Consequently, management practices implemented too early may fail to completely control late-emerging seedlings; whereas management practices implemented late will suffer from low efficacy (Marschner et al. 2024), and thus repeated application of pre-emergence herbicides were necessary. Besides, considering the significant influences of collection location on seed germination characteristics, weed management strategies of local or adjacent areas could be used for important references to improve the efficiency of integrated management against *Echinochloa* rice weeds in paddy fields (Boddy et al. 2012; Rezvani et al. 2021).

In summary, we collected 66 EC, 141 ECM, and 120 EG populations in 250 rice fields surveyed in East China, and average values of relative dominance of the three species did not

show significant differences. Thousand-seed weight of overall 327 *Echinochloa* populations showed significant and positive correlations with relative dominance, percentage germination and germination index. Significant interspecific and intraspecific variations in seed germination characteristics under different temperatures were testified. EC showed significantly the highest seed biomass and percentage germinations; and EG and ECM showed similar germination patterns with each other, under all temperature regimes treated (28/15, 30/20 and 35/25C). Percentage germination of three *Echinochloa* species increased with increasing temperatures treated, in which intraspecific variations were narrowed with increasing temperatures. The city where the population was collected had significant influences on the germination of *Echinochloa* species. At 30/20C and 35/25C, average germination percentage of populations collected from transplanted rice fields were significantly higher than that of populations collected from direct-seeded rice fields. Besides, the accumulated temperatures required for EC, ECM, and EG seeds germination at higher temperatures were significantly lower. Together the findings of this study suggested that management strategies against EC, ECM, and EG, such as stale-seed bed and pre-emergence chemical control, should be customizable according to the target populations, in particular when the temperatures are not high.

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Competing Interests. The authors declare no conflicts of interest.

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Table 1. The correlation between the latitude (Lat) and longitude (Lng) of the collection site and the germination indices of three *Echinochloa* species populations.

Temperatur e	Index	<i>Echinochloa</i> <i>crus-galli</i>		<i>Echinochloa</i> <i>crus-galli</i> var. <i>mitis</i> (Pursh)		<i>Echinochloa</i> var. <i>glabrescens</i> Munro ex Hook. f.	
		Lng	Lat	Lng	Lat	Lng	Lat
28/15C	GD ₅₀	-0.12	-0.20	0.07	-0.11	-0.01	-0.03
	GD ₉₀	-0.15	-0.09	0.03	-0.10	-0.07	0.04
	Percentage germinatio n	-0.02	0.09	0.01	0.16	-0.12	0.24*
30/20C	GD ₅₀	-0.13	-0.10	-0.07	-0.06	-0.05	0.06
	GD ₉₀	-0.12	-0.04	-0.10	-0.04	-0.07	0.08
	Percentage germinatio n	-0.08	0.19	0.06	0.09	-0.18*	0.24*
35/25C	GD ₅₀	-0.04	-0.19	-0.02	-0.02	-0.47*	0.36*
	GD ₉₀	0.07	-0.24	0.02	-0.05	-0.37*	0.39*
	Percentage germinatio n	-0.11	0.14	-0.08	0.09	0.15	-0.03

GD₅₀: the days required for 50% of total germination. GD₉₀: the days required for 90% of total germination. *: significant effect at $P < 0.05$. ^{NS}: no significant effect of latitude and longitude of the collection site.

Table 2. The effects (*F*-values) of species, planting method and city on three germination indices determined by general linear models.

Temperature	Index	Species	Planting method	City
28/15C	GD ₅₀	4.50*	0.17 ^{NS}	3.95*
	GD ₉₀	3.29*	0.16 ^{NS}	2.45*
	Percentage germination	22.78*	2.51 ^{NS}	3.94*
30/20C	GD ₅₀	2.18*	0.99 ^{NS}	2.06*
	GD ₉₀	2.25*	0.27 ^{NS}	1.56 ^{NS}
	Percentage germination	16.13*	14.01*	4.32*
35/25C	GD ₅₀	2.95*	0.13 ^{NS}	2.56*
	GD ₉₀	5.54*	2.65 ^{NS}	1.84*
	Percentage germination	17.06*	5.52*	1.84*

GD₅₀: the days required for 50% of total germination. GD₉₀: the days required for 90% of total germination. *: significant effect at $P < 0.05$. ^{NS}: no significant effect of the environmental factor.

Table 3. The accumulated temperature to 50% and 90% total germination of *Echinochloa* species at different temperature regimes.

Temperature	<i>Echinochloa crus-galli</i> var. <i>crus-galli</i> (L.) Beauv.		<i>Echinochloa crus-galli</i> var. <i>mitis</i> (Pursh) Peterm.		<i>Echinochloa glabrescens</i> Munro ex Hook. f.	
	TD ₅₀	TD ₉₀	TD ₅₀	TD ₉₀	TD ₅₀	TD ₉₀
28/15C	111.01±3.3 2a	159.21±7.3 7a	127.26±4. 16a	225.17±14. 05a	124.70±4. 01a	214.97±12. 95a
30/20C	116.08±1. 96a	153.02±4.4 7a	128.02±2. 40a	184.11±5.5 5b	130.28±2. 81a	205.24±9.2 2a
35/25C	74.25±1.5 4b	103.37±2.5 6b	76.41±1.0 9b	111.47±2.0 3c	80.16±1.3 2b	120.78±2.7 5b

TD: the accumulated temperature required for total germination. Different letters in the same column indicate significant differences among the three temperature regimes at $P < 0.05$.

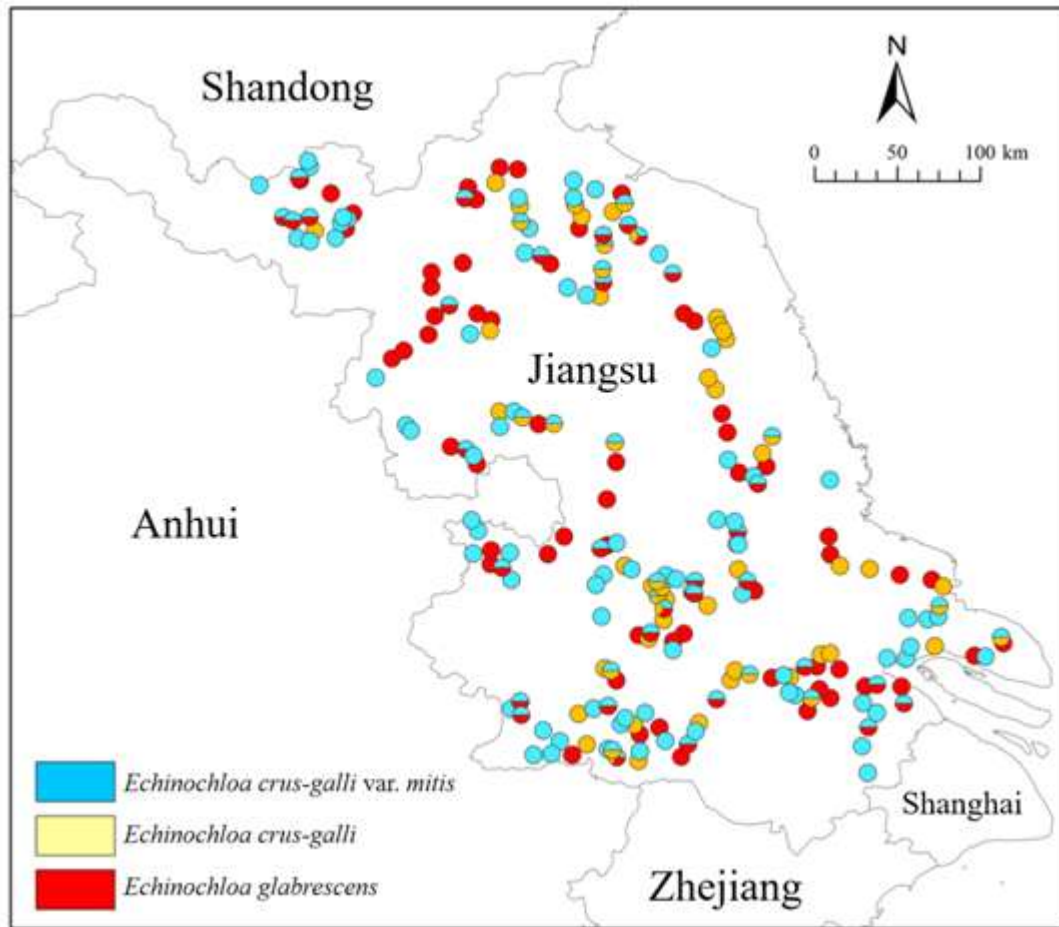


Figure 1. Collection sites of three *Echinochloa* species populations in rice fields.

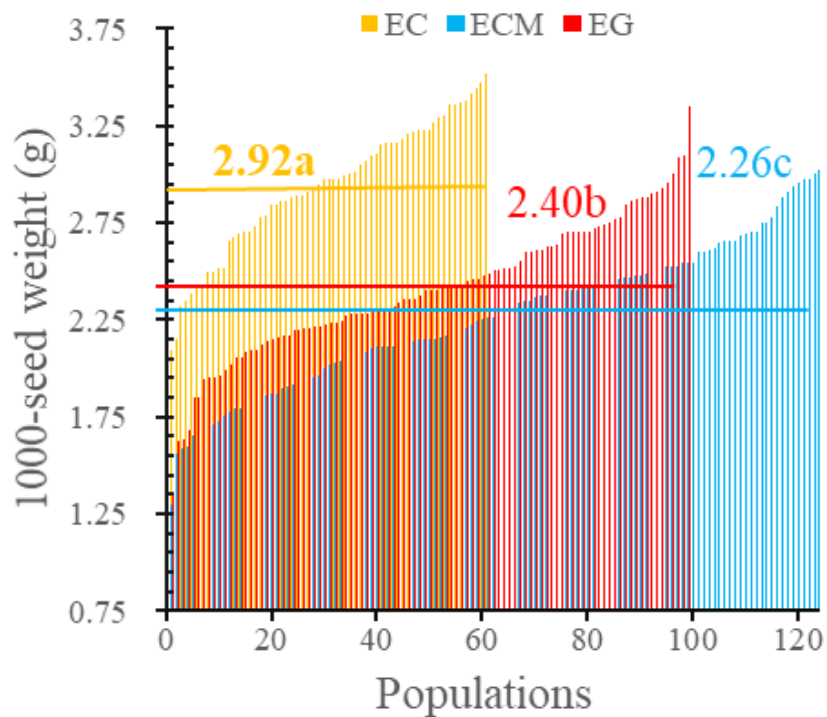


Figure 2. Thousand-seed weight of *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG). The horizontal line represented the average 1000-seed weight of three *Echinochloa* species. Different letters suggested significant differences among three *Echinochloa* species.

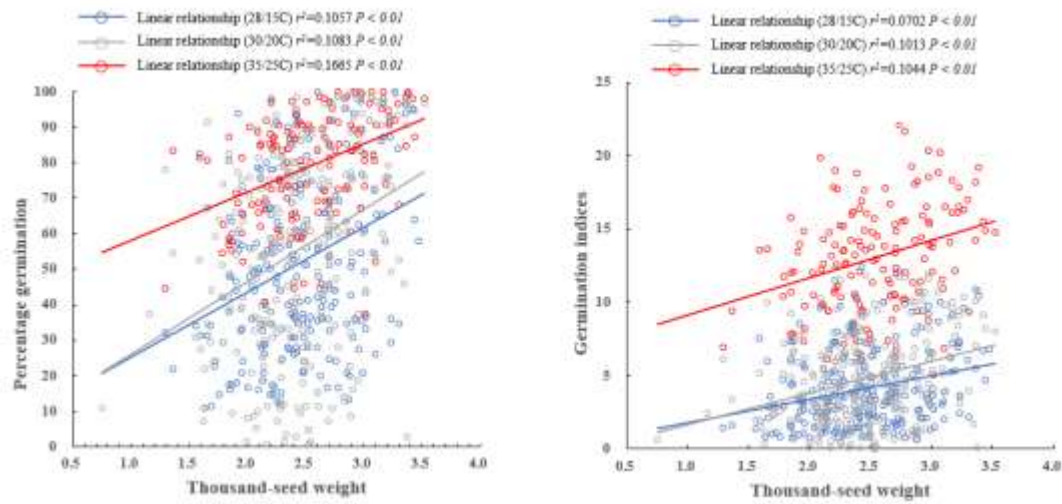


Figure 3. Correlations between 1000-seed weight, and percentage germination and germination index at different temperature regimes.

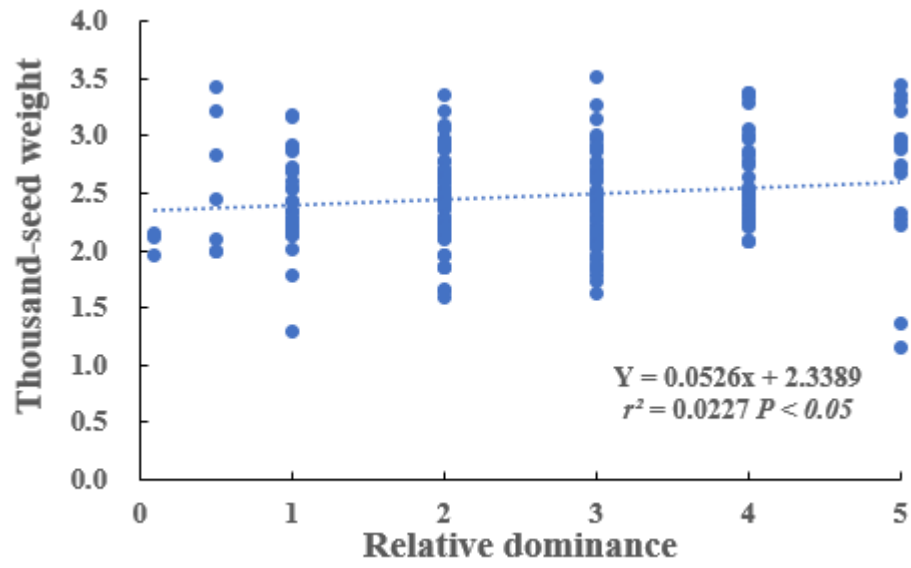


Figure 4. Correlations between 1000-seed weight and relative dominance.

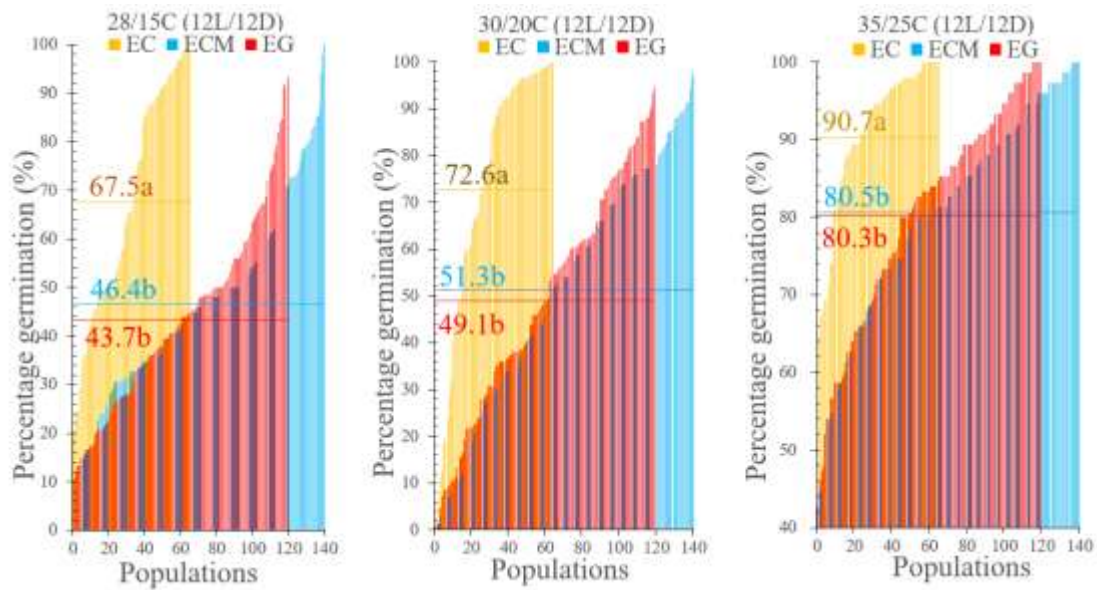


Figure 5. Percentage germination of *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG) at 28/15C, 30/20C and 35/25C (12L/12D). The horizontal lines represented the average percentage germinations of three *Echinochloa* species. Different letters suggested significant differences among three *Echinochloa* species in the same figure.

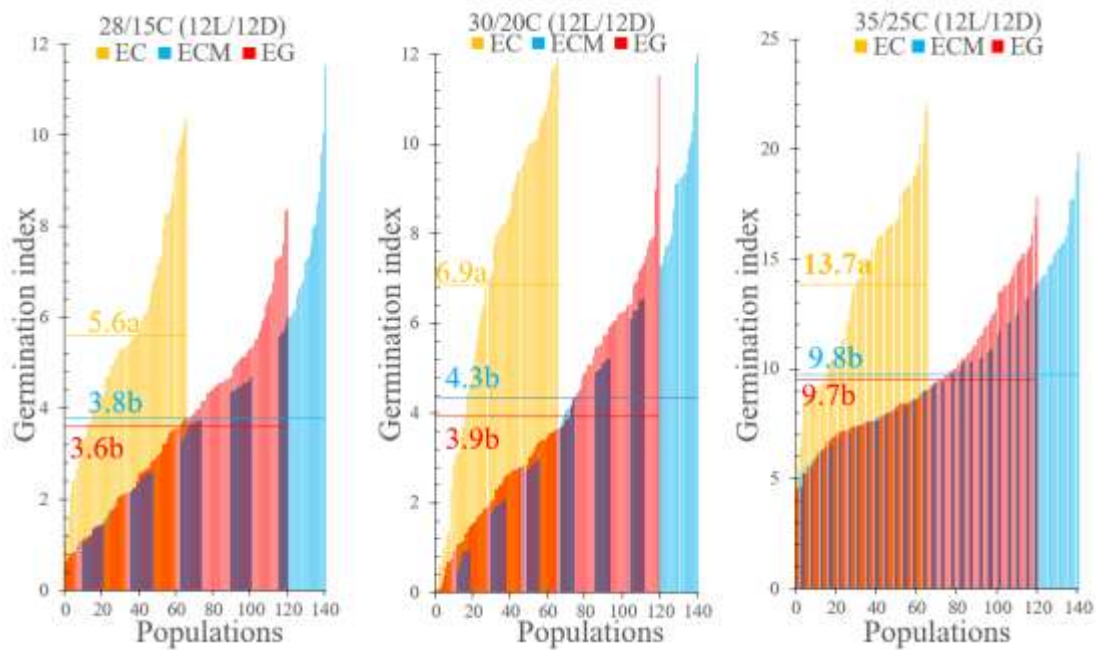


Figure 6. Germination index for *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG) at 28/15C, 30/20C and 35/25C (12L/12D). The horizontal lines represented the average germination indices of three *Echinochloa* species. Different letters suggested significant differences among three *Echinochloa* species in the same figure.

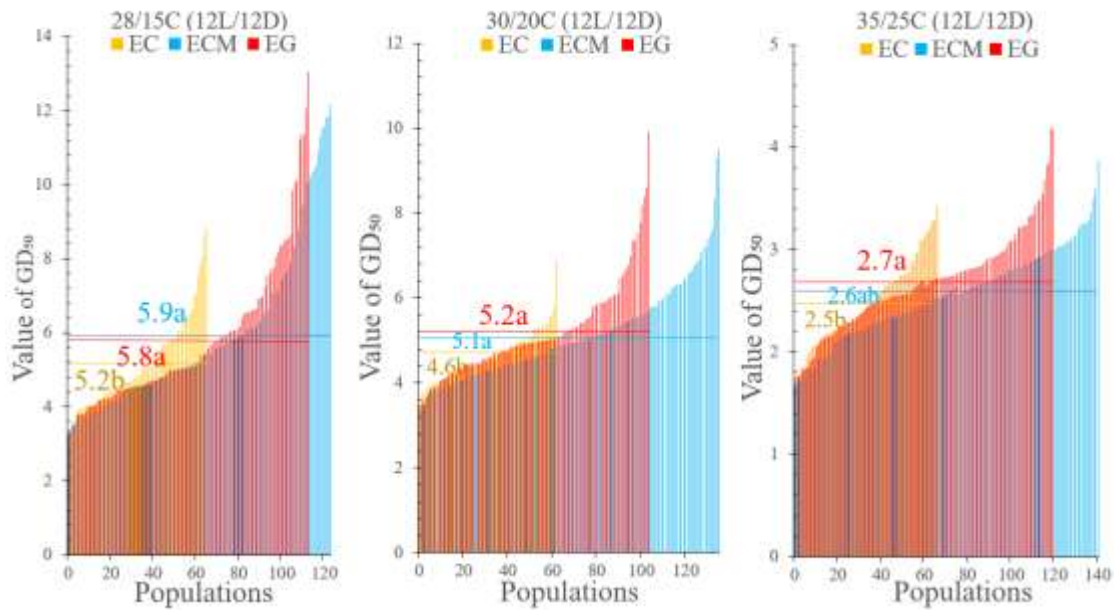


Figure 7. Number of days to 50% total germination rate (GD_{50}) of *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG) at 28/15C, 30/20C and 35/25C (12L/12D). The horizontal lines represented the average GD_{50} values of three *Echinochloa* species. Different letters suggested significant differences among three *Echinochloa* species in the same figure.

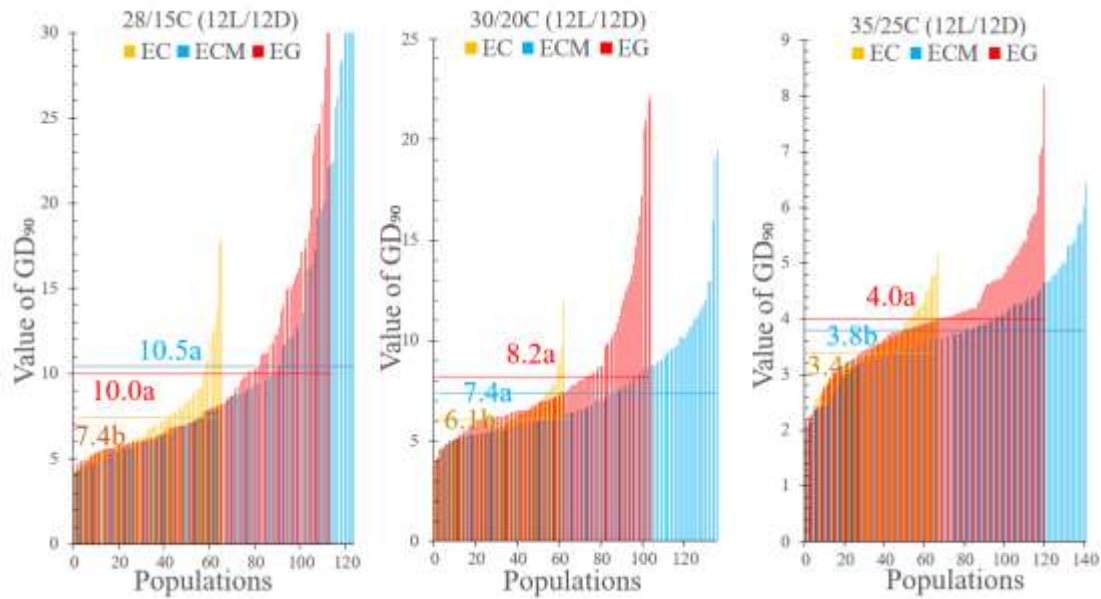


Figure 8. Number of days to 90% total germination rate (GD_{90}) for *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv. (EC), *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. (ECM), and *Echinochloa glabrescens* Munro ex Hook. f. (EG) at 28/15C, 30/20C and 35/25C (12L/12D). The horizontal lines represented the average GD_{90} values of three *Echinochloa* species. Different letters suggested significant differences among three *Echinochloa* species in the same figure.