

Local Adaptation Associated Phenotypic Divergence in Sympatric Weedy Rice Populations

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Abstract

Temperatures and photoperiods can profoundly affect plant growth and development, and play vital roles in the local adaptation of plant species. Weedy rice (*Oryza sativa* f. *spontanea*) is a conspecific weed of cultivated rice, occurring in the same rice fields (sympatry) of early and late rice-cultivation seasons. In this study, the contrasting patterns of air-temperature and daylength variation were identified between the early and late seasons in Leizhou, Guangdong Province of China, where lower air-temperatures and longer daylengths in the early seasons were found with more stressful conditions for weedy rice growth and development. Significant differences in plant heights, the number of tillers per plant, flowering time, and reproductive traits were detected between the corresponding early- and late-season weedy rice populations of the same rice fields in the early-season common garden experiment. The populations collected from the early season showed higher plant heights, more tillers, and earlier flowering time than those from the late season. However, such differences were not detected in the late-season common garden experiment. In addition, evident local adaptation represented by the traits such as plant heights, flowering time, and reproductive traits were only detected in the early-season weedy rice populations. These results suggest that the early-season weedy rice populations may have evolved adaptive to the early seasons. Altogether, these findings provided evidence for phenotypical divergence between sympatric weedy rice populations, most likely resulted from the local adaptation to the early seasons in a stressful environment, supporting the theory of sympatric divergence in ecological speciation.

1 INTRODUCTION

Temperature and photoperiod have profound effects on plant growth and development (Franklin, 2009; Ding et al., 2020), and contribute to seed germination, flowering time, and reproductive traits of plants (Penfield, 2008; Jackson, 2009). Generally, each species has a specific temperature range represented by minimum, maximum and optimum (Hatfield & Prueger, 2015). For example, the optimal temperature for rice cultivation is between 25 and 35 °C (Hussain et al., 2019), and temperature beyond optimum may have negative effects on rice growth and development. However, responses to temperatures differ among plant species throughout their life cycle and are primarily the phenological responses (Hatfield & Prueger, 2015). For instance, a prolonged period of cold, called the vernalization response, can promote plant to flower in *Arabidopsis thaliana* (Kim and Sung, 2014), and a high temperature can shorten the period of grain filling in rice (Kim et al., 2011). In addition, the defined range of maximum and minimum temperatures form the boundaries of observable growth, vegetative development increases as temperatures rise to the species optimum level (Hatfield & Prueger, 2015).

However, temperature is often an unreliable marker of seasonality, most plant species native to areas outside the tropics have evolved a second line of safeguarding them against misleading temperature conditions— photoperiod, which is defined as the developmental responses of plants to the change of daylength over the years (Korner, 2006). In other words, the response to photoperiod has evolved in plants because daylength is a reliable indicator of the time of year (Andres & Coupland, 2012; Kubota et al., 2014), enabling developmental events to be scheduled to coincide with particular environmental conditions (Jackson, 2009).

Photoperiod plays major roles in synchronization of flowering in plant populations and thus ensuring reproductive success, and preventing phenology from following temperature as a risky environmental signal for development (Korner, 2006). Noticeably, interaction between a temperature and photoperiod also plays important roles during the life of plant species (Franklin, 2009; Song et al., 2012). For example, the floral transition of plants always depends on the accurate measurement of changes in photoperiod and temperature, and thus photoperiod and temperature are two pivotal regulatory factors of plant flowering (Song et al., 2012).

Individual plants are sessile, and therefore have to develop the means to detect and respond to environmental changes as they occur. As a consequence, plants continuously monitor their surroundings and adjust their growth to daily and seasonal cues (Capovilla et al., 2015). Weed species, such as agricultural weeds, have been rapidly evolved to adapt to changes during farming practices (Vigueira et al., 2013; Mahaut et al., 2020). In the light of intrinsic capacity of rapid adaptation, weedy species that occur over a relatively short period of time become an appealing system to study evolutionary processes. Generally, the agricultural weed syndrome includes rapid growth, high nutrient-use efficiency, seed dormancy, efficient seed dispersal, crop mimicry, and herbicide resistance (Vigueira et al., 2013). Therefore, agricultural weeds must possess traits that permit them to survive and thrive in the recently created environment. The evolution of herbicide resistance is probably the most emblematic and well-documented case of rapid evolution in weeds (Baucom, 2019). In addition, climate change, such as temperature and moisture fluctuations, has direct effects on the survival, distribution and competition of weedy species in cropping system (Peters et al., 2014). For example, Xia et al. (2011) found seeds of weedy rice can germinate at a lower temperature than its co-occurred cultivated rice, and the germination ratio showed a latitudinal gradient pattern between weedy rice populations from north China down to the Jiangsu Province.

Weedy rice (*Oryza sativa* f. *spontanea*, WR, Figure S1a) is a noxious agricultural weed infesting worldwide rice fields (Delouche et al., 2007). It is a conspecific weed that belongs to the same biological species of cultivated rice (*O. sativa*), but with strong seed shattering and prolonged seed dormancy. In the typical tropic rice cultivation regions, such as Guangdong, Guangxi, and Hainan Provinces, rice is cultivated for two seasons, namely the early and late rice-cultivation seasons. In both the two seasons, weedy rice was found in the same rice fields (sympatry). Generally, phenological conditions, such as temperature and photoperiod, between the two seasons are considerably different. Differential genetic diversity and considerable genetic differentiation between the two-season WR populations were reported by Kong et al. (2021). Therefore, we believe that such considerable genetic differentiation is accompanied with certain phenotypic divergence between the two-season WR populations in the same rice fields, most likely because of the adaptive evolution in the weedy rice populations.

Common garden experiment is regarded as an efficient tool to study adaptation and the genetic bases of the adaptive traits by growing individuals from different populations in a common environment (de Villemereuil et al., 2016, 2020), and it has been used extensively with plant species (Albaugh et al., 2018; Groot et al., 2018). In this study, we conducted *in situ* common garden experiments to estimate the phenotypic divergence between the early- and late-season WR populations in early and late rice-cultivation seasons, respectively. The major questions addressed are as follows: (1) What are the patterns of temperature and photoperiod variation in different rice-cultivation seasons in Leizhou? (2) Do the vegetative and reproductive growth traits diverge between the sympatric two-season weedy rice populations? (3) Has the local adaptation developed in weedy rice populations? Answers of the above questions can support the genetic divergence between the sympatric two-season WR populations from another perspective, and provide solid evidence of ambient surroundings associated rapid adaptive evolution in plant species.

2 MATERIALS AND METHODS

2.1 Plant materials

A total of six WR populations occurring in the early (code as WRE) and late (WRL) rice-cultivation seasons were collected from three rice fields in Leizhou, Guangdong Province of China (Table S1). The geographic

distance between the sampled rice fields were >30 km. WR populations collected from the same field were treated as a population pair (e.g., CDE/L, DCE/L or HJE/L pair). About 60 randomly selected samples were collected from each WR population of either the early- or late-season rice field at the spatial distance intervals of >10 m to avoid the sampling of similar genotypes. Matured panicles from a WR plant were collected as an independent sample. The average duration of the early rice-cultivation seasons (ES) was from March 1st to June 25th, whereas that of late rice-cultivation seasons (LS) was from July 20th to November 5th.

2.2 Historical climate data collection and analysis

The 10-year air-temperature data (2011~2020) in Leizhou was collected from the Tianqi Database (<http://lishi.tianqi.com/leizhou/index.html>, in Chinese). The air-temperature data included the daily minimum and maximum temperatures (Table S2). The average of the minimum and maximum temperatures was defined as the daily average air-temperature. Also, the 10-year daylength data (2006~2015) was collected from the BMCX website (https://richurimo.51240.com/leigaozhen_richurimo/, in Chinese). The daylength data included the daily time of sunrise and sunset (Table S3). The differences between the time of sunrise and sunset were defined as the daylengths.

In addition, the WR growth period was artificially divided into four stages, corresponding to different rice growth phases: seedling (S), tillering (T), flowering (F), and ripening (R). The average values of each year in the ten years were used to estimate differences in air-temperatures and daylengths between the early and late rice-cultivation seasons at different stages.

2.3 Design of common garden experiments

Common garden experiments were conducted in a rice field in Leizhou, Guangdong Province of China (Figure S1) both in the early and late rice-cultivation seasons, respectively. In the experiments, 60 WR samples were included from each population and 10 well-developed seeds from each sample were used to determine the ratios of seed germination. Seeds were germinated in black boxes placed in the experimental rice field (Figure S1b). Thirty-day old seedlings (<3 seedlings from the same sample) were transplanted to the experimental rice field. In the experiment, each plot containing 36 seedlings was arranged with a 6 x 6 grid (Figure S1c) and 30 cm between the hills and rows. Six plots (replicates) were included for different treatments of each WR population. All plots were arranged in the experimental field with a complete randomized design with 60 cm spacing between the plots. About 80 days after seed germination, panicles were enclosed in mesh nylon bags to avoid accidental shattering of seeds (Figure S1d).

2.4 Measurements of fitness related traits

Vegetative growth traits, including plant height, number of tillers per plant, and leaf length/width were measured in the common garden experiments. Plant height was defined as the distance from the ground to the tip of the longest leaf, and number of tillers per plant referred to the total of branches emerged from the main stem culm of a plant. Leaf length and width of the main stem culm were also involved, including the top-first leaf (the first leaf on the top of main stem culm) and top-second leaf (the second leaf on the top of main stem culm). The leaf length was defined as the distance from leaf tip to the base, and the leaf width was measured at the middle of the leaf.

To detect the differences in vegetative growth between the early- and late-season WR populations at different stages, we measured these traits in every 20 days after germination (DAG), including the 20, 40, 60 and 80 DAG. At the 20 DAG, considering the elder seedlings are too weak to avoid the harms during the measurement, the measurements of leaf length and width were abandoned, therefore only plant height and number of tillers per plant were measured. After transplanting, the measurements of plant height, number of tillers, and leaf length/width for each plant individual were conducted in all planting plots at 40, 60 and 80 DAG. Experimental data obtained from the measurements and recording was mainly used to estimate differences in growth and development between the early- and late-season WR populations in the same rice-cultivation season, and to compare the performance of the same population in different rice-cultivation

seasons.

The flowering time pattern of each plant from beginning to end was recorded, and a dynamic pattern of flowering time was constructed in each population. The beginning of flowering was defined as the date of the first flower emerging, and the end of flowering was indicated by the rise of heavy panicles. The proportion of flowering plants per day was marked and recorded in each population, which was used to construct the flowering time patterns of weedy rice populations. To estimate more comprehensive differences in flowering time between the early- and late-season WR populations, the average flowering time in different phases, including 1%, 30%, 50% and 80% plants flowered, were also calculated to do further comparisons between the two-season WR populations.

The reproductive traits were closely associated with the flowering time in rice. Therefore, the number of seeds per plant, seed setting ratio and 100-seeds weight were measured for each plot. After harvesting and threshing, full seeds were separated by a seed air cleaning instrument (CFY-2, Top Cloud-agri Technology Company, Zhejiang, China), and seeds counting using electronic seed counter (PME, Shanco Instruments, Shanghai, China) and 100-seeds weighting using analytical balance.

2.5 Estimate magnitude of local adaptation

Fitness related traits, such as plant height, number of tillers, and reproductive traits, were used to quantify local adaptation (LA) of native WR populations in different rice-cultivation environment. In addition, the flowering time was also involved in this analysis based on the data of days to flowering, and early flowering was regarded as an adaptive trait. The quantitative measure of local adaptation was the relative fitness of the native population at a field site in a given year minus the relative fitness of a nonnative population at that site, following the equation from (Hereford, 2009):

$$LA = \frac{W_{\text{native}} - W_{\text{non-native}}}{\text{avg}(W)}$$

Where W represents the mean fitness of native and nonnative populations, and $\text{avg}(W)$ represents the mean fitness of all populations. In this study, the early-season WR populations were native in early rice-cultivation season, and the late-season WR populations were native in late rice-cultivation season. Generally, positive LA values indicate local adaptation in the native populations (Hereford, 2009). However, the negative LA values of flowering time also indicate local adaptation for early flowering in WR.

2.6 Statistical analyses of data

To estimate the effects of population (the corresponding early- and late-season WR populations), population pair (CD, DC and HJ from different collecting sites) and transplant season (the EARLY and LATE rice-cultivation season) on plant growth and development during the common garden experiments, two-way ANOVA (analysis of variance) was used to determine the factors affecting the plant growth and development significantly. Four groups of ANOVAs conducted: (1) estimation of the effects of population and population pair in the EARLY rice-cultivation season (Table 1), (2) estimation of the effects of population and population pair in the LATE rice-cultivation season (Table S4), (3) estimation of the effect of transplant season and population pair in the early-season weedy rice populations (Table S5), and (4) estimation of the effect of transplant season and population pair in the late-season weedy rice populations (Table S6). The first two groups of ANOVAs aimed to estimate the differences in growth and development traits, which were measured in common garden experiments, between the early- and late-season WR populations in the early and late rice-cultivation environments, respectively. The later two groups of ANOVAs aimed to estimate the differences in growth and development of the same population in different rice-cultivation seasons.

In addition, differences included in this study were tested based on the student t test (Blalock, 1972), and the method of two-tails and equal variance test of two samples were adopted. Two-way ANOVAs and t test were both performed using the software IBM SPSS Statistics ver. 22.0 for Windows (SPSS Inc., IBM Company Chicago, IL, USA, 2010).

3 RESULTS

3.1 Differences in air-temperature and daylength between the two rice-cultivation seasons

Different patterns of air-temperature variation were found between the early (ES) and late (LS) rice-cultivation seasons (Figure 1a, grey lines), based on the analysis of daily air-temperature data (10 years) of Leizhou in Guangdong Province, China. The average air-temperature raised gradually in ES, and decreased in LS. In addition, the average air-temperature was significant 1.7 lower in ES (25.9 *vs.* 27.6, $p < 0.001$) than that in LS (Figure 1b). Noticeably, much lower air-temperature was detected in the ES during the initial growth stages (Figure 1b). For example, during the seedling stage (S), the average air-temperature of ES was significant 8.2 lower (20.9 *vs.* 29.1, $p < 0.001$) than that of LS, and it was supported by the measured air- and soil-temperature in the common garden experiments (Figure S2). Similarly, average air-temperature was 4.6 lower (24.2 *vs.* 28.8, $p < 0.001$) in ES during the tillering stage (T). These results indicated the significant lower air-temperature in ES than that in LS, particularly during the initial growth stages.

Results also showed different patterns of daylength between ES and LS (Figure 1a, black lines). The average daylength increased gradually in ES, and decreased in LS. The average daylength was significant 0.45 h longer (12.76 *vs.* 12.31 h, $p < 0.001$) in ES than that in LS (Figure 1c). In addition, significant differences in daylength were detected at different rice growth stages, particularly during the flowering (F) and ripening stage (R) (Figure 1c). For example, the average daylength was 0.98 h longer (13.11 *vs.* 12.13 h, $p < 0.001$) in ES during the flowering stage, and was 1.81 h longer (13.37 *vs.* 11.56 h, $p < 0.001$) in ES during the ripening stage. These results suggested the significantly longer daylengths in ES than that in LS, particularly during the reproductive growth stages.

3.2 Effects of populations and population pairs on early-season WR growth and development based on two-way ANOVA

Analytical results showed that population (WRE or WRL) had significant ($p < 0.05$) effects on vegetative growth traits, including plant height, number of tillers per plant and leaf length/width, and reproductive growth traits, including flowering time and reproductive traits in the early rice-cultivation season (Table 1). However, no significant effects of population were detected in the late rice-cultivation season (Table S4). These results indicated possible significant differences in these vegetative and reproductive growth traits between the early- and late-season WR populations in the early rice-cultivation season. In addition, population pair (CDE/L, HJE/L and DCE/L) also showed significant effects on some of traits at different stages in both the early and late rice-cultivation season (Table 1, Table S4). Noticeably, a pronounced effect of population pair in the early season was detected, for example, the extremely significant effects ($p < 0.001$) on flowering time and reproductive traits (Table 1). These results showed some variations of the phenotypic differences estimated between the early- and late-season WR populations among different population pairs, and suggested that the micro-environments in different rice fields also played some roles in weedy rice growth and development.

In addition, transplant season (ES and LS) also had profound effects on almost all of growth and development traits in both the early- and late-season weedy rice populations (Table S5; Table S6). This result suggested that differences in ecological elements, such as temperature and daylength, between ES and LS may directly contribute to the different growth and development status of weedy rice, although in the same rice field.

3.3 Growth advantages in vegetative and reproductive traits of early-season WR

The significant differences ($p < 0.001$) in plant height were detected between the early- and late-season WR populations in the early-season common garden experiment (Figure 2), and the early-season WR populations were obviously taller than the late-season populations at different growth stages in all population pairs. In addition, significant differences were also detected in number of tillers per plant between the two-season WR populations at the early growth stages in the early season (Figure 3, $p < 0.001$). However, no such significant differences in plant height and number of tillers per plant were detected in the late-season common garden experiment (Figure 2; Figure 3). Furthermore, differences in the leaf length and width were also detected

(Figure S4; Figure S5). These results suggested significant differences in vegetative growth between the two-season WR populations in the early rice-cultivation environment, and the early-season WR populations showed advantages in vegetative growth.

Apparent differences in flowering time were observed between the WR population pairs (CDE/L and DCE/L) in the early-season common garden experiment (Figure 4). The early-season populations exhibited early flowering time compared to their corresponding late-season populations. In addition, results of flowering time at the stages of different proportions of flowered plants also supported the significant early flowering of the early-season populations in CDE/L and DCE/L population pairs (Figure 5). However, only minor differences in flowering time in CDE/L population pair were detected in the late-season common garden experiment (Figure S3), although no such differences in flowering was found in the early-season populations at different stages (Figure 5). These results indicated considerable divergences in flowering time between the two-season WR populations, and the early-season population was significant early flowering in native environment.

Also, results showed a relatively greater number of seeds per plant, higher seed setting ratios and 100-seeds weight in the early-season WR populations, in the early-season common garden experiment (Figure 6). For example, the early-season WR populations produced significant more seeds per plant than that of the late-season populations (Figure 6a), and had a significant higher ratio of seed sets than the corresponding late-season populations in CDE/L and DCE/L population pairs (Figure 6b). However, no such significant differences in reproductive traits were detected in the late-season common garden experiment (Figure 6a&b). These results suggested significant differences in reproductive traits between the two-season WR populations, and the early-season populations can produce more progenies than the late-season populations in the early rice-cultivation environment.

In addition, we found significant advantages in plant height (Figure 2), number of tillers per plant (Figure 3), and flowering time (Figure 5), reproductive traits (Figure 6) of all WR populations in the late rice-cultivation season (significance not shown), compared to that in the corresponding early season. These findings suggested that the late rice-cultivation environment may be more suitable for weedy rice growth, probably due to its high temperature and short daylength.

3.4 Evident local adaptation detected in the early-season WR populations

Evident local adaptation (LA) in the early-season WR populations was detected in the native early rice-cultivation environment (Table 2), based on the method recommended by Hereford (2009). For example, the average level of LA was 0.15 for plant height and 0.14 for reproductive traits. Generally, the positive values of LA indicate local adaptation for traits, but the early flowering was regarded as an adaptive trait in this study. Therefore, the negative value of -0.04 also indicated local adaptation for flowering time in the early-season WR populations. However, no such evident local adaptation was detected in the late-season WR populations in the native late rice-cultivation season (Table S7). These results indicated that ecological environment such as temperature and daylength played an important role in local adaptation of weedy rice (Figure 7).

In addition, the LA for plant height was stronger in population CDE (e.g., 0.32) and DCE (e.g., 0.21) than that in HJE (e.g., 0.14), as well as flowering time (Table 2). This result suggested more pronounced local adaptation of the early-season WR populations in CDE/L and DCE/L population pairs, likely associated with population histories of WR infesting cultivated rice field at different periods of times. Furthermore, the evident local adaptation for the number of seeds per plant and seed setting ratio were detected in all early-season WR populations, and it indicated that the local adaptation in the early rice-cultivation season may be conducive to the reproductive traits of the early-season WR populations.

4 DISCUSSIONS

4.1 The early rice-cultivation season is a stressful environment for weedy rice

Analytical results of 10-year climate data showed different variation patterns of air-temperature and

daylength between the early and late rice-cultivation seasons in Leizhou, and the 10-year average air-temperature of the early season was significant 1.7 lower than that in the late season, particularly during the initial rice growth stages (4.6~8.2). In addition, the 10-year average daylength of the early season was significant 0.45 hours longer than that in the late season, and it was up to 0.98~1.81 hours during the reproductive stages. Generally, low temperature and long daylength are environmental restrictions on rice performance, because rice is a short day and cold-sensitive plant (do Amaral et al., 2016; Wei et al., 2020). As for a conspecific weed that belongs to the same biological species of cultivated rice, weedy rice has the same responses to temperature and daylength. Therefore, these findings indicate that the low temperature and long daylength may be the primary stressful elements affecting weedy rice growth and development in the early-season environment.

The optimal temperature for rice cultivation is between 25 and 35 °C (Hussain et al., 2019), and temperature beyond optimum is harmful to rice and negatively affects growth, development and ultimately reduces the grain yield. In this study, lower average air-temperature was found during the seedling stage (20.9 °C) in the early season, as well as during the tillering stage (24.2 °C), than the optimum. By contrast, the average air-temperature was 29.1 and 28.8 °C, which fit the optimum, during the corresponding stages in the late season. Therefore, the early-season weedy rice had to experience the low temperature stress at the germination and seedling stages in the early season, and the low temperature may be a strong selective pressure to affect the growth and development of the early-season weedy rice in early stages. Altogether, above findings suggest that the early rice-cultivation season is a stressful environment with lower ambient temperatures compared to the corresponding late season, especially during the initial growth stages.

Generally, rice growth at the early stage is important for stable seedling establishment and subsequent vigorous vegetative growth (Sharifi, 2010), because seed germination, seedling emergence, stem elongation and individual survival are typically temperature dependent (Krishnan et al., 2011). During this period, the occurrence of low temperature stress inhibits seed germination, seedling establishment, and causes the retardation of rice growth. For example, apparent reduction in final seed germination ratio and the length of coleoptile and radical were observed when rice seeds were germinated at a temperature below optimum (Sharifi, 2010), and evidently reduced seedling growth and vigor of rice were also found under a low temperature condition (Rahul et al., 2017). In this study, significant reduction in plant height and number of tillers was found in almost all weedy rice populations during the early stages in the early-season common garden, compared to the same stages in late-season common garden. Therefore, these findings indicate that low temperature stress has direct negative effects on weedy rice growth, most likely caused by retardation in germination and seedling establishment during the initial stages. Low temperature stress not only lengthens the vegetative growth of rice, but also causes lagging in reproductive growth through delaying flowering time (Song et al., 2012). Our common garden experiments provided solid evidence of significant reduction in the number of seeds per plant, seed setting ratio and 100-seeds weight in weedy rice populations during the early-season common garden, compared to the high-temperature late season. Therefore, the early rice-cultivation season is a relative stressful environment for weedy rice growth and development because of its low ambient temperatures.

In addition, the daylength was significant longer in the early season than that in the corresponding late season, especially during the reproductive growth stages (~2 hours), based on the 10-year daylength data analyses in this study. Noticeably, the evident lagging in flowering time was found in almost all weedy rice populations in the early-season common garden, and it was about 10 days later in flowering time than that in the late-season common garden. Therefore, this finding supports the photoperiodical flowering response of rice in a long day environment, because rice is known as short day plant and long daylength environment usually delays the flowering time of rice (Wei et al., 2020). Therefore, long daylength is another constraint environmental factor affecting weedy rice growth and development in the stressful early rice-cultivation season. Previous studies suggest frequently interactions between temperature and photoperiod in plant flowering time regulation, and low temperature and long day environments are crucial causes of late flowering in rice (Franklin, 2009; Song et al., 2012). Altogether, we can conclude that the early rice-cultivation season is a stressful environment for weedy rice growth and development, because of its low temperature and long daylength.

4.2 Phenotypic divergences between sympatric weedy rice populations associated with local adaptation

Substantial divergence in vegetative and reproductive growth traits, including plant height, number of tillers, flowering time and reproductive traits, was detected between the early- and late-season weedy rice populations during the early-season common garden. Furthermore, the early-season populations had evident advantages in growth and development, such as higher plant height, more tillers and earlier flowering, compared to the corresponding late-season populations. However, no such divergence was detected in the late-season common garden experiment. Therefore, these findings demonstrate considerable phenotypic divergence between the sympatric two-season weedy rice populations in the stressful early rice-cultivation season. Previous study had detected considerable genetic divergence between the early- and late-season weedy rice populations (Kong et al., 2021), and our findings provided solid evidence to support the genetic divergence from phenotypes. In addition, evident local adaptation (LA) for these divergent traits was only detected in the early-season weedy rice populations in the stressful early-season common garden, based on the method adopted from (Hereford, 2009). Altogether, these findings not only provide evidence of growth and development divergence between the sympatric two-season weedy rice populations, but also demonstrate the occurrence of evident local adaptation in the early-season populations, which may be caused by the stressful environment in the early season.

The common garden experiment is generally used to test for local adaptation signal in traits of interest, such as life history traits and physiology, because it enables to unravel the genetic basis of complex phenotypes across various populations without the confounding effects of the corresponding environment (de Villemereuil et al., 2016, 2020). For example, physiological evidence for local adaptation to both freezing and drought stress in closely related American live oaks species was obtained from the common garden experiments (Cavender-Bares and Ramírez-Valiente, 2017). Additionally, van Boheemen et al. (2019) found the rapid and repeated local adaptation to climate in an invasive plant by examination of trait divergence in a common garden experiment. These studies demonstrate that local adaptation generally occurs in plant populations, when the plants expanded a new ecological niche or habitat and experienced some environmental stresses, such as low temperature and drought. Therefore, the evident local adaptation detected in the early-season weedy rice populations suggested that the early-season weedy rice may expand from the late season, and evolve in a rapid adaptive evolution process in the early season because of the stressful environment.

Plants often show differences in morphology within and between populations because of different local environments, which cause different selective pressures to shape adaptive genetic variation in plant individuals, and lead to heritable differences in plant phenotype (Groot et al., 2018). Generally, the phenotype of an individual is determined by the interactions between the environment and its genotype, which includes local adaptation (Des Marais et al., 2013). In previous study, considerable genetic divergence between the early- and late-season weedy rice populations in the same rice fields (Kong et al. 2021) was detected. Therefore, the phenotypic divergence between the two-season weedy rice populations is closely associated with the genetic divergence, which may be mainly caused by the local adaptation in the early-season populations. In addition, local adaptation is also assumed to occur under limited gene flow (Jacob et al., 2017), and it means the barriers to gene flow between populations are conducive to the maintenance of adaptive traits or alleles within population. Interestingly, the limited gene flow between the two-season weedy rice populations was also proposed by Kong et al. (2021), which was explained to maintain genetic diversity within populations. The finding of limited gene flow between the early- and late-season weedy rice populations provided evidence of the existence of two cryptical populations in the same rice field, and suggested that the weedy rice individuals, which adapted to low temperature and long daylength conditions, can survive and only retain in the early rice-cultivation season. Therefore, through local adaptation in the stressful environment and limited gene flow between the two seasons, the genetic and phenotypical divergence both emerged in weedy rice populations occurring in the same rice fields, which is a typical event of sympatric divergence in plant species.

4.3 Shift in flowering time contributes to sympatric divergence in weedy rice

Differences in flowering time were observed between the early- and late-season weedy rice populations, and the early-season populations showed early flowering phenotype in the early rice-cultivation season. In addition, evident local adaptation for early flowering time was also detected in the early-season weedy rice populations. These results suggested a divergent performance of flowering time between the sympatric two-season weedy rice populations, most likely caused by the local adaptation in the stressful early rice-cultivation season.

Generally, shift in flowering time is usually accompanied with local adaptation in flowering plants (Waser & Campbell, 2004), and have the potential to act as strong prezygotic reproductive barriers in plants. In some species, such as the grasses *Agrostis tenuis* and *Anthoxanthum odoratum*, heavy metals tolerant and intolerant races differ in seasonal time of flowering, and so are partially isolated reproductively at a pre-pollination stage (McNeilly & Antonovics, 1968). In addition, flowering time divergence was also found in some compelling examples of sympatric divergence. For instance, the two palm species (*Howea*) in Lord Howe Island segregate according to the acidity of the soil, and obvious disjunctions in flowering time were found between the two palms (Savolainen et al., 2006). In addition, two sister species of mountain rose (*Metrosideros*) endemic to Lord Howe Island also exhibited apparent divergence in flowering time because of their divergent ecological niches (Osborne et al., 2020). These studies provide reliable evidence for shifts in flowering time caused by local adaptation between sympatric plant populations, and demonstrate its important role in sympatric divergence of flowering plants. Therefore, the findings of local adaptation associated disjunctions in flowering time indicate a possible rapid adaptive evolution in weedy rice populations occurring in the same rice fields, and probably generate genetic and phenotypic divergence between the sympatric two-season weedy rice populations.

In addition, for agricultural weeds, the early flowering phenotype would likely result in the evolution of weed populations that display a shorter life cycle, allowing plants to set and shed seed prior to crop harvest (Ashworth et al., 2015). Therefore, we propose two possible reasons for the early-season weedy rice evolved an early flowering phenotype which causing divergent flowering time between the two-season weedy rice populations. First, the genes response to the stressful environment in the early rice-cultivation season are closely linked to the genes regulate flowering in weedy rice, or pleiotropy (Nosil et al., 2009), most likely caused by genetic variations. Second, to ensure reproduction success in the new environment, weedy rice shortened its growth period to complete its life cycle as soon as possible, most likely caused by epigenetic modification (Cabej, 2019). In other words, flowering time is crucial in determining the adaptation of weedy rice in different ecological environments, and may contribute to the reproductive isolation between sympatric plant populations because of genetic or epigenetic variations in certain adaptive genes. Therefore, it is necessary to identify the adaptive genes associated with divergent performances, such as flowering time, and investigate their mechanisms underlie local adaptation in the early-season weedy rice populations in the future work.

5 Conclusions

Differences in temperature and daylength were found between early and late rice-cultivation seasons in Leizhou, with a lower temperature and longer daylength in the early seasons than those in the late seasons. These findings indicated that some stressful ecological elements, which can affect weedy rice growth and development, probably present in the early rice-cultivation seasons. In addition, significant differences in plant heights, the number of tillers per plant, flowering time, and reproductive traits were detected between the early- and late-season weedy rice populations occurring in the same rice fields in the early rice-cultivation season, and the early-season populations showed evident growth and development advantages than the corresponding late-season populations. Noticeably, such phenotypical divergence was associated with the local adaptation in the early-season weedy rice populations, suggesting that the adaptive evolution had taken place in weedy rice populations. Altogether, findings of this study provided solid evidence of phenotypic divergence between the sympatric early- and late-season weedy rice populations, likely associated with the local adaptation to their ambient temperature and photoperiod.

Data Accessibility Statement

No additional data was used in this study.

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Tables

Table 1. Two-way ANOVA for WR populations transplanted in the EARLY rice-cultivation season

Stage	Population	Population	Population	Population pair	Population pair	Population p
20 days after germination	df	F-value	<i>p</i> -value	df	F-value	<i>p</i> -value
Plant height	1	310.084	0.000	2	1.010	0.365
Number of tillers	1	0.105	0.746	2	0.833	0.435
40 days after germination						
Plant height	1	102.788	0.000	2	2.046	0.147
Number of tillers	1	72.346	0.000	2	1.661	0.207
Top-first leaf length	1	4.458	0.043	2	12.486	0.000
Top-first leaf width	1	14.070	0.001	2	0.465	0.633
Top-second leaf length	1	48.062	0.000	2	9.518	0.001
Top-second leaf width	1	5.127	0.031	2	0.759	0.477
60 days after germination						
Plant height	1	73.012	0.000	2	10.752	0.000
Number of tillers	1	8.282	0.007	2	19.784	0.000
Top-first leaf length	1	33.493	0.000	2	1.193	0.317
Top-first leaf width	1	8.889	0.006	2	3.472	0.044
Top-second leaf length	1	60.119	0.000	2	7.693	0.002
Top-second leaf width	1	5.444	0.027	2	2.111	0.139
80 days after germination						
Plant height	1	51.059	0.000	2	3.104	0.060
Number of tillers	1	3.729	0.063	2	30.440	0.000
Top-first leaf length	1	0.284	0.598	2	4.757	0.016
Top-first leaf width	1	0.495	0.487	2	2.857	0.073
Top-second leaf length	1	14.317	0.001	2	4.290	0.023
Top-second leaf width	1	0.542	0.467	2	21.145	0.000
Flowering time						
Days for 1% plants to flower	1	12.300	0.001	2	27.964	0.000
Days for 30% plants to flower	1	65.147	0.000	2	86.491	0.000
Days for 50% plants to flower	1	49.082	0.000	2	39.619	0.000
Days for 80% plants to flower	1	32.213	0.000	2	17.578	0.000

Stage	Population	Population	Population	Population pair	Population pair	Population pair
Days for 100% plants to flower	1	0.729	0.400	2	12.611	0.000
Fecundity						
Number of seeds per plant	1	19.841	0.000	2	30.944	0.000
Seed setting ratio	1	19.030	0.000	2	22.563	0.000
100-seeds weight	1	7.050	0.013	2	30.360	0.000

df, degree of freedom.

Table 2. Local adaptation (LA) estimation of the early-season WR populations (WRE) in the early rice-cultivation season (ES)

Trait	Index	Local adaptation (LA)	Local adaptation (LA)	Local adaptation (LA)	Local adaptation (LA)
		CDE ³	HJE	DCE	DC
Plant height	20 DAG ¹	0.32	0.14	0.21	0.00
	40 DAG	0.15	0.09	0.13	0.00
	60 DAG	0.16	0.05	0.16	0.00
	80 DAG	0.15	0.02	0.17	0.00
Tiller numbers	20 DAG	0.00	0.00	0.00	0.00
	40 DAG	0.27	0.18	0.18	0.00
	60 DAG	0.11	0.10	0.03	0.00
	80 DAG	0.07	0.04	-0.02	0.00
Flowering time	1% ²	-0.06	-0.02	-0.09	0.00
	30%	-0.09	-0.01	-0.05	0.00
	50%	-0.07	-0.02	-0.04	0.00
	80%	-0.04	-0.01	-0.03	0.00
Fecundity	Number of seeds per plant	0.23	0.32	0.25	0.00
	Seed setting ratio	0.19	0.06	0.15	0.00
	100-seed weight	-0.02	0.04	0.08	0.00

¹ DAG, days after germination.

² %, proportion of flowering plants.

³ CDE, HJE, and DCE, the early-season WR populations collected from Chi Dou (CD), He Jia (HJ) and Dong Cun (DC), respectively.

Figure Legends

Figure 1. The differences in the average air-temperature and daylength between the EARLY and LATE rice-cultivation season. (a) Patterns of 10-year average daily air-temperature and daylength variation in the EARLY (ES) and LATE (LS) rice-cultivation seasons, respectively. Grey lines represent the air-temperature, and black lines for daylength. (b) Air-temperature differences between the two rice-cultivation seasons at different rice growth stages. White bars represent the early rice-cultivation season, and grey bars for the late season. (c) Daylength differences between the two rice-cultivation seasons at different rice growth stages. White bars represent the early rice-cultivation season, and black bars for the late season. S, seedling; T, tillering; F, flowering; R, ripening. ***, $p < 0.001$. Error bar, standard deviation.

Figure 2. Plant height of weedy rice populations at different stages in the EARLY (-E) and LATE (-L) rice-cultivation seasons. Bar plots show the plant height in CDE/L (a), HJE/L (b) and DCE/L (c) population pairs at different stages, respectively. *, $p < 0.05$, ***, $p < 0.001$.

Figure 3. Number of tillers per plant of weedy rice populations at different stages in the EARLY (-E) and LATE (-L) rice-cultivation seasons. Bar plots show the number of tillers per plant in CDE/L (**a**), HJE/L (**b**) and DCE/L (**c**) population pairs at different stages, respectively. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Figure 4. The flowering time patterns of weedy rice populations in the EARLY rice-cultivation season. Flowering time patterns within population pair are showed in (a) CDE/L, (b) HJE/L and (c) DCE/L, respectively. Horizontal axes represent the date of flowering, indicated by days after germination (DAG), and vertical axes represent the proportion of flowering plants. Curves of weedy rice populations belong to the same pair are plotted together to show the disjunction in flowering time between the early- and late-season weedy rice populations. Grey curves indicate the early-season weedy rice populations, and black curves indicate the late-season weedy rice populations.

Figure 5. Flowering time of weedy rice populations in the EARLY (-E) and LATE (-L) rice-cultivation at different stages. Bar plots show the flowering time when the proportion of flowering plants is determined as 1%, 30%, 50% and 80% in (a) CDE/L, (b) HJE/L and (c) DCE/L population pairs, respectively. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Figure 6. Reproductive traits of the early- and late-season weedy rice populations planting in the EARLY (-E) and LATE (-L) rice-cultivation seasons. (**a**) Grain per plant; (**b**) Seed setting ratio; (**c**) 100-seeds weight. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. CD, Chi Dou; HJ, He Jia; DC, Dong Cun. E/L-E, the early-/late-season populations planting in the EARLY rice-cultivation season; E/L-L, the early-/late-season populations planting in the LATE rice-cultivation season.

Figure 7. The model of local adaptation in weedy rice associated with ambient temperature and daylength. WRE, the weedy rice collected from the EARLY rice-cultivation seasons. WRL, the weedy rice collected from the LATE rice-cultivation seasons.

Supporting Information

Table S1. Information of the three weedy rice population pairs collected from Leizhou, Guangdong Province in China

Table S2. Average temperature of the early and late rice-cultivation seasons at different growth stages from 2011 to 2020

Table S3. Average daylength of the early and late rice-cultivation seasons at different growth stages from 2006 to 2015

Table S4. Two-way ANOVA for weedy rice populations transplanted in the LATE rice-cultivation season

Table S5. Two-way ANOVA of the early-season weedy rice populations (WRE) transplanted in different rice-cultivation seasons

Table S6. Two-way ANOVA of the late-season weedy rice populations (WRL) transplanted in different rice-cultivation seasons

Table S7. Local adaptation (LA) estimation of the late-season weedy rice populations (WRL) in the late rice-cultivation season

Figure S1. Landscape of common garden experiment. (**a**) Panicle morphology of WR in the rice field. (**b**) Seedlings cultivation in the black boxes. (**c**) Planting plot (6 x 6) in the planting field. (**d**) Matured panicles were packed with mesh nylon bag in the common garden experiments.

Figure S2. Average air- and soil-temperature during the seedling stage measured in the common garden experiments. White bars represent the EARLY rice-cultivation season (ES), and grey bars for the LATE rice-cultivation season (LS). Numbers on the top of bars are the average values of temperature. Significance estimations were based on student t-test (Blalock, 1972). ***, $p < 0.001$. Error bar, standard deviation.

Figure S3. The flowering time patterns of weedy rice populations in the LATE rice-cultivation season. The flowering time is represented by the proportion of flowering individuals per day, here we show the flowering time patterns in population pair CDE/L (a), HJE/L (b) and DCE/L (c), respectively. Flowering time variation curves of weedy rice populations belong to the same pair are plotted together to show the differences between the early- and late-season weedy rice populations. Grey curves represent the early-season weedy rice populations, and black curves for the late-season weedy rice populations.

Figure S4. Top-first leaf length and width of weedy rice populations at the different stages in the EARLY(-E) and LATE (-L) rice-cultivation seasons. (a, b, c) Top-first leaf length in population pair CDE/L, HJE/L, and DCE/L. (d, e, f) Top-first leaf width in population pair CDE/L, HJE/L, and DCE/L. Significance of differences was estimated using student t test (Blalock, 1972). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Figure S5. Top-second leaf length and width of weedy rice populations at the different stages in the EARLY(-E) and LATE (-L) rice-cultivation seasons. (a, b, c) Top-second leaf length in population pair CDE/L, HJE/L, and DCE/L. (d, e, f) Top-second leaf width in population pair CDE/L, HJE/L, and DCE/L. Significance of differences was estimated using student t test (Blalock, 1972). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Conflict of interest

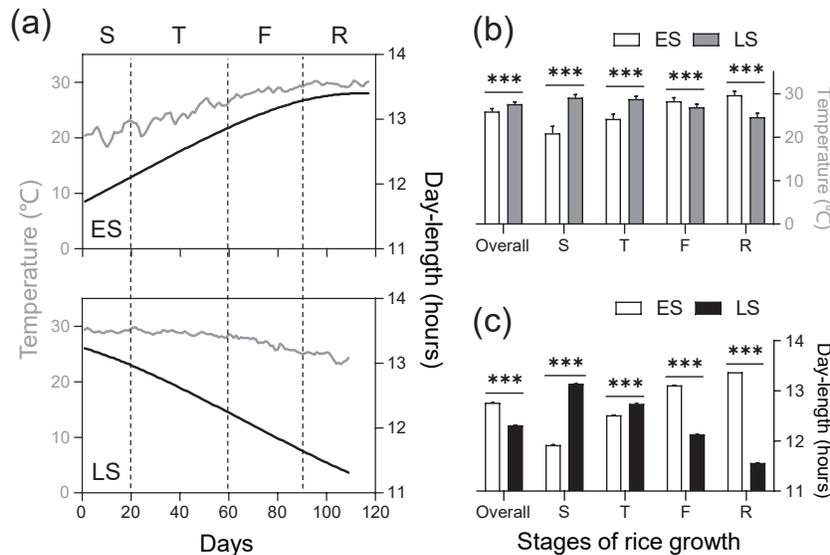
The authors declare no conflict of interests.

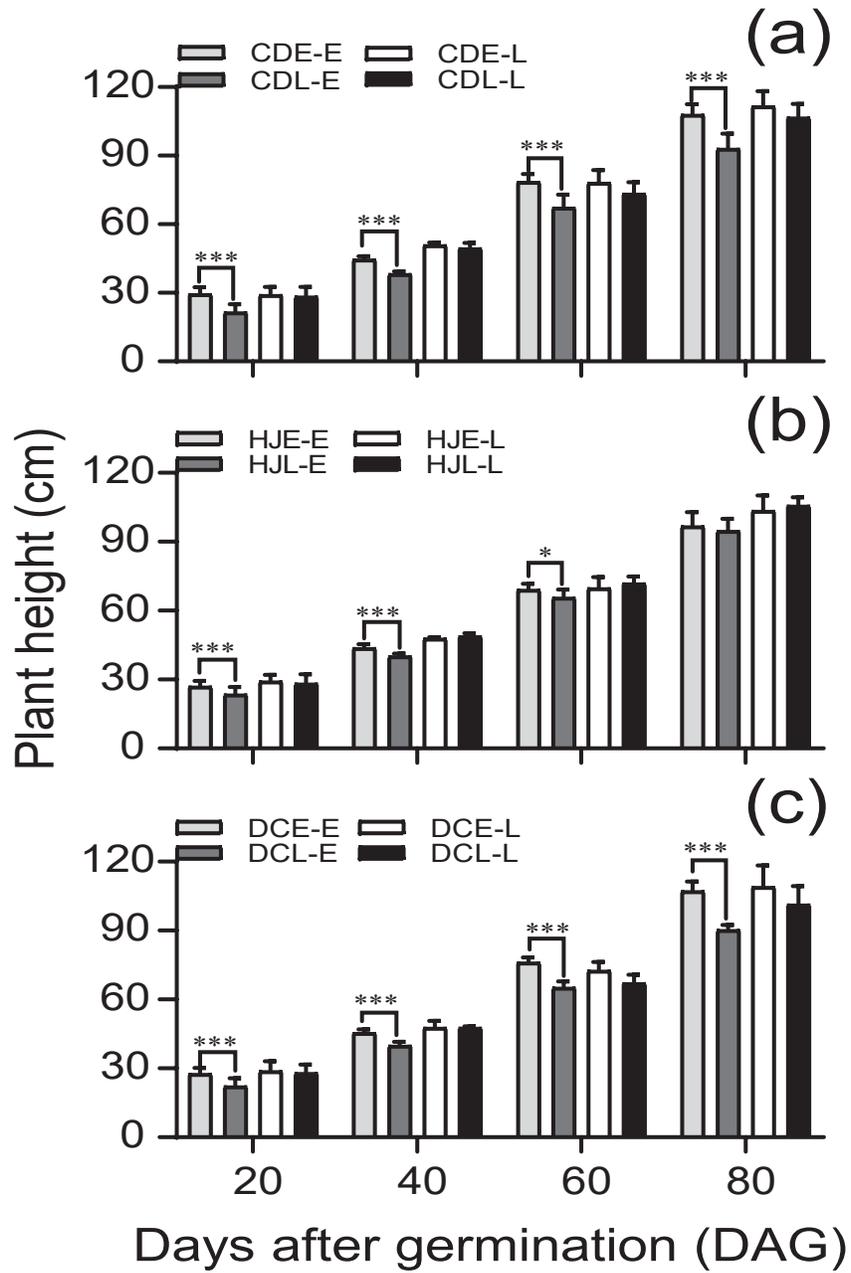
Author contribution

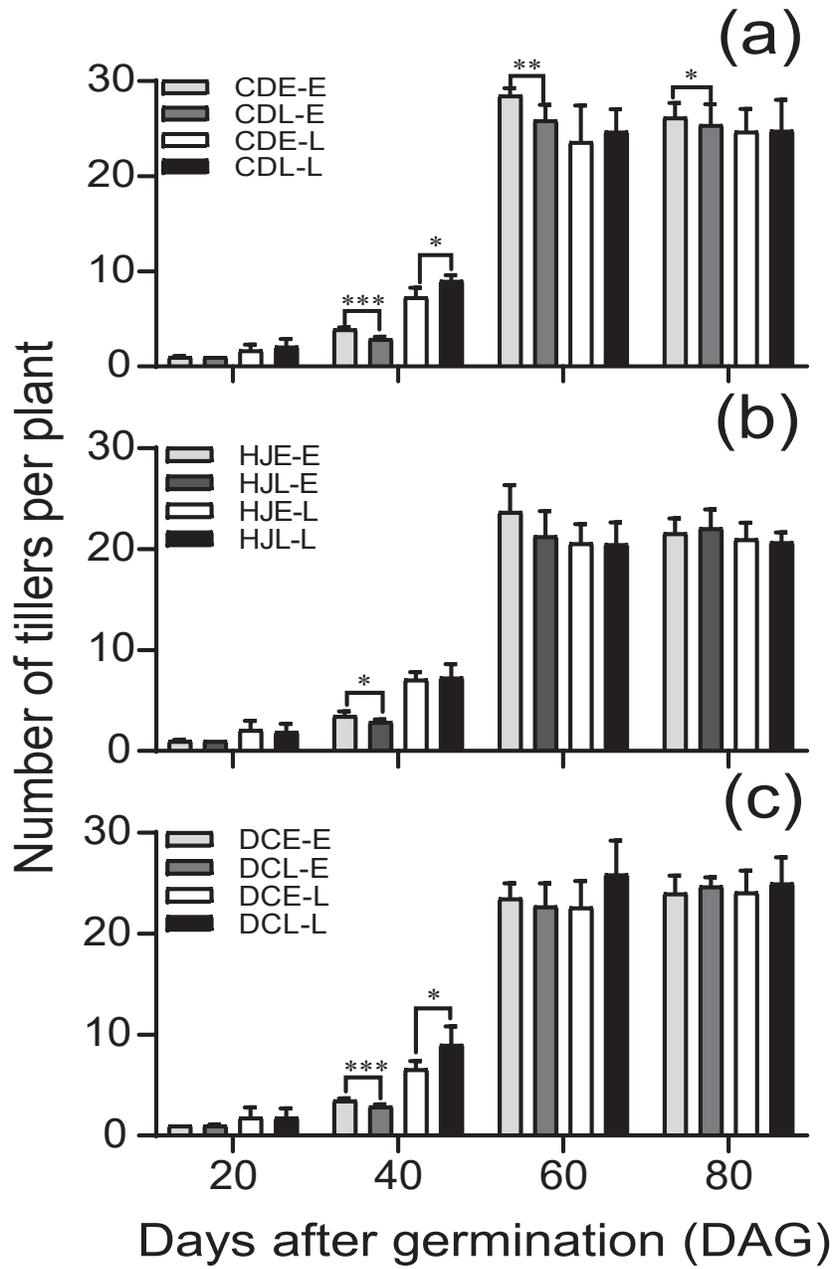
B.-R.L. conceived this study. Z.W. analyzed the data and wrote the manuscript. Z.W., X.-Q.J. and X.C. collected the plant materials. Z.W. and X.-Q.J. conducted the common garden experiments. All authors contributed in manuscript revision.

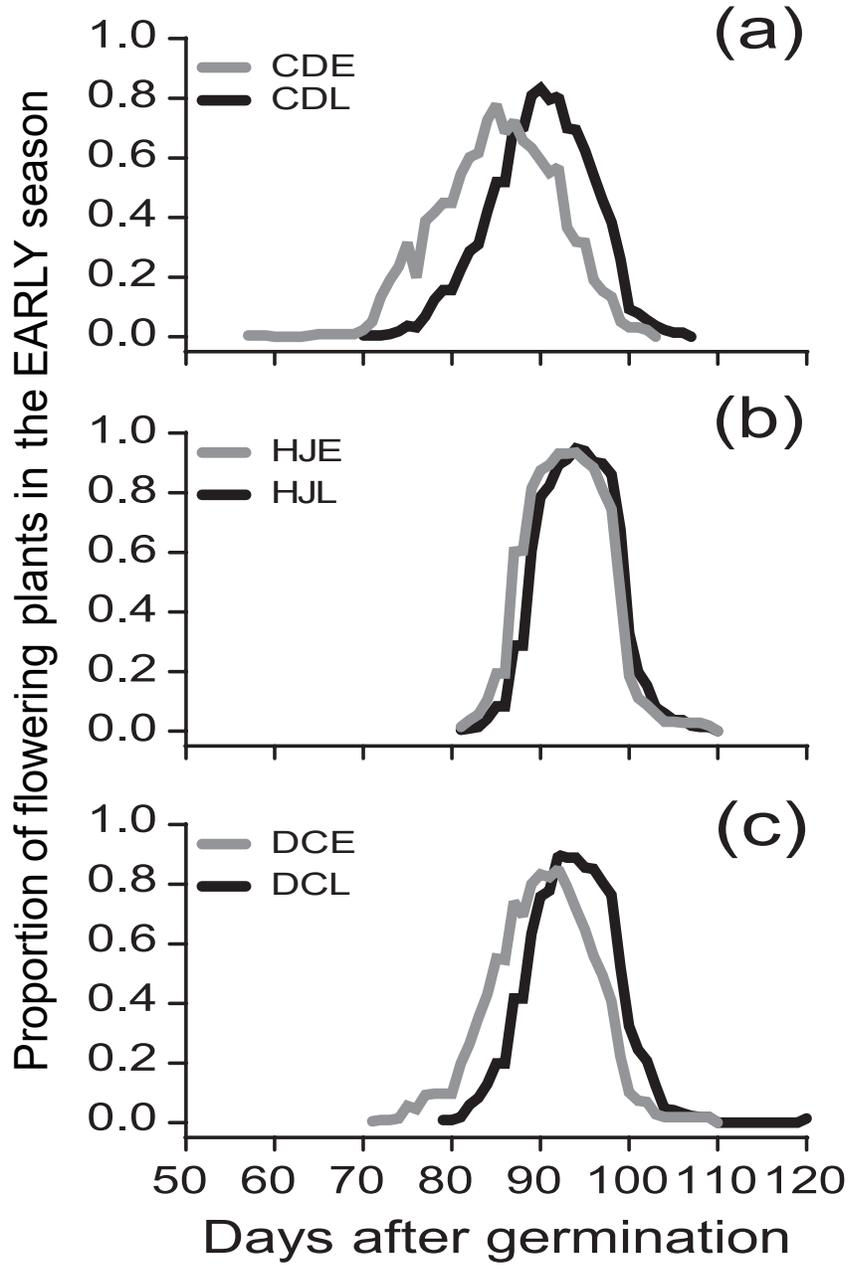
Acknowledgement

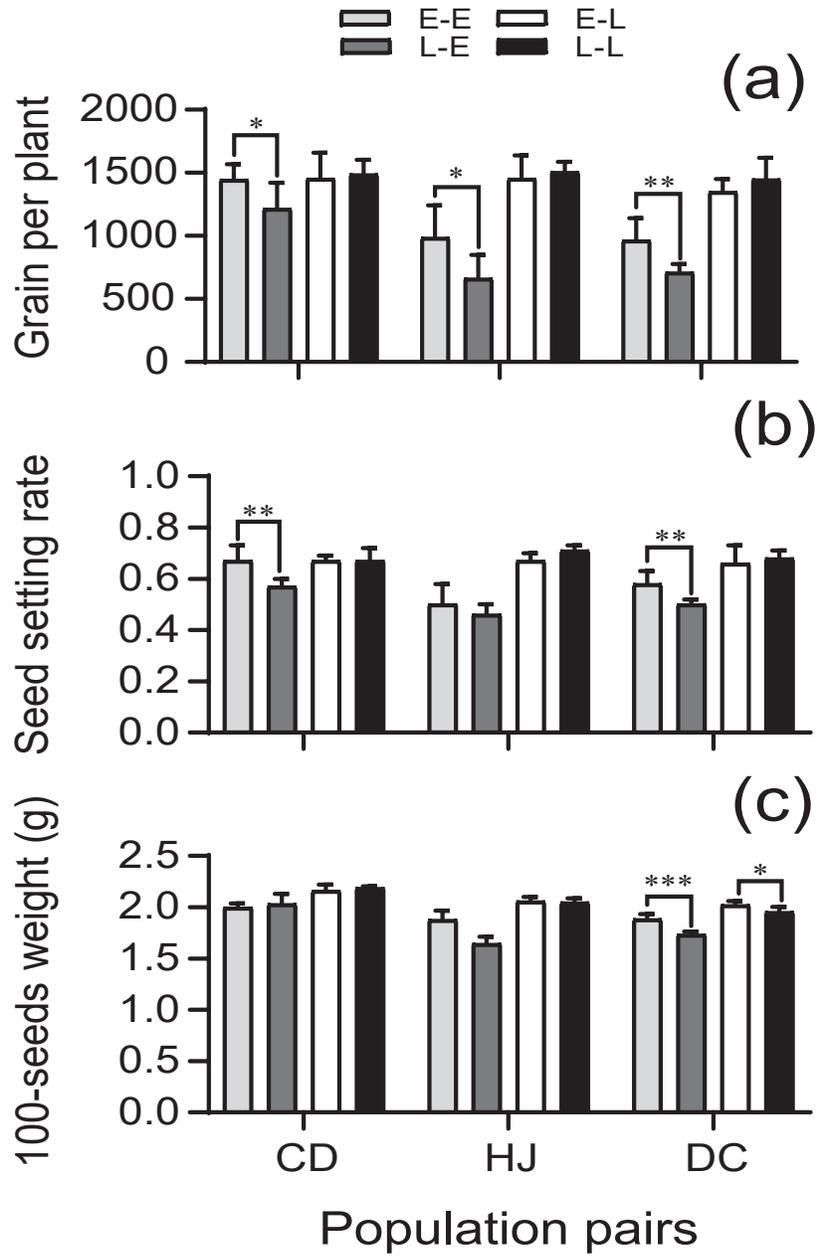
This research is supported by the National Natural Science Foundation of China (31770409, 32070245).

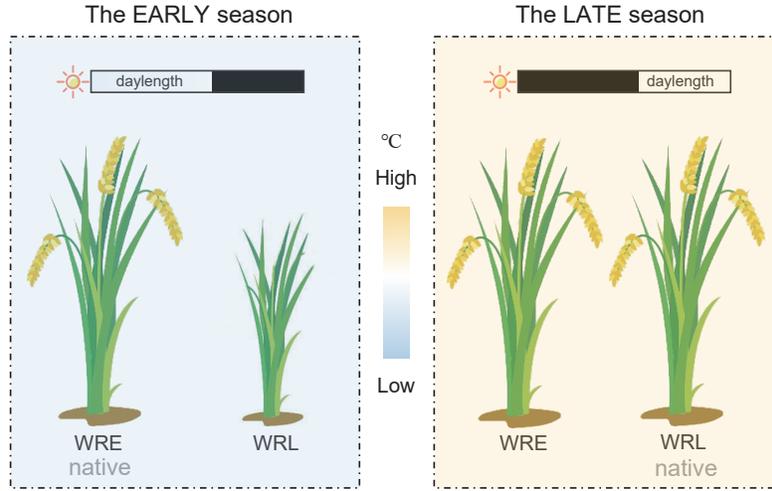












Local adaptation in the early-season weedy rice (WRE)