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Effect of environmental factors on seed germination and seedling emergence of weedy rice (*Oryza sativa* f. *spontanea*) in China

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Abstract

Weedy rice (*Oryza sativa* f. *spontanea* Auct. ex Backer) is a troublesome annual weed from the Gramineae family and infests rice (*Oryza sativa* L.) fields globally, with a notable presence in China. However, limited information is available regarding the effects of diverse environmental factors on its germination and emergence. A better understanding of the seed biology and ecology of weedy rice is crucial for developing effective weed management strategies. Experiments were conducted to evaluate the effects of temperature, light, soil burial depth, wheat (*Triticum aestivum* L.) crop residue amount, salt stress, osmotic stress, and radiant heat on the germination and seedling emergence of weedy rice. Weedy rice exhibited robust germination (>98%) when exposed to varying day/night temperatures (20/15 to 35/30 C) and remained unaffected by light conditions. Seedling emergence was not influenced within the top 5-cm soil layer, where 100% of the seedlings emerged. However, emergence decreased as the soil burial depth increased, eventually resulting in no emergence from a burial depth of 11 cm. The soil burial depth required for 50% of the maximum emergence was 8.3 cm. Seedling emergence ranged from 97% to 100% across different amounts of the wheat straw residue cover (0 to 10,000 kg ha^{-1}). The sodium chloride concentration and osmotic potential required for 50% were 230.8 mM and −0.5 MPa, respectively. No germination was observed when weedy rice seeds were exposed to >110 C pretreatment (radiant heat for 5 min), indicating that residue burning could reduce infestation of weedy rice. The insights gained from this study contribute valuable knowledge to enhance the integrated management of weedy rice in China.

Introduction

Weedy rice (*Oryza sativa* f. *spontanea* Auct. ex Backer), also known as red rice, poses a significant challenge in rice fields due to its competition with cultivated rice (*Oryza sativa* L.) and difficulty to control causing yield loss (Chauhan 2013; Ziska et al. 2015). Apart from barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and Chinese sprangletop [*Leptochloa chinensis* (L.) Nees], it has emerged as the most important malignant weed in rice fields in many Asian countries, such as Malaysia, Sri Lanka, Thailand, Vietnam, and the Philippines, particularly in regions where direct seeding of rice is prevalent (Juliano et al. 2020; Singh et al. 2013; Wang et al. 2023), and rice yield losses due to weedy rice infestation were reported to be from 16% to 74% (Chin 2001; Karim et al. 2004).Weedy rice also occurs widely in all rice-planting areas in China, with an incidence of 39% in 387 survey sites (Wang et al. 2023). There are several explanations for its prevalence. First, the popularization of direct-seeded rice has been identified as a crucial factor contributing to the emergence and proliferation of weedy rice (Chaudhary et al. 2023; Chung 2010; Toshiyuki 2018). This is due to the aerobic, partially or non-flooded conditions of direct-seeded rice compared with the flooded conditions of transplanted rice, as well as the absence of a crop-competitive advantage in direct-seeded rice compared with transplanted rice. Second, the absence of herbicides that are effective in selectively controlling weedy rice, given its physiological and biochemical similarity to rice, presents a significant challenge for management (Bertucci et al. 2019; Busi et al. 2017; Gross et al. 2010). Furthermore, the sharing of agricultural machinery services across provinces and cities poses a significant risk in the spreading of weedy rice between regions (Gao et al. 2018), further complicating the issue for growers.

Considering the absence of specific herbicides, integrated weed management strategies based on a profound understanding of weed biology are crucial for effective weed control (Mahajan and Chauhan 2020). Germination is a key component of weed establishment and is influenced by various environmental factors such as light, temperature, salt concentration, water stress, residue cover, and burial depth (Chauhan and Johnson 2010; Kibasa et al. 2022; Loura et al. 2020; Singh et al. 2021; Zhang et al. 2023). For instance, Dhanda et al. (2023) reported that the highest emergence (82%) of vipergrass [*Dinebra retroflexa* (Vahl) Panzer] was recorded under no-residue cover and significantly decreased from 72% to 28% as the chopped air-dried wheat (*Triticum aestivum* L. 'Spitfire') residue amounts increased from 1,000 to 4,000 kg ha^{-1}, and only 2% emergence was observed when the residue coverage amount was 8,000 kg ha^{-1}. Chauhan and Johnson (2008) found that the seedling emergence of goosegrass [*Eleusine indica* (L.) Gaertn.] was highest at 82% when sown on the soil surface but decreased gradually as the burial depth increased, and no seedlings emerged when the depth of soil burial was 8.0 cm. Therefore, knowledge of seed ecology has become valuable for developing comprehensive weed management strategies.

In China, approximately 3.69 million ha are affected by salinity, accounting for about 6.6% of the total cultivated soil area, greatly affecting crop productivity (Yang 2008; Yang et al. 2022). Tan et al. (2014) reported that germination of weedy rice declined with an increase in sodium chloride (NaCl) concentrations from 0 to 200 mM, about 68% germination was observed at 150 mM NaCl, and only 3% of seeds germinated at 200 mM NaCl. However, information on the effects of NaCl stress, light, burial depth, crop residue, osmotic stress, and heat on weedy rice germination/emergence is lacking. Chauhan (2012) reported that the occurrence and expansion of weedy rice could be effectively controlled through the implementation of deep-tillage practices. These practices entail burying seeds beyond their maximum emergence depth (>8 cm) and early flooding of fields, approaches that proved beneficial for thwarting the growth and spread of weedy rice. Fukuda et al. (2023) found that the germination rate of weedy rice in Japan increased with water availability and fluctuated between low and high temperatures. Nevertheless, little research has been conducted on the germination/seedling emergence of weedy rice collected in China in response to different environmental conditions. Therefore, this study aimed to evaluate the effect of environmental factors including temperature, light, soil burial depth, wheat crop residue, osmotic and salt stress, and radiant heat on seed germination and seedling emergence of weedy rice collected from paddy fields in China.

Materials and Methods

Seed Collection and Preparation

Weedy rice seeds used in this study were originally collected from several rice fields (ca. 600 ha in 16 different zones) in Taizhou, Jiangsu, in October 2022. Taizhou, located in central China, has been severely impacted by weedy rice infestations, with an overall weed infestation index exceeding 38% (Wang et al. 2023). This high rate of infestation has posed a significant threat to rice production, causing significant yield losses in the region. Rice varieties widely used in local production were 'Nanjing 9108', 'Nanjing 5055', 'Huaidao 5', and 'Taixiangjing 1402', all of which are *japonica* varieties. The weedy rice occurring in Taizhou paddy fields was awnless with yellow hulls and a red pericarp and was similar in morphology to *indica* rice. The collected seeds were dried under sunlight and then stored in a seed cabinet (Huruiming Instrument, Guangzhou, China) maintained at 4 C with 40% relative humidity. The average grain weight of weedy rice was 26.5 ± 0.2 mg per seed. Weedy rice seeds were taken out of the seed cabinet and sun-dried for several days before the commencement of the experiments. More than 98% of seeds germinated in a preliminary test. Experiments were carried out from April to October 2023 in the laboratory and screenhouse of Jiangsu Agricultural Sciences, China (32.03°N, 118.84°E).

Experiment 1. Effect of Temperature and Light on Germination

Twenty-five seeds of weedy rice were evenly arranged in a 9-cm-diameter petri dish (BKMAM Trading, Changde, Hunan, China) with two pieces of Whatman No. 1 filter paper (Whatman International, Ltd., Maidstone, Kent, UK) moistened with 5 ml of distilled water. Petri dishes were sealed using Parafilm® PM-996 (Bemis Company, Inc., Sheboygan Falls, WI, USA) to avoid water loss and subsequently incubated under four alternating day/night temperature regimes (20/15, 25/20, 30/25, and 35/30 C) in both light/dark (12/12-h) and continuous-dark (24-h) conditions with four replicates for each temperature by light combination. The petri dishes for the continuous-dark treatment were covered with two layers of aluminum foil before being placing in incubators (Dhanda and Chauhan 2022). Germination was recorded at 14 d after sowing. The emergence of radicles was defined as seed germination (Rehman et al. 2011).

Experiment 2. Effect of Soil Burial Depth on Seedling Emergence

An assessment was conducted in a screenhouse in May 2023 to determine the effect of seed burial depth on the emergence of weedy rice. In each 15-cm-diameter plastic pot, 25 seeds were sown on the soil surface (0 cm) or at varying depths of 1, 3, 5, 7, 9, and 11 cm. The soil used in this study was obtained from paddy fields belonging to Jiangsu Agricultural Sciences and sieved through a 3-mm sieve before being used to fill the pots. The pots were subirrigated to maintain adequate water. Weedy rice seeds were incubated at 30/20 C (day/night) with a 12/12-h light/dark cycle. Seedling emergence was recorded daily, and the experiment was terminated at 21 d after sowing; there was no germination after this period.

Experiment 3. Effect of Wheat Crop Residue Amount on Seedling Emergence

Weedy rice emergence and biomass were determined in a screenhouse by sowing 25 seeds on the soil surface in a 15-cm-diameter plastic pot, using the same soil as the soil burial depth experiment mentioned earlier. The wheat straw residue used in this study was manually obtained from a wheat field. The wheat variety was 'Yangmai 23', which is widely cultivated locally. To match the length of mechanically harvested wheat straw, it was chopped to approximately 3 to 5 cm in length. The chopped wheat straw was air-dried finely (<10% moisture content) and used to cover on the soil surface at rates of 0, 2,000, 4,000, 6,000, 8,000, and 10,000 kg ha^{-1}. Seedling emergence data were recorded at 21 d after sowing, and the aboveground biomass of weedy rice was also measured after drying in an oven for 4 d at 70 C. This experiment was carried out in a randomized complete block design with four replications for each treatment.

Experiment 4. Effect of Salt Stress on Germination

An experiment was conducted to evaluate the effect of salinity on seed germination by using varying concentrations of NaCl (0, 25, 50, 100, 150, 200, 250, and 300 mM). Twenty-five seeds were added to each petri dish, with two filter papers moistened with 5 ml of salt solution according to the treatment. Petri dishes were sealed with Parafilm® and placed in an incubator at 30/20 C in a day/night cycle with a 12/12-h light/dark cycle. Germination data were recorded at 21 d after sowing.

Experiment 5. Effect of Osmotic Stress on Germination

To evaluate the effect of water stress on weedy rice germination, an experiment was established by evenly distributing 25 seeds in 9-cm-diameter petri dishes containing solutions with osmotic potentials of 0, −0.1, −0.2, −0.4, − 0.6, −0.8, or −1.0 MPa, corresponding to 0.0, 93.6, 132.4, 187.2, 229.2, 264.7, or 295.9 g of polyethylene glycol 8000 dissolved in 1 L of distilled water, respectively (Michel and Radcliffe 1995). The seeds were incubated at 30/20 C (light/dark), and germination data were recorded at 21 d after sowing.

Experiment 6. Effect of Radiant Heat on Germination

To evaluate the influence of radiation heat on seed germination, 1,300 bulk seeds were selected. The seeds were subsequently divided into 52 paper bags, with 25 seeds placed in each bag. The bags were labeled with the corresponding 13 treatments (30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 C), and each treatment had four replicates. These heat treatments were chosen to simulate temperatures during crop straw burning in a field (Kibasa et al. 2022). Each treatment of 100 selected seeds was subjected to a specific temperature in an oven for 5 min. After preheating, the seeds were placed in petri dishes, and the petri dishes were placed in an incubator set at alternating day/night (12/12-h) temperatures of 30/20 C. Germination was determined at 21 d after sowing.

Statistical Analysis

All experiments were conducted in two runs spanning from April to October 2023.

All experiments were conducted in a randomized complete block design, and each treatment was replicated four times. The data collected from these runs were aggregated due to the absence of significant differences ($P > 0.05$) between experimental runs and treatments. Despite attempts at transformation, the homogeneity of variance remained unaltered, and the residuals exhibited a normal distribution. Consequently, ANOVA was carried out on the nontransformed germination percentage values using SPPSS software (v. 25.0 SPSS, Chicago, IL, USA). Before the statistical analysis, data variance was evaluated by examining residuals to ensure uniformity. Nonlinear regression analysis (with $r > 0.99$) (SigmaPlot v. 10.0, Systat Software, San Jose, CA, USA) was utilized when appropriate; otherwise, the LSD at the 5% probability level was employed to differentiate means. The seedling emergence of weedy rice in response to different environmental conditions, including seed burial depths, straw residue amounts, NaCl, water stress, and radiant heat was described using a three-parameter sigmoid model. The model was:

$$E = E_{max}/\{1 + \exp[-(x - T_{50})/E_{rate}]\} \quad [1]$$

In the model, E is the total seedling emergence (%) at time x, E_{max} is the maximum seedling emergence (%), T_{50} is the time to reach 50% of maximum seedling emergence, and E_{rate} is the slope.

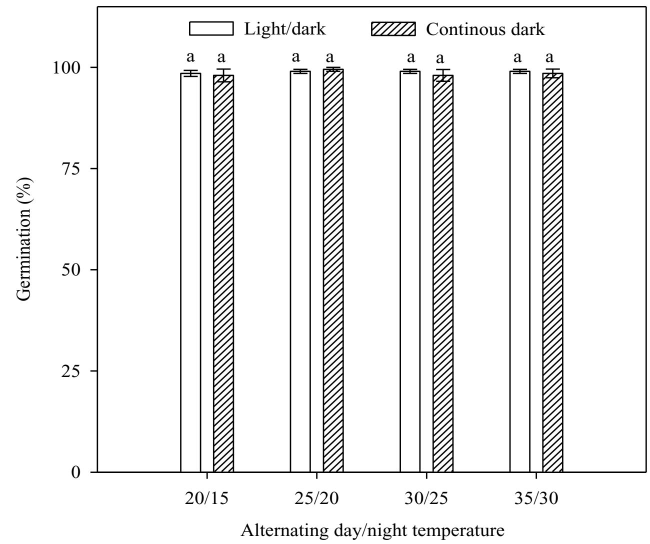

Figure 1. Seed germination of weedy rice in response to temperature and light. Seeds were incubated for 14 d under different temperature and light conditions. Different letters above a column indicate a significant difference at the 0.05 probability level. Error bars denote ± standard errors of the mean ($n = 8$).

Results and Discussion

Germination of Weedy Rice Response to Temperature and Light

The study revealed that weedy rice germination was not affected by the tested temperature and light regimes, consistently showing greater than 98% germination (Figure 1). The germination ability of weedy rice under all tested temperature regimes indicates that weedy rice can germinate throughout the rice-growing season in China, demonstrating its adaptability to a range of temperature conditions. Moreover, the germination response of weedy rice to continuous darkness indicates that it was not sensitive to light and could still germinate regardless of being buried at shallow soil depths or under a closed rice canopy. Similar findings on germination between light/dark regimes have been studied for other weed species, such as Alkali barnyardgrass [*Echinochloa crus-galli* var. *zelayensis* (Kunth) Hitchc.] and three-lobe morningglory (*Ipomoea triloba* L.), which do not exhibit significant differences in germination between light/dark regimes (Chauhan and Abugho 2012; Zhang, et al. 2023). The very high germination ability of weedy rice at 15 to 35 C (light/dark [12/12-h] and continuous dark [24-h]) darkness/light) indicates that it has ability to germinate in rice-growing areas in China under a wide range of temperature conditions.

Seedling Emergence in Response to Burial Depth

The seedling emergence of weedy rice showed a sigmoid relationship with seed burial depth (Figure 2). Highest seedling emergence (100%) was observed when weedy rice seeds were sown at a burial depth less than 5 cm; however, a decline occurred as the seed burial depth exceeded 7 cm, and no seedlings emerged from a depth of 11 cm. The model predicted the soil burial depth for 50% seedling inhibition to be 8.3 cm (Figure 2). When seeds were on the surface, the time for 50% seedling emergence (T_{50}) was 3.8 d, whereas increases in burial depth protracted emergence, causing the time for 50% emergence to increase (Table 1).

Table 1. Parameter estimates (E_{max}, maximum emergence [%]; T_{50}, time to reach 50% of maximum emergence [d]; and E_{rate}, slope) of a three-parameter sigmoid model fit to the seedling emergence to the different soil burial depths in Figure 2

Soil burial depth	Parameter estimates[a]			
	E_{max}	T_{50}	E_{rate}	R^2
cm	%	d		
0	99.9 a	3.82 f	0.01 e	0.99
1	100.0 a	5.30 e	0.26 d	0.99
3	99.5 a	5.93 d	0.34 d	0.99
5	100.0 a	7.74 c	0.66 b	0.99
7	79.2 b	8.46 b	0.50 c	0.99
9	34.3 c	10.42 a	1.34 a	0.98
11	—[b]			

[a]Different letters in the same column indicate a statistical difference at $P < 0.05$.
[b]A dash (—) indicates that no seedlings emerged when seeds were planted at 11-cm depth.

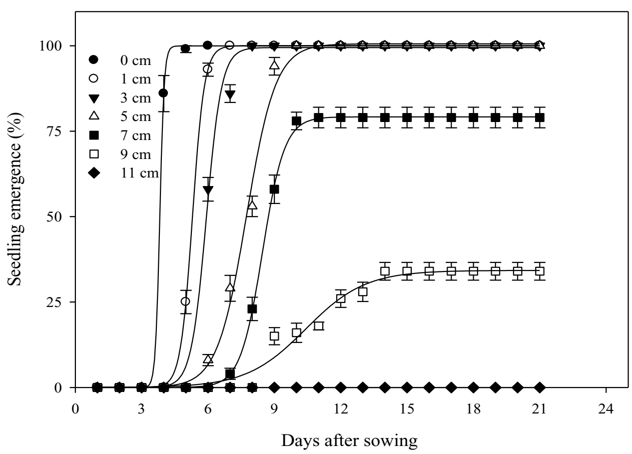

Figure 2. Emergence of weedy rice seedlings in response to different burial depths (cm). Seedling emergence was assessed until 21 d after sowing. The line represents a three-parameter sigmoid model fit to the data. Error bars denote ± standard errors of the mean ($n = 8$).

Generally, seedlings emerging from deep soil may be inhibited due to the lack of light and small seed sizes (Chauhan and Leon 2014). However, as mentioned earlier, weedy rice does not need light to germinate. Lightweight seeds, such as *D. retroflexa* (2.2 mg per seed), may have difficulty emerging from even a 2-cm soil burial depth (Dhanda et al. 2023). However, heavier seeds, such as weedy rice (25.4 mg per seed), can emerge from a 9-cm soil burial depth. Heavier seeds have more carbohydrate reserves than lightweight seeds, which is conducive to their emergence from a greater soil burial depth (Grundy et al. 2003).

The findings on seed burial depth on weedy rice seedling emergence suggest that tillage practices affect seedling emergence; that is, shallow tillage may increase seedling emergence of weedy rice, while deep tillage may be conducive to burying seeds below the minimum emergence zone (≥11 cm, 0% emergence). However, it is important to practice subsequent tillage operations at a shallow depth to prevent previously buried seeds from being brought to or near the soil surface (Kurstjens and Perdok 2000).

Effect of Wheat Straw Residue Amount on Seedling Emergence and Shoot Biomass

Rice–wheat rotations are the main cropping system in China (Timsina and Connor 2001). Crop residues used as mulch are very useful resources when left in the field. Previous studies have shown that crop residues (i.e., rice, corn [*Zea mays* L.], and wheat) hinder light penetration and reduce soil temperatures through shading, which can cause a decrease or the complete absence of emergence of several weed species (Chauhan and Abugho 2014; Chauhan et al. 2012; Davis 2007; Ranaivoson et al. 2018; Zhang et al. 2023). Contrary to our expectations, the emergence of weedy rice seedlings was not greatly influenced by varying amounts of wheat straw residue (0 to 10,000 kg ha^{-1}), ranging from 97% to 100% emergence (Figure 3). This suggests that adding wheat straw residue up to 10,000 kg ha^{-1} may not effectively suppress the emergence of weedy rice seedlings in fields under conservation agricultural systems in China. In China, conservation agriculture is primarily adopted to improve soil fertility and increase organic carbon sequestration (Wu et al. 2019; Xia et al. 2014; Zhao et al. 2015).

For weedy rice seedling shoot biomass, no significant effect was observed with wheat straw residue ≤8,000 kg ha^{-1}, but a significant reduction occurred at the maximum wheat straw residue amount (10,000 kg ha^{-1}) compared with the absence of wheat straw residue (0 kg ha^{-1}). This indicates that wheat straw residue produced under current wheat yields is not very effective for weedy rice management in China.

Effect of NaCl on Seedling Germination of Weedy Rice

Weedy rice exhibited its highest germination (98.8%) in the 0 mM NaCl (control) treatment and significantly decreased to 30% at 250 mM (Figure 4). It was determined from the fitted model that a salt concentration of 231 mM NaCl could lead to 50% inhibition of weedy rice germination.

Salt stress causes varied changes in seed germination for different weed species. The concentration for 50% inhibition of germination for wild bushbean [*Macroptilium lathyroides* (L.) Urb.], for example, was 149 mM NaCl (Chauhan and Leon 2014), but the NaCl concentration for feather fingergrass (*Chloris virgata* Sw.) was 25 mM NaCl (Fernando et al. 2016). The fact that weedy rice seeds can germinate at high salt concentrations indicates their ability to grow in China and other countries. In Asia, Lafitte et al. (2006) reported that there is a considerable area (21.5 million ha) of high-salinity soil. Zeng and Shannon (2000) found that salinity levels ranging from 3.4 to 7. 9 dS m^{-1} in water and soil were able to cause serious reduction in yield components, while very high salinity (>11.5 dS m^{-1}) lead to total failure of rice. In salt-affected rice-growing areas, apart from salt stress, weedy rice infestation may add to a substantial decrease in rice yield.

Effect of Osmotic Potential on Seedling Germination of Weedy Rice

The osmotic potential at < −0.8 MPa did not significantly affect weedy rice germination, with >99% germination at −0.4 MPa. However, as water stress increased, the germination of weedy rice decreased, reaching 30% at −0.6 MPa, with no observed germination at −1.0 MPa (Figure 5).

The osmotic potential for 50% inhibition of weedy rice seed germination was −0.54 MPa. These findings suggest that high water stress may cause a reduction in weedy rice seed germination, because seeds are unable to obtain a critical moisture threshold level for imbibition (Bittencourt et al. 2017). However, weedy rice will infest rice fields under adequate soil moisture or mild water stress conditions typically required for rice production.

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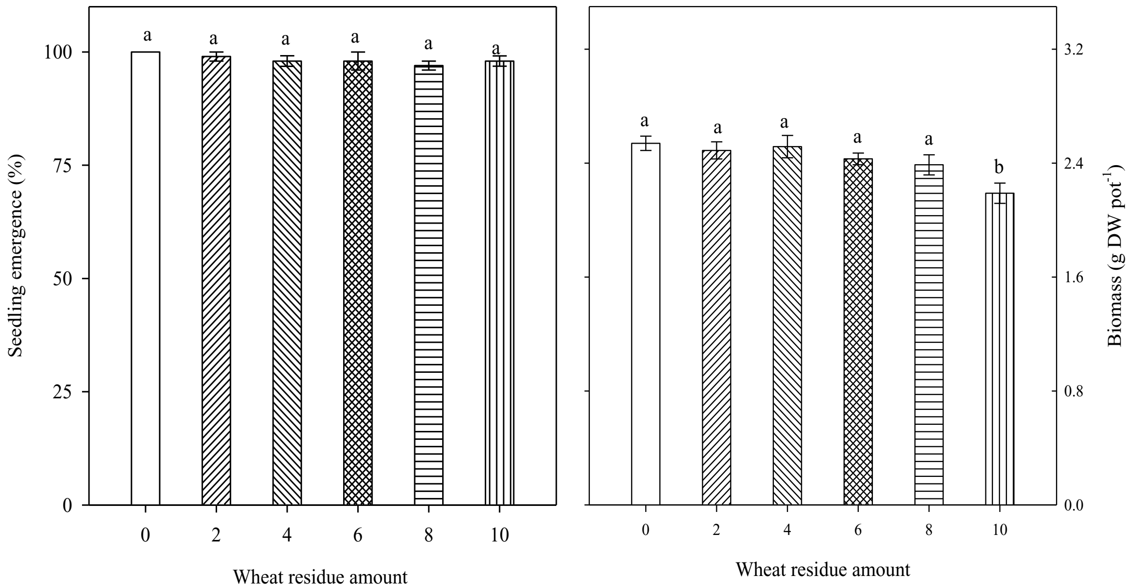

Figure 3. Effect of wheat straw residue amount on seedling emergence and shoot biomass of weedy rice. Seedling emergence and shoot biomass were assessed until 21 d after sowing. Error bars denote ± standard errors of the mean ($n = 8$).

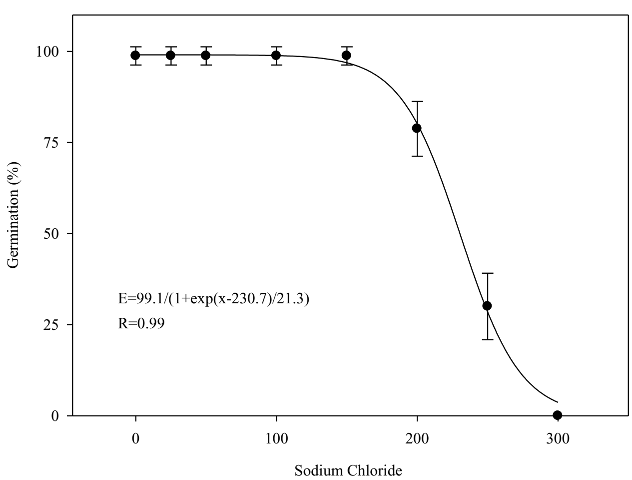

Figure 4. Effect of sodium chloride on germination of weedy rice incubated at 30/20 C day/night temperature in a 12/12-h for 21 d. The line represents a three-parameter sigmoid model fit to the data. Error bars are standard errors of mean ($n = 8$).

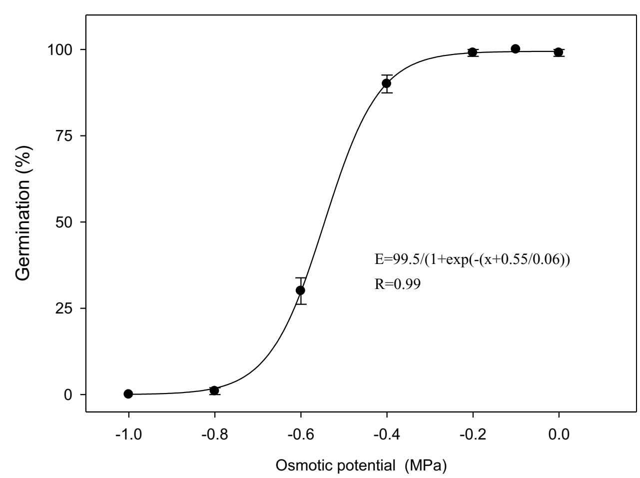

Figure 5. Effect of osmotic potential on weedy rice germination at 21 d after incubation at 30/20 C day/night temperature. The line represents a three-parameter sigmoid model fit to the data. Error bars are standard errors of mean ($n = 8$).

Effect of Radiant Heat on Germination of Weedy Rice Seeds

Weedy rice germination was 100% after a pretreatment of 5 min at a temperature of 70 C, 89% at 80 C, 5% at 100 C, and 0% at 110 C (Figure 6). The germination percentage of weedy rice at 80 C (89%) indicates that it can tolerate radiant heat for a short period, as weedy rice germination was completely inhibited at temperatures higher than 110 C. Iizumi and Iwanami (1965) reported maximum temperatures of 390, 520, 388, 178, 116, and 90 C at 20, 50, 100, 150, 200, and 500 mm, respectively, below the soil surface during residue burning. Therefore, burning may help in managing weedy rice when seeds are in the topsoil layer. The temperature for 50% inhibition of weedy rice germination estimated from the fitted model was 90 C.

Burning residue in the fields can eliminate weed seeds and other pests when they are present on or near the soil surface (Virto et al. 2007). Windrow burning has proven to be a highly effective method for eliminating weed seeds in paddocks (Walsh and Newman 2007). However, the practice of burning crop residue may kill microbes and pollute the air, thus causing environmental destruction (Lin and Begho 2022). Moreover, elevated temperatures may result in a decrease in soil organic matter content, leading to soil degradation (Sarkar et al. 2020). Therefore, these aspects should also be taken into account when formulating weed management strategies for burning crop residue.

In summary, this study provides insights into the germination and emergence behaviors of weedy rice under various environmental conditions. Weedy rice collected in China demonstrates a high potential for germination across a wide temperature range and is not influenced by light conditions. High seedling emergence of weedy rice was observed on the soil surface (0 to 7 cm) and under wheat straw residue conditions ranging from 0 to 10,000 kg ha^{-1}, suggesting that a no-till system or wheat residue mulching has limited efficacy in suppressing weedy rice emergence. Additionally,

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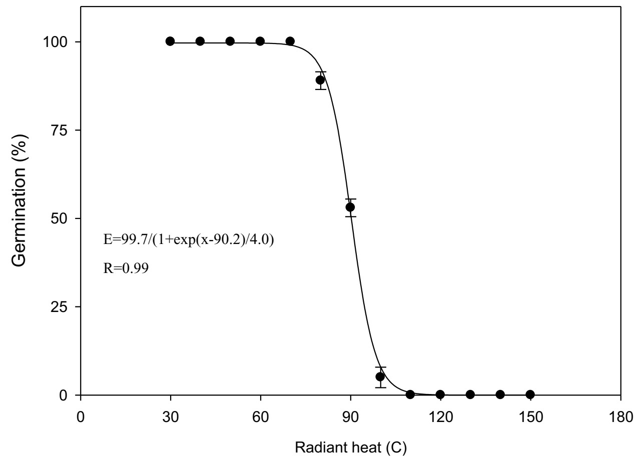

Figure 6. Effect of radiant heat for 5 min on germination of weedy rice seeds placed in an incubator at 30/20 C (day/night) for 21 d. The line represents a three-parameter sigmoid model fit to the data. Error bars denote ± standard errors of the mean ($n = 8$).

the research revealed that weedy rice demonstrated a considerable tolerance to water scarcity and high salt levels, emphasizing its adaptability to adverse conditions. Weedy rice failed to germinate after exposure to radiant heat of 110 C (for 5 min), suggesting that burning residues may be an effective strategy for managing weedy rice, particularly when seeds are on the soil surface.

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Competing interests. No competing interests have been declared.

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