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# Impact of Soil Burial Depths on Survival of Weedy Rice Seeds: Implications for Weed Management

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**Abstract:** Weedy rice (*Oryza sativa* f. *spontanea*) is a noxious weed infesting rice fields worldwide and causes great yield losses for cultivated rice. Effective management of this weed is essential for the world's rice production. Yet, the management of weedy rice is challenging. One of the reasons is that shattered weedy rice seeds stored in soil often trigger great weed proliferation in the succeeding crop seasons. To study the survival of weedy rice seeds in soil seedbanks, we conducted 90-day soil burial experiments at different soil depths from 0–25 cm, using weedy rice seeds from Jiangsu Province in China. Results from two independent experiments under the rice field and laboratory conditions indicated significant differences in seed death ratios (SDRs) and induced seed dormancy ratios (ISDRs) of weedy rice at different soil burial depths. Weedy rice seeds exposed to the soil surface (0 cm burial treatment) had the highest SDRs and lowest ISDRs. An evident pattern of quickly declining SDRs with increased soil burial depths was identified from this study, suggesting rapid losses of seed viability on the surface and in shallow layers of soil. Our findings provide a useful guide for designing strategies to effectively control weedy rice by maintaining shattered seeds on the surface or in shallow layers of soil. The practices can easily be achieved through adopting the no-till farming system, which can substantially minimize viable weedy rice seeds as an important component in comprehensive weed management strategies.

**Keywords:** no-till farming; rice field; soil seed burial experiment; seed viability; soil seedbank; weed management

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## 1. Introduction

The challenge of global food security triggered by the continued increases in world human populations, in addition to the global climate change, poses tremendous threats to the world's socio-economic development and stability [1–4]. Consequently, the demand for substantially increased crop production worldwide has become critical for resolving global food security problems. Asian cultivated rice (*Oryza sativa* L., referred to as rice hereafter) is an important world cereal crop providing staple foods for nearly one half of the global population [5,6]. Therefore, rice production plays an essential role in ensuring global food security. However, the infestation and outbreaks of weeds in rice fields cause a tremendous reduction in grain yield of rice because weeds compete with cultivated rice for resources such as water, nutrients, and light in the field [5,7–11]. Thus, the effective management of weeds infesting rice fields provides a key solution for maintaining high rice production.

Weedy rice (*O. sativa* f. *spontanea*), also referred to as red rice, is a noxious weed that infests rice fields worldwide, causing substantial reductions in the yield of cultivated rice [5,9]. As a conspecific weed, meaning that it belongs to the same biological species as cultivated rice, weedy rice not only causes significant losses in grain yield, but also influences the commercial quality of rice when weedy rice seeds with red pericarps mix with crop grains. Furthermore, weedy rice exhibits many similar phenotypic and physiological characteristics to cultivated rice across different growth stages, except for its weediness traits such as seed shattering and dormancy, thus rendering its identification and control more difficult [5,12]. In recent decades, the decline in agricultural labor and the shift of rice cultivation techniques from transplanting to direct-seeding have further exacerbated the infestation of weedy rice in rice ecosystems worldwide [5,10,13]. As a result, the Food and Agriculture Organization of the United Nations (FAO) has identified weedy rice as one of the most destructive rice-field weeds threatening the production of cultivated rice [14].

Various weed management measures have been implemented to contain weedy rice infestations in rice fields. To date, there is still no single effective method that can significantly reduce weedy rice populations [8–10,14], and no choice of proper herbicides that can specifically kill weedy rice without damaging cultivated rice available [15]. A plausible explanation is that most measures overemphasize the above-ground management of weedy rice with less attention given to weedy rice seeds stored in soil seedbanks [15–17]. Unlike cultivated rice, weedy rice is characterized by a strong seed shattering ability, enabling the dropped seeds to accumulate in soil seedbanks for a long period of time [5,18,19]. Such features can facilitate the emergence and proliferation of weedy rice populations in paddy fields in the following seasons [19,20]. Furthermore, recent studies have indicated that weedy rice seeds originating from temperate rice planting regions typically lack primary seed dormancy [21,22]. However, soil burial can induce secondary seed dormancy in these weedy rice seeds [22], although the relationship between soil burial depths and the ratios of seed dormancy is still unclear. With these advantages, weedy rice seeds stored in soil seedbanks can easily escape from adverse environmental conditions, which can facilitate the survival, reproduction, and dissemination of weed rice in rice fields. Thus, understanding the survival of weedy seeds is very important, including their death and induced dormancy associated with their burial depths in soil seedbanks. Based on the survival of weedy rice seeds at different depths in soil, farmers can design useful strategies, such as no-till or alternatively deep plough after cultivated rice is harvested, to effectively control weedy rice.

To investigate the impact of soil burial depths on the survival or viability of weedy rice seeds with solid experimental data, we studied the death and induced seed dormancy of weedy rice seeds from eight populations buried in soil under the rice field and laboratory conditions. The primary objectives of this study are to address the following questions: (1) What is the impact of soil burial depths on the seed death ratios in different weedy rice populations? (2) What is the impact of soil burial depths on the induced seed dormancy ratios in different weedy rice populations that do not have primary seed dormancy? (3) Is the pattern of the death and induced secondary dormancy ratios of weedy rice seeds consistent between the natural rice field and laboratory conditions? The answers to these questions can help us to understand the impact of different field management methods or farming systems, such as adopting no-tillage or plough/tillage farming in rice fields after rice harvest, on the survival of weedy rice seeds in soil seedbanks. The obtained knowledge can facilitate designing more effective strategies for weedy rice control and management under different farming practices.

## 2. Materials and Methods

### *2.1. Weedy Rice Seeds Used in the Experiments*

Eight weedy rice populations each containing more than 60 mature plants or individuals were randomly collected from rice production areas in Jiangsu Province, China (Table 1). All samples were collected in rice fields with direct-seeding cultivation systems in the autumn of 2020. The spatial distances between the sampled weedy rice populations were >5 km and those between sampled weedy rice plants within a weedy rice population were >5 m. The estimated collecting area for each weedy rice population in a rice field was about 2500 $m^2$. The collected weedy rice seeds were stored under dry conditions at room temperature for more than two weeks for post-ripeness. The seeds were tested for germination ratios as a pre-experiment to ensure that nearly all seeds were viable and no primary dormancy.

**Table 1.** Weedy rice populations included in the seed burial experiments with information on their locations in Jiangsu Province, China.

| Sampling Site | Population Code | Longitude | Latitude | Altitude (m) |
|---|---|---|---|---|
| Nanjing | NJ | 119°2′ N | 32°1′ E | 22.0 |
| Zhenjiang | ZJ | 119°30′ N | 32°6′ E | 26.3 |
| Xuzhou | XZ | 119°59′ N | 34°29′ E | 21.6 |
| Nantong | NT | 121°1′ N | 31°51′ E | 6.3 |
| Yangzhou | YZ | 119°38′ N | 32°30′ E | 11.6 |
| Suqian | SQ | 111°44′ N | 34°44′ E | 13.2 |
| Wuxi | WX | 121°35′ N | 32°41′ E | 4.2 |
| Lianyungang | LYG | 121°30′ N | 33°57′ E | 21.3 |

Two sets of weedy rice seeds with equal numbers were randomly selected from the sampled weedy rice. Each set contained >6000 well-developed seeds sampled randomly from 60 plants (~100 seeds/plant) in a weedy rice population. One set of the weedy rice seeds was included in the seed burial experiment under the rice field condition, and another set was included in the experiment under the laboratory condition. These seeds were used to measure seed death ratios (SDRs) and induced seed dormancy ratios (ISDRs) after the seeds were buried in soil to understand the survival of weedy rice seeds at different depths in soil seedbanks.

### 2.2. Designs of Soil Seed Burial Experiments

#### 2.2.1. Soil Seed Burial Experiment in the Rice Field

To investigate the SDR and ISDR of weedy rice samples at different soil depths under the field condition, we conducted the seed burial experiments with the prepared weedy rice seeds at six different depths: 0 cm, 5 cm, 10 cm, 15 cm, 20 cm, and 25 cm. The six soil burial depths were simulating different tillage methods after cultivated rice was harvested when weedy rice seeds shattered into soil. All the weedy rice seeds were buried in a rice field located at the experimental site of Fudan University (Jiangwan, 121°5′ N, 31°3′ E) in Shanghai, China. The seed burial experiment lasted for 90 days starting in the early winter of 2021, given that the pilot experiment indicated >90% of the seeds buried on the surface of soil were dead after 75 days. The recorded average temperature during the experiment in the field was ~8.1 °C (ranging between −4.2 °C and 14.6 °C), whereas the recorded average humidity was ~65% (ranging between 29 and 97%). Thirty weedy rice seeds were placed in a nylon-net-bag as a replicate, and each soil depth treatment included ten replicates. Consequently, a total of 8 populations × 6 soil depths × 10 replicates × 30 seeds = 14,400 seeds were included in the seed burial experiment under the rice field condition. The layout of all replicates representing the eight weedy rice populations of the six treatments followed the randomized complete block design in the experiments.

#### 2.2.2. Soil Seed Burial Experiment in the Laboratory

We designed an additional soil seed burial experiment in the laboratory as a control for a consistent condition, because the climatic conditions (i.e., temperature and humidity) in the rice field were greatly variable, which may affect the obtained results. To investigate the SDRs and ISDRs of weedy rice samples at different soil depths under the laboratory condition, we buried another set of weedy rice seeds in soil placed in plastic containers (70 × 48 × 39 cm). The soil used in the laboratory experiment was collected from the same rice field at the experimental site of Fudan University. Weedy rice seeds were also buried at six different depths: 0 cm, 5 cm, 10 cm, 15 cm, 20 cm, and 25 cm, in containers filled with soil that were placed in a dark growth chamber. The temperature and humidity in the growth chamber were controlled to ~8 °C and ~70%, respectively. The experimental designs followed those of the field experiments.

*2.3. Calculation of the Seed Death Ratios and Dormancy Ratios*

Ninety days after weedy rice seeds were buried, all seeds of the two independent experiments were dug up from the soil burial treatments. The collected seeds were soaked in 75% ethanol solution for ~2 min to kill fungi and bacteria, and then cleaned with running deionized-water for ~10 min. All the cleaned seeds were subjected to germination on moist filter papers placed in petri-dishes at ~28 °C in a dark growth chamber. The 2,3,5-triphenyltetrazolium chloride (TTC) staining method [22] was employed to determine ungerminated but still viable seeds by staining the vertical-cut embryos of the seeds.

The seed death ratio (SDR) was calculated as the number of dead seeds divided by the total number of seeds subjected to germination. The dead seeds were determined as the ungerminated but unstained (pale embryo) seeds treated by the TTC staining method 10 days after germination. The induced seed dormancy ratio (ISDR) was calculated as the number of ungerminated but viable (red embryo) seeds by TTC staining 10 days after germination divided by the total number of seeds subjected to germination.

*2.4. Data Analyses*

Two-way ANOVAs were conducted to determine the effect of soil burial depths (D), weedy rice populations (P), and their interaction (D × P) on SDR and ISDR [22]. When the ANOVA data showed significant differences ($p < 0.05$), the Duncan test was conducted to determine differences among the six seed burial depths in soil for SDR and ISDR. Linear and quadratic regressions were conducted to analyze the correlations between soil burial depths and SDR/ISDR, respectively. All statistical analyses were conducted using the software SPSS Statistics ver. 20 (2011, IBM Corp., Endicott, NY, USA).

## 3. Results

*3.1. Effect of Soil Burial Depths on Seed Death and Induced Seed Dormancy in the Rice Field and Laboratory Experiments*

Our results evidently showed that the germination ratios of all untreated weedy rice seeds (control) were very high (96.6–100.0%, Table S1), suggesting that weedy rice seeds from Jiangsu Province were viable with no primary seed dormancy. After 90 days of burial, no seed germination was observed in the soil. Results from the two-way ANOVA indicated significant effects ($p < 0.001$) of different soil burial depths (D) on the seed death ratios (SDRs) and induced seed dormancy ratios (ISDRs) of weedy rice, both in the rice field and laboratory experiments (Table 2). These results indicated the necessity to further analyze the actual impact of soil burial depths on the survival of weedy rice seeds in soil seedbanks. Additionally, the value of $\eta^2_{partial}$, which was a parameter to evaluate the magnitude of the effect in each factor, also confirmed that the depths of soil burial played very important roles in the survival of weedy rice seeds stored in seed soil banks ($\eta^2_{partial} > 0.8$).

**Table 2.** Two-way ANOVA of the seed death ratios (SDRs) and induced seed dormancy ratios (ISDRs) of weedy rice seeds from different populations buried at different depths (0~25 cm) in the rice field and laboratory experiments.

| | | | SDR | | | ISDR | | |
|---|---|---|---|---|---|---|---|---|
| | | **df** † | $F$ | $p$ | $\eta^2_{partial}$ | $F$ | $p$ | $\eta^2_{partial}$ |
| Field experiment | Soil-burial depth (D) | 5 | 711.7 | <0.001 | 0.892 | 428.8 | <0.001 | 0.832 |
| | Population (P) | 7 | 9.1 | <0.001 | 0.128 | 19.2 | <0.001 | 0.237 |
| | Interaction (D × P) | 35 | 17.3 | <0.001 | 0.583 | 9.5 | <0.001 | 0.434 |
| Laboratory experiment | Soil-burial depth (D) | 5 | 384.4 | <0.001 | 0.816 | 457.8 | <0.001 | 0.841 |
| | Population (P) | 7 | 44.4 | <0.001 | 0.419 | 9.3 | <0.001 | 0.131 |
| | Interaction (D × P) | 35 | 5.5 | <0.001 | 0.310 | 7.6 | <0.001 | 0.381 |

† Df, degree of freedom; $F$, ratio of the mean-square value for that source of variation to the residual mean square; $p$, $p$-value; $\eta^2_{partial}$, a parameter to evaluate the magnitude of the effect in each factor (0–1.000).

In addition, different weedy rice populations (P) also had great variation in reacting to different depths of soil. The two-way ANOVA also showed significant effects ($p < 0.001$) of different soil burial depths (D), weedy rice populations (P), and their interaction (D × P) on SDR and ISDR in the two sets of experiments (Table 2).

### *3.2. Death Ratios and Induced-Dormancy Ratios of Buried Weedy Rice Seeds in the Rice Field Experiment*

The seed death ratios (SDRs) and induced-dormancy ratios (ISDRs) of all weedy rice seeds buried at different soil depths varied significantly in the rice field experiment (Figures 1 and 2). In general, the observed SDRs of the buried weedy rice seeds were extremely high (87.3–98.7%) on the surface (0 cm) of the soil, whereas those of deeply buried seeds (25 cm) showed the lowest SDRs (0.0–5.3%). Obviously, the SDRs declined rapidly with increases in soil burial depths from 0–25 cm, although with a considerable variation among populations at the depths between 5 and 20 cm (Figure 1). The reason for the observed variation was most likely due to different genetic backgrounds among populations responding to the changing environment. Further linear regression analysis indicated a significant negative correlation ($R^2 = 0.708$, $p < 0.001$) between the SDRs and soil burial depths (Figure 3). Different from the SDR pattern, the observed ISDRs of the buried weedy rice seeds were very low (0.0–2.7%) at the surface (0 cm) of the soil. The ISDRs increased quickly on the soil surface (0 cm) to the peaks (10–15 cm) and then declined quickly with increases at soil depths from 10/15–25 cm (Figure 2). Further quadratic regression analysis indicated a parabolic correlation ($R^2 = 0.782$, $p < 0.001$) between ISDRs and soil burial depths (Figure 4).

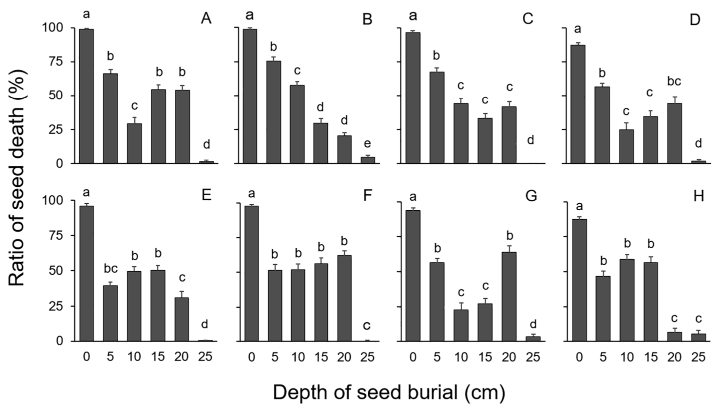


**Figure 1.** The seed death ratios (SDRs) of weedy rice from different populations after soil burial at different depths in the field experiments. (**A**–**H**) represent different weed rice populations: Nanjing, Zhenjiang, Xuzhou, Nantong, Yangzhou, Suqin, Wuxi, and Lianyungang. The small letters above the columns indicate the level of significance ($p < 0.05$) based on the post-hoc comparison by the Duncan test. Error bars represent the standard error of mean ($n = 10$ replicates).

In addition, our results also showed significant variation in SDRs and ISDRs among the eight weedy rice populations at the various soil burial depths. However, there was no consistent variation pattern detected (Table S2). Altogether, the SDR and ISDR results demonstrated much less opportunities for weed rice seeds to survive when the seeds were exposed to the surface layers or shallow layers of the soil for all the weedy rice populations, and vice versa. This conclusion was based on the highest seed death ratios and the lowest induced seed dormancy ratios obtained at the soil surface (0 cm) treatment showing much less variation from the rice field experiment.

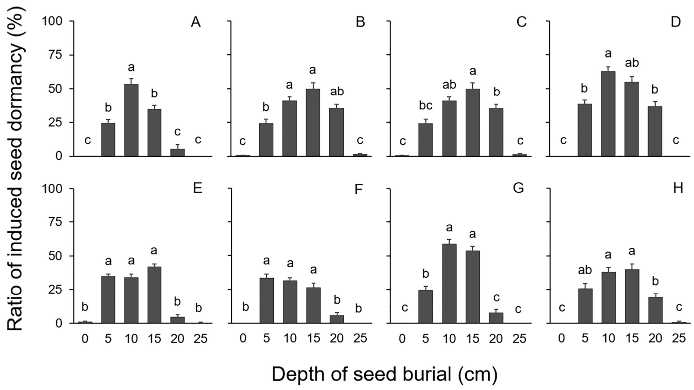


**Figure 2.** The induced seed dormancy ratios (ISDRs) of weedy rice from different populations after soil burial at different depths in the field experiments. (**A**–**H**) represent different weed rice populations: Nanjing, Zhenjiang, Xuzhou, Nantong, Yangzhou, Suqin, Wuxi, and Lianyungang. Small letters above the columns indicate the level of significance ($p < 0.05$) based on the post-hoc comparison by the Duncan test. Error bars represent standard error of mean ($n$ = 10 replicates).

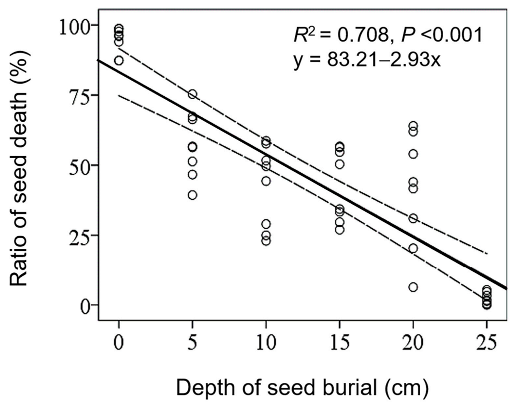


**Figure 3.** Results of regression between the seed death ratios (SDRs) and the depth of seed burial in the field experiments. Empty dots represent the average of SDR in different populations at corresponding burial depths. The solid curve is the regression line and dashed lines indicate 95% confidence intervals.

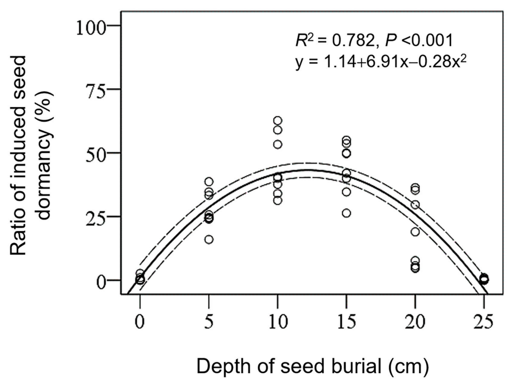


**Figure 4.** Results of regression between the induced seed dormancy ratios (ISDRs) and the depth of seed burial in the field experiments. Empty dots represent the average of ISDR in different populations at corresponding burial depths. The solid curve is the regression line and dashed lines indicate 95% confidence intervals.

### 3.3. Death Ratios and Induced Dormancy Ratios of Buried Weedy Rice Seeds in the Laboratory Experiment

Our results from the laboratory experiment also showed that SDRs and ISDRs of all weedy rice seeds buried at different soil depths varied significantly (Figures 5 and 6). The observed SDRs of the buried weedy rice seeds were high (38.3–69.7%) on the surface (0 cm) of the soil, whereas those of deeply buried seeds (25 cm) showed the lowest SDRs (3.7–12.3%, Figure 5). However, the SDR values on the surface were generally much lower than those obtained from the rice field experiment (Figures 1 and 5). Similar to the pattern obtained from the rice field experiment, the SDR values of buried weedy rice seeds declined rapidly with increases in soil burial depths from 0–25 cm. Further linear regression analysis indicated a significant negative correlation ($R^2$ = 0.778, $p$ < 0.001) between the SDRs and soil burial depths (Figure 7).

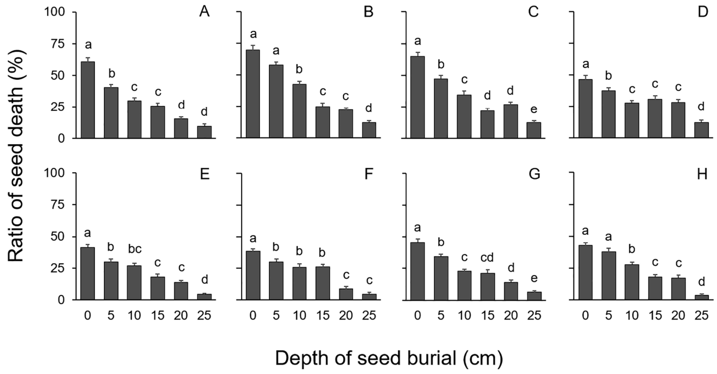


**Figure 5.** The seed death ratios (SDRs) of weedy rice from different populations after soil burial at different depths in the laboratory experiments. (**A**–**H**) represent different weed rice populations: Nanjing, Zhenjiang, Xuzhou, Nantong, Yangzhou, Suqin, Wuxi, and Lianyungang. The small letters above the columns indicate the level of significance ($p < 0.05$) based on the post-hoc comparison by the Duncan test. Error bars represent the standard error of mean ($n$ = 10 replicates).

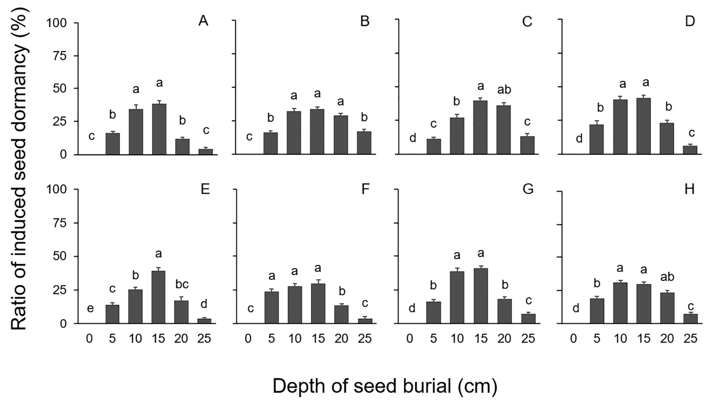


**Figure 6.** The induced seed dormancy ratios (ISDRs) of weedy rice from different populations after soil burial at different depths in the laboratory experiments. (**A**–**H**) represent different weed rice populations: Nanjing, Zhenjiang, Xuzhou, Nantong, Yangzhou, Suqin, Wuxi, and Lianyungang. Small letters above the columns indicate the level of significance ($p < 0.05$) based on the post-hoc comparison by the Duncan test. Error bars represent standard error of mean ($n$ = 10 replicates).

The observed ISDRs of the buried weedy rice seeds did not suggest induced dormancy at the surface (0 cm) of the soil, but increased quickly to the peak (~15 cm). After that peak, the ISDRs declined quickly with increases in soil depths from 15–25 cm (Figure 6). Noticeably, the ISDRs obtained from the laboratory experiment (19.1% on average) were generally lower than those obtained from the rice field experiment (22.5% on average, Figures 2 and 6). Further quadratic regression analysis indicated a parabolic correlation

($R^2 = 0.843$, $p < 0.001$) between ISDRs and soil burial depths (Figure 8). Furthermore, the SDRs and ISDRs of weedy rice soil buried seeds also varied significantly among weedy rice populations although there was no evident pattern detected (Table S3).

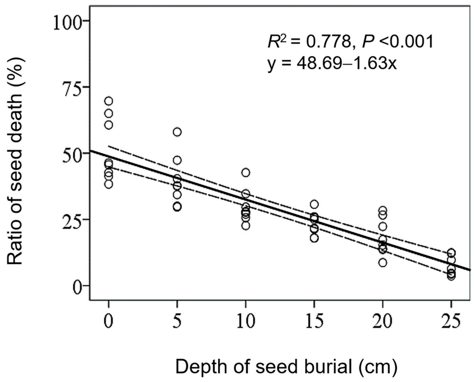


**Figure 7.** Results of regression between the seed death ratios (SDRs) and the depth of seed burial in the laboratory experiments. Empty dots represent the average of SDR in different populations at corresponding burial depths. The solid curve is the regression line and dashed lines indicate 95% confidence intervals.

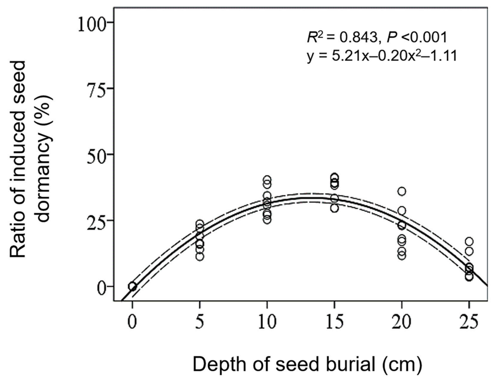


**Figure 8.** Results of regression between the induced seed dormancy ratios (ISDRs) and the depth of seed burial in the laboratory experiments. Empty dots represent the average of ISDR in different populations at corresponding burial depths. The solid curve is the regression line and dashed lines indicate 95% confidence intervals.

## 4. Discussion

### *4.1. The Survival Dynamics of Weedy Rice Seeds Stored at Different Depths in Soil Seedbanks*

Understanding the survival dynamics of weedy rice seeds shattered from matured plants and stored in soil seedbanks can facilitate the design of effective strategies for weedy rice management and control [20,23]. The vertical locations or depths of weedy rice seeds stored in soil will considerably determine the fate of weedy rice seeds, which can be greatly affected by different cultivation practices such as ploughing and tilling after the crop is harvested [15–17,23]. Therefore, it is important to generate knowledge regarding the survival of weedy rice seeds in different locations of soil seedbanks by establishing a well-designed investigation system. Seed germination results obtained from this study indicated that weedy rice seeds collected from Jiangsu Province are highly viable with no detectable primary dormancy. Therefore, the initial condition of the weedy rice seeds included in this study has established an ideal system with clear baseline data for the seed burial experiments to investigate the survival dynamics of weedy rice seeds in different soil depths under both the rice field and laboratory conditions.

Our results obtained from the two sets of experiments indicated the highest seed death ratios (SDRs) of weedy rice in the soil surface treatments (0 cm). However, the lowest induced seed dormancy ratios (ISDRs) were detected both in the soil surface treatments and the deep soil burial treatments (25 cm) in the two independent experiments. These results clearly demonstrate that the survival or longevity of weedy rice seeds becomes very poor on the soil surface after they are shattered from the matured plants. In other words, the seeds of weedy rice can easily die or lose their viability when they shatter on the surface of soil, because of their high SDRs and low ISDRs under the conditions. The generated knowledge will be useful for farmers to design effective methods for weedy rice control by keeping the shattered weedy rice seeds on the surface of soil and to adopt the no-till cultivation system [15–17]. Noticeably, the values of SDRs and ISDRs were generally lower in the laboratory experiment than those in the rice field experiment, although the overall variation patterns remained nearly identical in the two experiments. The differences in SDRs and ISDRs detected between the rice field and laboratory experiments are most likely due to differences in the environment, where the outdoor conditions such as the temperatures and moistures in the rice field were much more fiercely fluctuated than those in the laboratory (see Section 2.2 in Materials and Methods). The environmental conditions in the field experiment are close to the rice production situation, which is more severe for weedy rice seeds to survive.

The reasons for weedy rice seeds expiring easily when they are exposed to soil surface conditions are likely due to several factors. First, the environmental conditions of soil surfaces or in very shallow layers of soil are much harsher and more fiercely fluctuate. For example, radical temperature and moisture changes usually cause greater losses of viability and depletions of shattered weedy rice seeds in soil seedbanks. This explanation gains supports from previously published studies for weedy rice and other weedy species [20,24,25]. Second, the oxygen concentration that determines the respiration and energy consumption of seeds can also play a critical role in determining the death and induced seed dormancy ratios of weedy rice seeds. It is reported that the survival capacity or longevity of weedy rice seeds can increase with gradually reduced oxygen concentration in deep soil where the weed seeds become less active [26–28]. Obviously, shattered weedy rice seeds stored in deep soil are relatively well protected under relatively milder and less fiercely fluctuating environmental conditions. Third, the survival of weedy seeds shattered to the ground can also be threatened by fungi or bacteria, and even animals, that are more active on the surface of soil [29,30], likely causing the high proportions of mortality for weedy rice seeds. As observed in this study, particularly in the natural rice field experiment, many seeds in the surface treatments became moldy and rotten during the seed burial experiment, resulting in extremely high SDRs.

Unexpectedly, results from the two soil burial experiments in the rice field and laboratory indicated very low ISDRs when weedy rice seeds were exposed to the soil surface (0 cm), although very low ISDRs were also detected in the deep soil-burial treatments (25 cm). Our results further showed the highest ISDRs of weedy rice seeds in the medium-depth soil burial treatment (10–15 cm). The low ISDRs detected for weedy rice seeds exposed to the soil surface are more likely due to the reasons that many seeds already died in the soil surface treatment (0 cm). It is difficult to identify whether the seeds died before or after dormancy was induced in this study. Nevertheless, the obtained results suggest that the fluctuation of environmental conditions, such as temperature, moisture, photoperiod, and oxygen concentration together play an important role in determining the viability and induced dormancy of weedy seeds in soil, as reported in previous studies [31,32]. That is the reason why weedy rice seeds survived poorly when exposed to the surface and shallow layers of the soil with much more fiercely fluctuating conditions.

### *4.2. Implications of Seed Survival Dynamics in Different Soil Depths for Weedy Rice Control and Management*

The survival or longevity of shattered weedy seeds in soil seedbanks in the rice field can greatly impact the magnitude of weedy rice infestation in the next or subsequent rice cultivation seasons [5,20,23]. Therefore, it is very important to understand the factors, including the physical locations of seeds in soil, which can determine the survival dynamics of weedy rice seeds stored in the soil seedbanks after the harvest of cultivated rice [20,23]. Results obtained from our seed burial experiments both in the rice field and laboratory in this study indicate a clear pattern of the survival dynamics of weedy rice seeds at different vertical positions or depths in soil seedbanks. Obviously, the survival dynamics of weedy rice seeds in soil seedbanks were substantially determined by the combined factors of the seed death ratios (SDRs) and induced seed dormancy ratios (ISDRs). In other words, the high SDRs combined with the low ISDRs together determine the low survival or viability of weedy rice seeds in soil seedbanks. Our findings regarding the high mortality of the seeds that are exposed to the surface or shallow layers of soil can be very useful to facilitate farmers' decision-marking or the design for weedy rice management of shattered seeds in soil seedbanks, as an important component of overall weed control strategies [5,15].

The general message from this study is that the maintenance of shattered weedy rice seeds on the soil surface or in shallow soil layers can significantly reduce their populations in soil seedbanks in the rice field. This message provides useful knowledge or a basis for the design of practical strategies for the efficient control of weedy rice in rice ecosystems. For example, farmers can apply the no-till system for rice cultivation, which will eradicate most of the shattered weedy rice seeds remaining on the surface or in shallow layers of soil in rice fields. Sometimes, farmers mainly focus more on the control of above-ground plants for weedy rice management and may only pay little attention to the fate of shattered weedy rice seeds in soil seedbanks. Consequently, the control of weedy rice in the rice field becomes exceedingly challenging if the focal point remains only weed plants that can easily be seen by farmers [15–17,23]. We propose that measures for the effective reduction of weedy rice seeds stored in soil seedbanks should be taken into serious consideration within the comprehensive strategies for weedy rice control. Undoubtedly, if a substantial quantity of viable weedy rice seeds persists in soil seedbanks, weedy rice infestation will become very severe in subsequent rice cultivation seasons [5,20]. Therefore, to manage the survival of weedy rice seeds, namely to significantly reduce and eradicate viable weedy rice seeds, is critical for the efficient control of weedy rice in the rice field.

As demonstrated in this study, the survival dynamics of weedy rice seeds in soil seedbanks are significantly determined by the combination of their SDRs and ISDRs. In other words, the minimum number of viable weedy rice seeds is determined by the combination of the highest seed mortality and the lowest induced seed dormancy ratios. In our experiments, the highest seed mortality and lowest induced seed dormancy were detected when weedy rice seeds were exposed to the surface or in the shallow layers of the soil. Therefore, we propose to maintain weedy rice seeds on the surface or in the shallow layers in the soil as an important component for comprehensive weed management strategies in the rice field. The generated knowledge from this study about maintaining weedy rice seeds on the surface or in shallow layers will provide a useful practice for reducing and eradicating viable seeds. We believe that the continued reduction of shattered weedy rice seeds, together with the effective control of the above-ground weedy plants using effective weeding measures, such as applying suitable herbicides and mechanical tools, can eliminate or substantially minimize weedy rice populations in following rice cultivation seasons.

The control practices of weedy rice seeds by maintaining shattered seeds on the soil surface or in shallow soil layers can easily be achieved through adopting appropriate rice farming systems [5,15,20,23]. In practice, there are serval choices of such rice farming systems to be considered. One of the practices is that farmers can adopt the no-tilling farming system for rice cultivation, which allows shattered weedy rice seeds to be maintained on the soil surface after rice is harvested [33,34]. Under such a circumstance, the shattered seeds may quickly expire, which is particularly true when environmental conditions more fiercely fluctuate. In addition, the strict practices of the no-tilling farming system will prevent the shattered weedy rice seeds from getting into the deep soil. However, results obtained from our experiments in this study suggested that weed rice seeds were much more viable in deep soil, which can easily be achieved by deep plough or tillage. Therefore, such farming practices (deep plough and tillage) will offer more opportunities for the shattered seeds to survive and remain viable in deep soil for a longer period [20]. If the plough or tillage of the field cannot be circumvented, farmers can adopt shallow plough or tillage to their rice fields just before the following crop seasons. This practice allows weedy rice seeds to remain in the shallow layers of the soil for a certain period, which achieves the high seed death ratios, in addition to the reduction of probabilities for induced secondary seed dormancy.

In addition to the reduction of viable seeds for weedy rice control, there are more benefits from the adoption of no-till farming in rice ecosystems. For example, no-till farming can conserve the agricultural field from soil erosion and restore soil fertility [33,34]. The no-till farming practice can also save time for the rotation of following crops, where the upcoming crops can be sown to the field slightly before cultivated rice completely matures [35], smoothing the crop rotation. Furthermore, the no-till farming system will block the shattered weedy rice seeds accumulated from different years in the deep soil seedbanks and they will never have opportunities to germinate. Therefore, we strongly recommend the no-till or shallow tillage farming system in rice ecosystems because of the efficient control of the viable weedy rice seeds as an important component for weedy rice control and other benefits. Our observation of quickly reduced lost seed viability when seeds are exposed to the soil surface in the crop field is probably not only limited to weedy rice, but also true for the shattered seeds of many other weedy species. In other words, the no-till farming system is probably useful for minimizing the viable seeds of many other weedy species stored in soil [20,24]. Therefore, no-till farming may likely provide a useful system for overall weed control in rice ecosystems, in addition to other benefits such as soil and water preservation, although evidence is needed from further studies.

## 5. Conclusions

In summary, we found based on our soil burial experiments in this study that weedy rice seeds exposed to the surface and shallow layers of soil exhibited extremely high death ratios and low induced dormancy ratios. The variation pattern of seed death and induced seed dormancy was essentially the same between the two independent experiments although values in the laboratory experiment were generally low. Given that the more fluctuating conditions in the natural rice field experiment are close to the realistic rice cultivation environment, knowledge generated from this study provides useful implications for the effective control of weedy rice. We therefore recommend to substantially minimize or eradicate weedy rice seeds in the seedbanks of rice fields to circumvent the extensive infestation of weedy rice in the following crop seasons. As an important component of the comprehensive strategy for weedy rice control, it is practicable to minimize or eradicate weedy rice seeds by adopting the no-till farming system, in which the shattered weedy seeds will rapidly lose their viability when exposed to the soil surface or in shallow layers of the soil. The no-till farming system may provide a useful solution for the comprehensive control of weedy rice and possibly also other weeds in rice fields, in addition to the benefits of preserving soil and water in agroecosystems.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14061281/s1, Table S1: Average germination and dormancy ratios of weedy rice seeds in different populations (each represented by three replicates) before soil burial experiments, Table S2: Duncan test on the seed death ratios and induced seed dormancy ratios of weedy rice populations from Jiangsu Province in China at different soil burial depths in the rice field experiment, Table S3: Duncan test on the seed death ratios and induced seed dormancy ratios of weedy rice populations from Jiangsu Province in China at different soil burial depths in the laboratory experiment.

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