

# Seed germination comparison between *japonica* and *indica* weedy rice from China

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## ABSTRACT

Weedy rice (*Oryza sativa* L. f. *spontanea* Roshev.), a pervasive weed in rice cultivation systems, exhibits significant genetic and phenotypic diversity, particularly between its two major subspecies-*japonica* and *indica* weedy rice. Seed germination characteristics play a critical role in the establishment, persistence, and competitive ability of weedy rice in agricultural ecosystems. This study aimed to compare the germination behavior of *japonica* and *indica* weedy rice under varying environmental conditions, including temperature, pH, NaCl stress, seed buried depth and moisture regimes. The combination of seed morphology comparison and PCR-based methods has enabled accurate identification of two subspecies of weedy rice. Among the ten populations selected for this study, only one was identified as *japonica* weedy rice. Seeds of both subspecies were subjected to controlled laboratory experiments to evaluate germination rates in response to environmental factors. Results revealed distinct differences between *japonica* and *indica* weedy rice, with *japonica* weedy rice exhibiting lower germination rates, while *indica* weedy rice demonstrated higher germination rates and adaptability to a broader range of conditions under all treatments. Weedy rice germination responses to environmental factors were population-specific, especially for *indica* weedy rice. Meanwhile, the germination behavior of *indica* weedy rice and *japonica* weedy rice often corresponded to that of *indica* cultivated rice and *japonica* cultivated rice, respectively. These findings suggest that weedy rice prioritizes rapid establishment to outcompete coexisting cultivated rice. The study highlights the importance of understanding subspecies-specific germination traits for developing targeted weed management strategies. The research provides valuable insights into the ecological success of weedy rice and offers practical implications for managing its impact on rice production systems.

**Key words:** Environmental factors, *japonica* and *indica* weedy rice, seed germination behavior, seeds morphology, subspecies identification.

## INTRODUCTION

Weedy rice (*Oryza sativa* L. f. *spontanea* Roshev.), also known as red rice, is a close relative of cultivated rice and a significant agricultural weed that poses a major threat to global rice production (Svizzero, 2021). The damage caused by weedy rice in paddy fields is more severe under dry direct seeding conditions compared to water direct seeding (Ushiki et al., 2005; Busi et al., 2017; Wang et al., 2019). Weedy rice exhibits remarkable genetic and phenotypic diversity, often mimicking cultivated rice in its early growth stages, which complicates its identification and management (Qiu et al., 2017). Phylogenetic and demographic analyses indicate that Chinese weedy rice underwent independent de-domestication from cultivated rice and experienced a strong genetic

bottleneck (Qiu et al., 2017). Its ability to compete aggressively for resources, adapt to diverse environments, e.g., drought and burial depth (Nadir et al., 2017), low temperature (Fukuda et al., 2023), hyperosmotic solutions (Wang et al., 2019), salt stress (Hakim et al., 2011), etc. The following persistence of weedy rice in agricultural systems has made it a challenging problem for farmers worldwide.

Among its many adaptive traits, seed germination characteristics play a critical role in its survival, spread, and persistence in agroecosystems (Zhang et al., 2025). Unlike cultivated rice, weedy rice often exhibits asynchronous germination, prolonged dormancy, and the ability to germinate under a wide range of environmental conditions. These traits enable it to evade control measures and persist in the soil seed bank for extended periods. The genetic basis of these traits has been studied extensively, revealing that weedy rice populations have diverse genetic backgrounds, often involving contributions from both cultivated and wild rice varieties (Sun et al., 2019). Additionally, seed shattering contributes to the persistence of the soil seed bank, which contains a massive reservoir of weedy rice seeds (Zhang et al., 2019).

In most cases, weedy rice can be broadly classified into two subspecies: *Indica* weedy rice and *japonica* weedy rice (Wang et al., 2019). These two subspecies differ in morphology and genetic background, leading to distinct performances in various traits. However, distinguishing between *indica* and *japonica* types of weedy rice can be challenging. Molecular and genetic markers provide the most accurate and reliable means of differentiating between *indica* and *japonica* types (Xu et al., 2012; Zhang et al., 2012). These methods are particularly useful when morphological traits overlap or are ambiguous. Several techniques can be employed to distinguish between these two types, including simple sequence repeats (SSR) markers, single nucleotide polymorphisms (SNP) markers and PCR-based assays (Ushiki et al., 2005; Xu et al., 2012).

The introduction of herbicide-resistant rice varieties, such as those resistant to imidazolinone herbicides, initially offered an effective approach to control weedy rice (Sudianto et al., 2013). These plants were designed to tolerate multiple herbicides, reducing the likelihood of resistance development and providing more effective control of weedy rice populations (Fartyal et al., 2018). However, the evolution of herbicide resistance in weedy rice has become a significant concern, as it could lead to increased weediness and persistence in rice fields. Studies have shown that weedy rice populations could evolve resistance through both target-site and non-target-site mechanisms, further complicating control measures (Yean et al., 2021). Moreover, gene flow from these resistant cultivars to weedy rice has led to the emergence of herbicide-resistant weedy rice populations, complicating management strategies and increasing production costs for farmers (Chen, 2004; Shivrain et al., 2009; Busconi et al., 2012; Merotto et al., 2016; Dauer et al., 2017). This resistance not only undermines the efficacy of herbicides but also poses a threat to biodiversity and the economic stability of rice farming communities (Merotto et al., 2016).

Therefore, understanding the germination behavior of *indica* and *japonica* weedy rice is essential for developing effective management strategies and mitigating its impact on rice cultivation. Comparative studies on the germination characteristics of its various subspecies remain limited. This study aims to systematically compare the germination differences between *indica* and *japonica* weedy rice under multiple environmental stresses such as temperature, salinity, pH, moisture and burial depth, and to explore their corresponding relationship with cultivated rice. The findings of this study will enhance our understanding of weedy rice ecology and support sustainable rice production systems in the face of this persistent weed threat.

## MATERIALS AND METHODS

### Plant materials

In 2022, seeds from ten weedy rice (*Oryza sativa* L. f. *spontanea* Roshev.) populations distributed across various districts in Shanghai, China, were collected (Table 1). Two rice cultivars, *japonica* rice 'Nipponbare' and *indica* rice '9311', were used as comparison in this study. All seeds were cultivated and propagated in 2023 to obtain the new seeds with consistent quality. Decayed and underdeveloped seeds were removed, and viable seeds were used for subsequent experiments in 2024.

**Table 1.** Collection information of ten weedy rice populations.

Populations	Collection site	Location
PDSY	Shuyuan village, Pudong districts	121°51'59.083" E, 30°55'40.537" N
FXYX	Yongxin village, Fengxian districts	121°26'25.994" E, 30°51'9.526" N
JSYH	Yuhui village, Jinshan districts	121°0'19.940" E, 30°54'17.885" N
JDCJ	Changjing village, Jiading districts	121°9'48.676" E, 31°20'38.468" N
QPAG	Aiguo village, Qingpu districts	121°0'6.599" E, 31°2'26.426" N
MHLX	Lianxing village, Minhang districts	121°33'3.388" E, 31°5'37.558" N
BSSF	Sifang village, Baoshan districts	121°20'21.048" E, 31°25'45.977" N
PDRY	Renyi village, Pudong districts	121°38'26.761" E, 31°3'30.560" N
CMCJ	Changjiang Farm, Chongming districts	121°32'14.932" E, 31°40'22.519" N
FXZH	Zhuanghang town, Fengxian districts	121°23'5.50" E, 30°53'20.82" N

### Seeds morphology comparison and subspecies identification of weedy rice

**Seeds morphology comparison.** Among the ten weedy rice populations, only one population (FXYX) exhibited distinct seed morphology compared to the others. Two representative populations, FXYX and FXZH, were selected for seed morphology comparison, with 'Nipponbare' and '9311' used as references. The morphology of seeds with and without hulls was photographed. Additionally, the 1000-seed weight of four populations was measured. A completely random block design was adopted. Each group was set with four replicates, and 1000 seeds were independently counted for each replicate. Weigh and record using an electronic analytical balance with an accuracy of 0.01 g. Make sure the seeds are balanced to room temperature in the desiccator before weighing.

**Subspecies identification.** Polymerase chain reaction (PCR)-based methods were employed to identify the subspecies of weedy rice (Shen et al., 2004; Chen et al., 2020). First, *Pst I-12* gene fragment was used to distinguish *indica* rice and *japonica* rice. A 69 bases-deletions in the open reading frame (ORF) of the *indica* rice (137 bp) was detected by specific primers, compared to *japonica* rice (206 bp) (Chen et al., 2020). The PCR methods were conducted as previously described (Chen et al., 2020).

### *Indica* and *japonica* indices determination

Although the accuracy of this method exceeds 90% (Chen et al., 2020), further experiments were conducted to confirm the accurate subspecies. Next, Insertion/Deletions (InDels) markers were used to determine the precise index of *indica* or *japonica*. Based on the previous report and preliminary screening, 41 pairs of InDels primers that produced stable PCR amplification with clear electrophoretic bands were selected for subspecies identification (Shen et al., 2004). The DNA extraction was performed using a Plant Genomic DNA kit (Tiangen Biotech, Beijing, China), following the manufacturer's instructions. The PCR mixture contained 0.5 ng template DNA, 0.4 µL each primer (10 µM), 5 µL 2×Rapid taq Master Mix (Vazyme Biotech, Nanjing, China), and ddH<sub>2</sub>O to a final volume of 10 µL. Amplification was conducted as follows: 5 min at 95 °C for DNA denaturation; 35 cycles of 30 s at 95 °C for DNA denaturation, 30 s at 60 °C for annealing, and 30 s at 72 °C for DNA elongation; and a final elongation for 7 min at 72 °C. The PCR products were separated by 1% agarose gel electrophoresis and visualized under ultraviolet light. The band patterns of each population were recorded.

Samples with band positions consistent with 9311 at each InDel locus were classified as homozygous *indica* (II); those consistent with 'Nipponbare' were classified as homozygous *japonica* (JJ); and those with bands matching both 'Nipponbare' and '9311' were classified as hybrid *indica-japonica* (IJ). The *indica* and *japonica* indices were calculated using the following equations:

$$\begin{aligned} \textit{indica} \text{ index} &= (\text{Numbers of II} \times 2 + \text{Numbers of IJ} \times 1) / 82 \times 100\%; \\ \textit{japonica} \text{ index} &= (\text{Numbers of JJ} \times 2 + \text{Numbers of IJ} \times 1) / 82 \times 100\%. \end{aligned}$$

### Evaluating the effect of environmental factors on seed germination of weedy rice

For burial depth and soil moisture content assays, a soil cultivation method was used, where seeds were cultivated in pure soil (clay, pH = 7.3). For other environmental factors, the Petri dish method was employed. Sodium chloride (NaCl), polyethylene glycol 6000 (PEG6000), HCl, and NaOH (analytical grade) were purchased

from Sinopharm Reagent, Shanghai, China. Four replicates were used for each treatment, and all tests were repeated twice. For the soil cultivation method, the number of germinated seeds was recorded at 5 and 10 d after treatment; for the Petri dish method, germination was recorded at 3 and 7 d after treatment.

Temperature conditions for seed germination assays. Twenty-five seeds per replicate for each population were evenly placed in Petri dish ( $\varnothing$  9 cm  $\times$  1.5 cm) lined with two layers of filter paper lined with two layers of filter paper. Distilled water (pH = 6.50) was added (10 mL) every 2 d to maintain moisture. Petri dishes were incubated under white fluorescent lamps (photosynthetic photon flux density of  $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at alternating temperatures of 5/10 °C, 10/15 °C, 15/20 °C, 20/25 °C, 25/30 °C, and 30/35 °C in 12/12 h photoperiod. The optimal temperature for germination was determined to be 25/30 °C in 12/12 h photoperiod, and this condition was used for subsequent experiments.

NaCl stress for seed germination assays. NaCl was dissolved in the aforementioned distilled water to assess the effects of salt stress on seed germination. The NaCl concentrations were set at 17.1, 34.2, 51.3, 68.4, 102.6 and 136.8 mM. Other culture conditions were consistent with the previous description.

The pH conditions for seed germination assays. The pH of distilled water was adjusted to 3.50, 4.50, 5.50, 6.50, 7.50, 8.50, and 9.50 using previously described methods (Chachalis and Reddy, 2000). Other culture conditions were consistent with the previous description.

Osmotic stress for seed germination assays. The water potential was calculated using the following equation (Michel and Kaufmann, 1973):

$$\text{Water potential} = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T$$

where T is 27.5 °C and C is concentration of PEG6000 ( $\text{g L}^{-1}$  distilled water). Six treatments were conducted using PEG6000 concentrations of 50, 100, 150, 200, 250, and 300  $\text{g L}^{-1}$ , corresponding to osmotic potentials of -0.05, -0.14, -0.28, -0.47, -0.70, and -0.99 MPa, respectively. Other culture conditions were consistent with previous description.

Soil moisture content for seed germination assays. A total of 100 g pure soil was placed in plastic cups ( $\varnothing$  7 cm  $\times$  10 cm). Water was added in increments of 10, 20, 30, 40, and 50 g, resulting in soil moisture contents of 10%, 20%, 30%, 40%, and 50%, respectively. Fifteen seeds per replicate were placed on the soil surface, and the cups were sealed with Parafilm to conserve moisture. Other culture conditions were consistent with previous description.

Burial depth of seeds for seed germination assays. The effect of burial depth on seed germination was tested in plastic pots (7 cm  $\times$  7 cm  $\times$  12 cm). Seeds were buried at depths of 10, 7.5, 5.0, 2.5, and 0 cm. Adequate water was supplied from the bottom of the pots to ensure normal germination. Other culture conditions were consistent with previous description.

## Data analysis

The seed germination rate was calculated as:

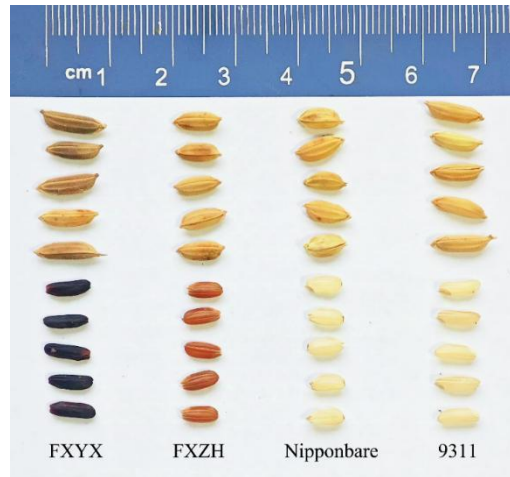
$$\text{Germination rate (\%)} = (\text{Number of germinated seeds}/25) \times 100\%.$$

Average germination rates for each population and the 1000-seed weight of the four populations were analyzed for significance using SPSS Statistics 27.0 (IBM, Armonk, New York, USA) with Duncan's test ( $P = 0.05$ ). Nonsignificant differences were observed between the two repeated experiments, and the results showed consistent trends.

## RESULTS

### Morphological difference between *indica* and *japonica* weedy rice

Figure 1 illustrates the differences in seed color and shape between representative weedy rice and cultivated rice populations. The hull color of the weedy rice populations (FXYX and FXZH) appeared darker than that of the cultivated rice ('Nipponbare' and '9311'), particularly for the FXYX population. Without the hull, the seed color of the FXYX population was dark red or nearly black, while the FXZH population was red. In contrast, the cultivated rice seeds were faint yellow. The seed shape of the FXYX population was longer and slenderer, showing partial similarity to '9311', whereas the FXZH population was shorter and rounder, closely resembling 'Nipponbare'.



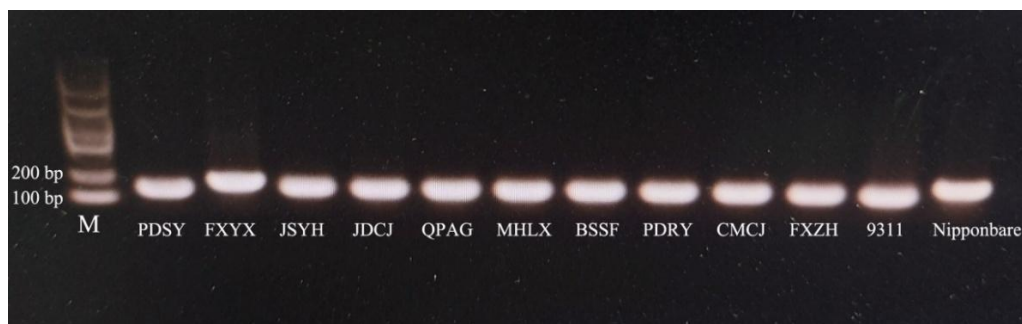
**Figure 1.** Seed morphology comparison of representative weedy rice populations and cultivated rice populations.

The 1000-seed weights for FXYX, FXZH, 'Nipponbare', and '9311' were 25.19, 23.31, 26.03, and 29.03 g, respectively. Significant differences ( $P < 0.05$ ) were detected among these four populations.

#### Accurate classification of the subspecies in weedy rice

Figure 2 shows that the PCR products of all cultivated and weedy rice populations produced single, distinct bands. Among these, only the bands for FXYX and 'Nipponbare' were longer (over 200 bp) compared to the other weedy rice populations and '9311' (approximately 130 bp). The PCR results confirmed that the FXYX population was *japonica* weedy rice, while the remaining weedy rice populations were *indica* weedy rice.

Further analysis using InDel markers provided detailed subspecies indices for the weedy rice populations. The selected 41 pairs of primers did not produce bands indicative of hybrid *indica* and *japonica* types; all PCR products were single bands. The band patterns were analyzed compared to 'Nipponbare' and '9311'. The numbers of II (homozygous *indica*) and JJ (homozygous *japonica*) types, along with the corresponding *indica* and *japonica* indices, are presented in Table 2. The results confirmed that 'Nipponbare' and '9311' were standard *japonica* (100.00% *japonica* index) and *indica* (100.00% *indica* index) rice, respectively. The FXYX population was identified as a typical *japonica* weedy rice with a high *japonica* index (95.12%), while the other populations were classified as *indica* weedy rice with *indica* indices ranging from 78.05% to 95.12%.



**Figure 2.** Agarose gel electrophoresis of *Pst I-12* gene fragments in different weedy rice and cultivated rice populations. Only the bands for FXYX and 'Nipponbare' were longer (over 200 bp) compared to the other weedy rice populations and '9311' (approximately 130 bp), indicated the FXYX population was *japonica* weedy rice, while the remaining weedy rice populations were *indica* weedy rice. Lanes show DNA fragments amplified from representative samples. M: DL2000 Plus DNA Marker.

**Table 2.** Index of *indica* and *japonica* in weedy rice and cultivated rice.

Populations	Homozygous <i>indica</i>		Homozygous <i>japonica</i>		Hybrid subspecies of <i>indica</i> and <i>japonica</i> (IJ)	Index of <i>indica japonica</i>	
	subspecies (II)	Nr	subspecies (JJ)	Nr		%	%
PDSY	33	33	8	8	0	80.49	19.51
FXYX	2	2	39	39	0	4.88	95.12
JSYH	33	33	8	8	0	80.49	19.51
JDCJ	39	39	2	2	0	95.12	4.88
QPAG	34	34	7	7	0	82.93	17.07
MHLX	34	34	7	7	0	82.93	17.07
BSSF	36	36	5	5	0	87.80	12.20
PDRY	32	32	9	9	0	78.05	21.95
CMCJ	35	35	6	6	0	85.37	14.63
FXZH	39	39	2	2	0	95.12	4.88
9311	41	41	0	0	0	100.00	0.00
Nipponbare	0	0	41	41	0	0.00	100.00

**Indica weedy rice germinated better than *japonica* weedy rice under multiple environmental factors**

The *indica* weedy rice displayed stronger germinate ability than *japonica* weedy rice under different temperature setting. The effect of temperature on the seed germination in cultivated rice and weedy rice is shown in Table 3. Under the optimal temperature setting of 25/30 °C with a 12/12 h photoperiod, nine out of ten populations exhibited 100% seed germination rates 7 d after treatment, which was significant higher compared to the FXYX (96%) populations. At lower temperatures (10-15 °C and 5-10 °C), few weedy rice seeds germinated by 3 d after treatment. Across almost all temperature treatments, the *japonica* weedy rice population (FXYX) showed the lowest germination rate compared to the nine *indica* weedy rice populations. A similar trend was observed in cultivated rice, where the germination rate of *japonica* rice ('Nipponbare') was significantly lower than that of *indica* rice ('9311'). In summary, the germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different temperature treatments.

**Table 3.** Seed germination rates of different types of weedy rice under different temperature. Values are shown as means of three replicates and the standard error is shown in the brackets; different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

Temperature conditions	Seed germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
30-35 °C	98 <sup>a</sup>	76 <sup>f</sup>	98 <sup>a</sup>	99 <sup>a</sup>	85 <sup>cd</sup>	97 <sup>a</sup>	92 <sup>b</sup>	100 <sup>a</sup>	89 <sup>bc</sup>	89 <sup>bc</sup>	81 <sup>e</sup>	73 <sup>f</sup>
3 d	(1.15)	(2.31)	(1.15)	(1.00)	(1.91)	(1.00)	(1.63)	(0)	(1.00)	(1.00)	(1.00)	(1.00)
30-35 °C	100 <sup>a</sup>	92 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	99 <sup>a</sup>	100 <sup>a</sup>	98 <sup>a</sup>	84 <sup>c</sup>
7 d	(0.00)	(0.00)	(0)	(0)	(0)	(0)	(0)	(0.00)	(1.00)	(0)	(1.15)	(2.31)
25-30 °C	95 <sup>b</sup>	76 <sup>d</sup>	96 <sup>b</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	96 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	91 <sup>c</sup>	100 <sup>a</sup>	95 <sup>b</sup>	91 <sup>c</sup>
3 d	(1.00)	(0.00)	(0.00)	(0)	(1.15)	(0.00)	(0)	(0)	(1.91)	(0)	(1.91)	(1.91)
25-30 °C	100 <sup>a</sup>	96 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	93 <sup>c</sup>
7 d	(0)	(1.63)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.91)
20-25 °C	91 <sup>abc</sup>	43 <sup>e</sup>	96 <sup>ab</sup>	90 <sup>bc</sup>	88 <sup>c</sup>	91 <sup>abc</sup>	97 <sup>a</sup>	74 <sup>d</sup>	63 <sup>e</sup>	90 <sup>bc</sup>	89 <sup>c</sup>	56 <sup>f</sup>
3 d	(1.91)	(1.91)	(0)	(2.00)	(1.63)	(3.00)	(1.91)	(3.83)	(1.00)	(2.00)	(1.00)	(2.31)
20-25 °C	96 <sup>ab</sup>	81 <sup>d</sup>	100 <sup>a</sup>	97 <sup>a</sup>	96 <sup>ab</sup>	100 <sup>a</sup>	100 <sup>a</sup>	96 <sup>ab</sup>	96 <sup>ab</sup>	97 <sup>a</sup>	97 <sup>a</sup>	92 <sup>bc</sup>
7 d	(1.63)	(1.00)	(0)	(1.00)	(1.63)	(0)	(0)	(2.31)	(2.31)	(1.00)	(1.00)	(2.31)
15-20 °C	49 <sup>c</sup>	9 <sup>e</sup>	68 <sup>b</sup>	39 <sup>d</sup>	34 <sup>e</sup>	16 <sup>f</sup>	51 <sup>c</sup>	16 <sup>f</sup>	16 <sup>f</sup>	80 <sup>a</sup>	67 <sup>b</sup>	3 <sup>h</sup>
3 d	(1.00)	(1.00)	(2.31)	(2.51)	(1.15)	(0)	(1.00)	(0)	(0)	(0)	(1.00)	(1.00)
15-20 °C	98 <sup>ab</sup>	76 <sup>d</sup>	100 <sup>a</sup>	90 <sup>c</sup>	95 <sup>abc</sup>	97 <sup>ab</sup>	96 <sup>ab</sup>	94 <sup>bc</sup>	67 <sup>e</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	90 <sup>c</sup>
7 d	(1.15)	(2.31)	(0)	(2.00)	(1.00)	(1.00)	(1.63)	(2.00)	(2.51)	(1.00)	(1.00)	(2.00)
10-15 °C	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	43 <sup>a</sup>	2 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	15 <sup>c</sup>	20 <sup>b</sup>	0 <sup>d</sup>
3 d	(0)	(0)	(0)	(1.91)	(1.15)	(0)	(0)	(0)	(0)	(1.91)	(1.63)	(0)
10-15 °C	41 <sup>d</sup>	19 <sup>e</sup>	41 <sup>d</sup>	83 <sup>a</sup>	50 <sup>c</sup>	7 <sup>f</sup>	47 <sup>c</sup>	48 <sup>c</sup>	18 <sup>e</sup>	82 <sup>a</sup>	75 <sup>b</sup>	46 <sup>c</sup>
7 d	(1.91)	(1.00)	(1.00)	(1.91)	(1.15)	(1.00)	(1.00)	(1.63)	(2.58)	(1.15)	(1.00)	(1.15)
5-10 °C	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	12 <sup>a</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	13 <sup>a</sup>	9 <sup>b</sup>	0 <sup>c</sup>
3 d	(0)	(0)	(0)	(1.63)	(0)	(0)	(0)	(0)	(0)	(1.91)	(1.00)	(0)
5-10 °C	12 <sup>bc</sup>	0 <sup>d</sup>	0 <sup>d</sup>	21 <sup>a</sup>	13 <sup>bc</sup>	0 <sup>d</sup>	10 <sup>bc</sup>	9 <sup>c</sup>	3 <sup>d</sup>	19 <sup>a</sup>	14 <sup>b</sup>	9 <sup>c</sup>
7 d	(1.63)	(0)	(2.22)	(1.00)	(2.52)	(0)	(1.15)	(1.00)	(1.91)	(1.00)	(1.15)	(2.52)

### ***Indica* weedy rice displayed stronger tolerance to NaCl treatment**

The seed germination rate of all populations decreased with increasing NaCl concentration (Table 4). Weedy rice exhibited high NaCl tolerance, with germination rates reaching 80% under 136.8 mM NaCl. Across almost all NaCl stress treatments, the *japonica* weedy rice population (FXYX) showed the lowest germination rate compared to the nine *indica* weedy rice populations. A similar pattern was observed in cultivated rice, where the germination rate of *japonica* rice ('Nipponbare') was significantly lower than that of *indica* rice ('9311'). The germination rate of *indica* rice '9311' was comparable to that of *indica* weedy rice under different NaCl concentrations, highlighting the strong germination rate of *indica* weedy rice. Unlike the results from temperature treatments, the *japonica* weedy rice population (FXYX) displayed a significantly higher germination rate than *japonica* rice ('Nipponbare') under NaCl stress. In summary, the germination rate under NaCl stress, in descending order, was: *indica* rice and *indica* weedy rice > *japonica* weedy rice > *japonica* rice.

**Table 4.** Seed germination rates of different types of weedy rice under different NaCl concentrations. Values are shown as means of three replicates and the standard error is shown in the brackets; different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

NaCl treatment	Germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
17.1 mM	100 <sup>a</sup>	82 <sup>e</sup>	98 <sup>ab</sup>	95 <sup>abc</sup>	95 <sup>abc</sup>	97 <sup>ab</sup>	88 <sup>d</sup>	93 <sup>bc</sup>	91 <sup>cd</sup>	100 <sup>a</sup>	95 <sup>abc</sup>	70 <sup>f</sup>
3 d	(0)	(2.00)	(1.15)	(1.00)	(2.51)	(1.00)	(2.83)	(1.00)	(11.91)	(0)	(1.00)	(1.15)
17.1 mM	100 <sup>a</sup>	96 <sup>ab</sup>	100 <sup>a</sup>	98 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	92 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	88 <sup>c</sup>
7 d	(0)	(0)	(0)	(1.15)	(0)	(0)	(2.83)	(0)	(0)	(0)	(0)	(3.26)
34.2 mM	97 <sup>ab</sup>	79 <sup>d</sup>	95 <sup>abc</sup>	90 <sup>c</sup>	92 <sup>bc</sup>	97 <sup>ab</sup>	82 <sup>d</sup>	91 <sup>c</sup>	84 <sup>d</sup>	100 <sup>a</sup>	95 <sup>abc</sup>	52 <sup>e</sup>
3 d	(1.00)	(1.00)	(1.00)	(2.00)	(2.31)	(1.00)	(1.15)	(3.00)	(2.31)	(0)	(1.91)	(2.31)
34.2 mM	100 <sup>a</sup>	93 <sup>ab</sup>	100 <sup>a</sup>	98 <sup>a</sup>	98 <sup>a</sup>	98 <sup>a</sup>	93 <sup>ab</sup>	97 <sup>a</sup>	91 <sup>ab</sup>	100 <sup>a</sup>	98 <sup>a</sup>	83 <sup>b</sup>
7 d	(0)	(1.91)	(0)	(2.00)	(2.00)	(2.00)	(3.00)	(3.00)	(1.00)	(0)	(1.15)	(1.00)
51.3 mM	93 <sup>b</sup>	74 <sup>e</sup>	92 <sup>b</sup>	91 <sup>b</sup>	93 <sup>b</sup>	89 <sup>bc</sup>	81 <sup>d</sup>	84 <sup>cd</sup>	82 <sup>d</sup>	100 <sup>a</sup>	95 <sup>ab</sup>	57 <sup>f</sup>
3 d	(1.91)	(2.00)	(2.31)	(1.91)	(1.31)	(4.12)	(1.00)	(2.31)	(1.15)	(0)	(1.00)	(1.91)
51.3 mM	97 <sup>ab</sup>	90 <sup>bcd</sup>	100 <sup>a</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	93 <sup>abcd</sup>	90 <sup>bcd</sup>	89 <sup>cd</sup>	87 <sup>de</sup>	100 <sup>a</sup>	95 <sup>abc</sup>	81 <sup>e</sup>
7 d	(1.00)	(3.83)	(0)	(1.00)	(1.00)	(2.52)	(3.83)	(1.00)	(3.00)	(0)	(1.00)	(3.00)
68.4 mM	90 <sup>b</sup>	66 <sup>e</sup>	86 <sup>bc</sup>	91 <sup>b</sup>	86 <sup>bc</sup>	89 <sup>b</sup>	77 <sup>d</sup>	82 <sup>cd</sup>	80 <sup>d</sup>	100 <sup>a</sup>	90 <sup>b</sup>	53 <sup>f</sup>
3 d	(1.15)	(2.00)	(1.15)	(1.91)	(3.46)	(1.91)	(1.91)	(2.00)	(1.63)	(0)	(1.15)	(1.00)
68.4 mM	97 <sup>ab</sup>	82 <sup>d</sup>	95 <sup>ab</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	92 <sup>bc</sup>	87 <sup>cd</sup>	84 <sup>d</sup>	84 <sup>d</sup>	100 <sup>a</sup>	93 <sup>b</sup>	74 <sup>e</sup>
7 d	(1.00)	(3.46)	(1.00)	(1.00)	(1.00)	(2.31)	(2.52)	(2.31)	(1.63)	(0)	(1.91)	(1.15)
102.6 mM	86 <sup>b</sup>	61 <sup>e</sup>	81 <sup>c</sup>	86 <sup>b</sup>	86 <sup>b</sup>	86 <sup>b</sup>	74 <sup>d</sup>	81 <sup>c</sup>	47 <sup>g</sup>	98 <sup>a</sup>	82 <sup>bc</sup>	53 <sup>f</sup>
3 d	(3.46)	(1.00)	(1.00)	(1.15)	(1.15)	(1.15)	(1.15)	(1.00)	(1.00)	(1.15)	(1.15)	(1.00)
102.6 mM	91 <sup>b</sup>	82 <sup>d</sup>	95 <sup>ab</sup>	90 <sup>bc</sup>	94 <sup>ab</sup>	89 <sup>bc</sup>	84 <sup>cd</sup>	84 <sup>cd</sup>	73 <sup>e</sup>	98 <sup>a</sup>	84 <sup>cd</sup>	69 <sup>e</sup>
7 d	(1.91)	(1.15)	(1.00)	(2.58)	(2.58)	(1.00)	(2.83)	(1.63)	(2.52)	(1.15)	(2.31)	(1.00)
136.8 mM	84 <sup>b</sup>	59 <sup>d</sup>	77 <sup>c</sup>	85 <sup>ab</sup>	74 <sup>c</sup>	74 <sup>c</sup>	73 <sup>c</sup>	75 <sup>c</sup>	41 <sup>f</sup>	89 <sup>a</sup>	77 <sup>c</sup>	47 <sup>e</sup>
3 d	(1.63)	(1.00)	(1.91)	(1.00)	(1.15)	(1.15)	(1.00)	(1.91)	(1.00)	(1.00)	(1.91)	(1.91)
136.8 mM	87 <sup>bc</sup>	81 <sup>ab</sup>	86 <sup>c</sup>	90 <sup>b</sup>	90 <sup>b</sup>	85 <sup>cd</sup>	82 <sup>d</sup>	81 <sup>d</sup>	70 <sup>e</sup>	94 <sup>a</sup>	84 <sup>cd</sup>	57 <sup>f</sup>
7 d	(1.00)	(1.00)	(1.15)	(1.15)	(1.15)	(1.00)	(1.15)	(1.00)	(2.00)	(1.15)	(2.31)	(1.00)

### ***Indica* weedy rice had a higher germination rate than *japonica* under different pH conditions**

Weedy rice demonstrated strong adaptability to a wide range of pH conditions (3.5 to 9.5), although extreme acidic and alkaline conditions inhibited seed germination (Table 5). The optimal pH for seed germination was 6.5. The germination rate of weedy rice and cultivated rice under different pH conditions followed a similar trend to that observed under different temperature treatments. Specifically, the *japonica* weedy rice population (FXYX) exhibited a lower germination rate compared to the *indica* weedy rice populations; the germination rate of *japonica* cultivated rice ('Nipponbare') was significantly lower than that of *indica* cultivated rice ('9311'); the germination rate of *indica* cultivated rice ('9311') was comparable to that of *indica* weedy rice under different pH conditions, reflecting the strong germination rate of *indica* weedy rice; the *japonica* weedy rice population (FXYX) displayed a germination rate similar to that of *japonica* cultivated rice ('Nipponbare'). In summary, the germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different pH treatments. Nonsignificant difference in germination rate was detected between cultivated rice and weedy rice belonging to the same subspecies.

**Table 5.** Seed germination rates of different types of weedy rice under different pH conditions. Values are shown as means of three replicates and the standard error is shown in the brackets; the different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

pH	Germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
3.50	66 <sup>d</sup>	30 <sup>f</sup>	54 <sup>e</sup>	62 <sup>d</sup>	76 <sup>c</sup>	75 <sup>c</sup>	54 <sup>e</sup>	92 <sup>a</sup>	51 <sup>e</sup>	75 <sup>c</sup>	85 <sup>b</sup>	53 <sup>e</sup>
3 d	(1.15)	(1.15)	(2.00)	(1.15)	(1.63)	(1.91)	(1.15)	(1.63)	(1.91)	(1.00)	(1.91)	(1.91)
3.50	82 <sup>c</sup>	74 <sup>de</sup>	72 <sup>e</sup>	70 <sup>e</sup>	83 <sup>c</sup>	86 <sup>bc</sup>	77 <sup>d</sup>	93 <sup>a</sup>	70 <sup>e</sup>	83 <sup>c</sup>	89 <sup>ab</sup>	75 <sup>de</sup>
7 d	(1.15)	(3.46)	(1.63)	(1.15)	(1.00)	(1.15)	(1.91)	(1.00)	(1.15)	(1.00)	(1.00)	(1.91)
4.50	91 <sup>abc</sup>	59 <sup>g</sup>	93 <sup>a</sup>	85 <sup>de</sup>	87 <sup>cd</sup>	82 <sup>e</sup>	87 <sup>cd</sup>	95 <sup>a</sup>	81 <sup>e</sup>	92 <sup>ab</sup>	88 <sup>bcd</sup>	65 <sup>f</sup>
3 d	(1.00)	(1.91)	(1.00)	(1.00)	(1.00)	(1.15)	(1.00)	(2.52)	(1.00)	(1.63)	(1.63)	(1.91)
4.50	97 <sup>a</sup>	87 <sup>bc</sup>	95 <sup>a</sup>	93 <sup>a</sup>	95 <sup>a</sup>	86 <sup>bc</sup>	91 <sup>ab</sup>	97 <sup>a</sup>	93 <sup>a</sup>	97 <sup>a</sup>	93 <sup>a</sup>	83 <sup>c</sup>
7 d	(1.91)	(1.00)	(1.91)	(2.51)	(1.91)	(1.15)	(1.00)	(1.91)	(2.51)	(1.91)	(1.91)	(1.00)
5.50	95 <sup>ab</sup>	68 <sup>d</sup>	95 <sup>ab</sup>	95 <sup>ab</sup>	91 <sup>b</sup>	91 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	82 <sup>c</sup>	94 <sup>b</sup>	95 <sup>ab</sup>	68 <sup>d</sup>
3 d	(1.00)	(1.63)	(1.00)	(1.00)	(1.91)	(3.00)	(0)	(0)	(1.15)	(2.00)	(1.00)	(1.63)
5.50	97 <sup>ab</sup>	89 <sup>c</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	97 <sup>ab</sup>	100 <sup>a</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	99 <sup>a</sup>	95 <sup>b</sup>	83 <sup>d</sup>
7 d	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.91)	(0)	(0)	(2.00)	(1.00)	(1.00)	(1.00)
6.50	96 <sup>a</sup>	82 <sup>c</sup>	96 <sup>a</sup>	95 <sup>a</sup>	98 <sup>a</sup>	96 <sup>a</sup>	97 <sup>a</sup>	97 <sup>a</sup>	90 <sup>b</sup>	95 <sup>a</sup>	95 <sup>a</sup>	85 <sup>c</sup>
3 d	(1.63)	(1.15)	(1.63)	(1.00)	(2.00)	(1.63)	(1.91)	(1.00)	(1.15)	(1.91)	(1.00)	(1.00)
6.50	98 <sup>a</sup>	94 <sup>b</sup>	97 <sup>a</sup>	98 <sup>a</sup>	98 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	98 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	93 <sup>b</sup>
7 d	(1.15)	(1.15)	(1.00)	(1.15)	(1.15)	(0)	(0)	(0)	(1.15)	(0)	(0)	(1.91)
7.50	96 <sup>ab</sup>	72 <sup>e</sup>	94 <sup>b</sup>	90 <sup>c</sup>	93 <sup>bc</sup>	95 <sup>ab</sup>	93 <sup>bc</sup>	98 <sup>a</sup>	83 <sup>d</sup>	95 <sup>ab</sup>	95 <sup>ab</sup>	58 <sup>f</sup>
3 d	(0.00)	(1.63)	(1.15)	(1.15)	(1.00)	(1.00)	(1.00)	(1.15)	(1.00)	(1.00)	(1.00)	(1.15)
7.50	98 <sup>ab</sup>	87 <sup>c</sup>	97 <sup>a</sup>	97 <sup>ab</sup>	95 <sup>b</sup>	100 <sup>a</sup>	96 <sup>b</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	85 <sup>c</sup>
7 d	(1.15)	(1.00)	(1.00)	(1.00)	(1.00)	(0)	(1.63)	(0)	(1.15)	(0)	(1.15)	(1.00)
8.50	95 <sup>a</sup>	66 <sup>d</sup>	88 <sup>bc</sup>	87 <sup>c</sup>	93 <sup>a</sup>	93 <sup>a</sup>	93 <sup>a</sup>	94 <sup>a</sup>	63 <sup>d</sup>	94 <sup>a</sup>	92 <sup>ab</sup>	49 <sup>e</sup>
3 d	(1.00)	(1.15)	(2.83)	(3.00)	(1.00)	(1.00)	(1.00)	(1.15)	(1.00)	(1.15)	(0)	(1.00)
8.50	96 <sup>ab</sup>	81 <sup>d</sup>	93 <sup>b</sup>	98 <sup>a</sup>	93 <sup>b</sup>	98 <sup>a</sup>	96 <sup>ab</sup>	98 <sup>a</sup>	87 <sup>c</sup>	98 <sup>a</sup>	95 <sup>ab</sup>	77 <sup>d</sup>
7 d	(0)	(1.00)	(1.00)	(1.15)	(1.00)	(1.15)	(1.63)	(1.15)	(3.00)	(1.15)	(1.00)	(1.91)
9.50	85 <sup>cd</sup>	57 <sup>f</sup>	84 <sup>cd</sup>	86 <sup>bcd</sup>	84 <sup>cd</sup>	81 <sup>d</sup>	93 <sup>a</sup>	88 <sup>abc</sup>	62 <sup>e</sup>	91 <sup>ab</sup>	84 <sup>cd</sup>	48 <sup>g</sup>
3 d	(1.00)	(1.00)	(1.63)	(2.00)	(1.63)	(1.00)	(1.91)	(1.63)	(2.00)	(1.91)	(2.31)	(1.63)
9.50	89 <sup>bcd</sup>	70 <sup>f</sup>	86 <sup>d</sup>	91 <sup>bc</sup>	91 <sup>bc</sup>	85 <sup>d</sup>	96 <sup>a</sup>	92 <sup>ab</sup>	75 <sup>e</sup>	93 <sup>ab</sup>	87 <sup>cd</sup>	73 <sup>ef</sup>
7 d	(1.00)	(1.15)	(1.15)	(1.91)	(1.00)	(1.91)	(1.63)	(2.31)	(1.91)	(1.91)	(1.91)	(1.00)

#### ***Indica* weedy rice had a higher germination rate than *japonica* under water potential stress**

The seed germination rate of all populations decreased with increasing intensity of osmotic stress (Table 6). The germination rate of weedy rice and cultivated rice under different osmotic stress conditions followed a similar trend to that observed under different temperature and pH treatments. Specifically, the *japonica* weedy rice population (FXYX) exhibited a lower germination rate compared to the *indica* weedy rice populations; the germination rate of *japonica* cultivated rice ('Nipponbare') was significantly lower than that of *indica* cultivated rice ('9311'); the germination rate of *indica* cultivated rice ('9311') was comparable to that of *indica* weedy rice under different osmotic stress conditions, reflecting the strong germination rate of *indica* weedy rice; the *japonica* weedy rice population (FXYX) displayed a germination rate similar to that of *japonica* cultivated rice ('Nipponbare'). In summary, the germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different osmotic stress conditions. Nonsignificant difference in germination rate was detected between cultivated rice and weedy rice belonging to the same subspecies.

**Table 6.** Seed germination rates of different types of weedy rice under different water potential stress. Values are shown as means of three replicates and the standard error is shown in the brackets; the different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

Water potential	Germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
-0.05 MPa	98 <sup>a</sup>	62 <sup>e</sup>	94 <sup>ab</sup>	97 <sup>a</sup>	83 <sup>c</sup>	83 <sup>c</sup>	83 <sup>c</sup>	89 <sup>bc</sup>	74 <sup>d</sup>	93 <sup>ab</sup>	87 <sup>c</sup>	75 <sup>d</sup>
3 d	(1.15)	(2.00)	(1.15)	(1.91)	(1.91)	(2.51)	(1.91)	(1.00)	(1.15)	(1.91)	(3.00)	(1.91)
-0.05 MPa	99 <sup>a</sup>	87 <sup>c</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	89 <sup>c</sup>	95 <sup>ab</sup>	86 <sup>c</sup>	96 <sup>ab</sup>	92 <sup>bc</sup>	100 <sup>a</sup>	98 <sup>ab</sup>	87 <sup>c</sup>
7 d	(1.00)	(3.00)	(0)	(1.15)	(2.52)	(1.00)	(1.15)	(1.63)	(3.65)	(0)	(1.15)	(2.52)
-0.14 MPa	95 <sup>a</sup>	53 <sup>g</sup>	91 <sup>ab</sup>	89 <sup>bc</sup>	83 <sup>ab</sup>	77 <sup>e</sup>	83 <sup>d</sup>	84 <sup>d</sup>	56 <sup>g</sup>	86 <sup>cd</sup>	84 <sup>d</sup>	64 <sup>f</sup>
3 d	(1.00)	(1.91)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.63)	(2.83)	(1.15)	(1.63)	(1.63)
-0.14 MPa	98 <sup>a</sup>	82 <sup>e</sup>	98 <sup>a</sup>	98 <sup>a</sup>	89 <sup>cd</sup>	89 <sup>cd</sup>	87 <sup>d</sup>	93 <sup>bc</sup>	89 <sup>cd</sup>	97 <sup>ab</sup>	95 <sup>ab</sup>	81 <sup>e</sup>
7 d	(1.15)	(1.15)	(2.00)	(2.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.91)
-0.28 MPa	88 <sup>ab</sup>	27 <sup>f</sup>	89 <sup>a</sup>	83 <sup>bc</sup>	83 <sup>bc</sup>	75 <sup>d</sup>	81 <sup>c</sup>	82 <sup>c</sup>	41 <sup>e</sup>	81 <sup>c</sup>	78 <sup>cd</sup>	39 <sup>e</sup>
3 d	(1.63)	(1.91)	(1.00)	(1.91)	(1.91)	(1.00)	(1.00)	(2.00)	(1.91)	(2.52)	(1.15)	(2.52)
-0.28 MPa	93 <sup>a</sup>	84 <sup>c</sup>	94 <sup>a</sup>	91 <sup>ab</sup>	85 <sup>c</sup>	87 <sup>bc</sup>	84 <sup>c</sup>	91 <sup>ab</sup>	82 <sup>cd</sup>	95 <sup>a</sup>	87 <sup>bc</sup>	78 <sup>d</sup>
7 d	(1.91)	(2.83)	(2.58)	(1.91)	(1.91)	(1.00)	(2.31)	(1.00)	(1.15)	(1.00)	(1.00)	(2.00)
-0.47 MPa	71 <sup>a</sup>	6 <sup>h</sup>	50 <sup>c</sup>	66 <sup>b</sup>	44 <sup>d</sup>	13 <sup>g</sup>	29 <sup>e</sup>	42 <sup>d</sup>	10 <sup>gh</sup>	28 <sup>ef</sup>	67 <sup>ab</sup>	24 <sup>f</sup>
3 d	(2.52)	(2.00)	(1.15)	(2.00)	(1.63)	(1.91)	(1.00)	(1.15)	(1.15)	(1.63)	(1.91)	(1.63)
-0.47 MPa	86 <sup>a</sup>	65 <sup>e</sup>	75 <sup>bc</sup>	80 <sup>ab</sup>	70 <sup>cde</sup>	68 <sup>de</sup>	76 <sup>bc</sup>	79 <sup>b</sup>	67 <sup>de</sup>	57 <sup>f</sup>	70 <sup>cde</sup>	72 <sup>cd</sup>
7 d	(1.15)	(1.91)	(1.91)	(2.83)	(1.15)	(2.31)	(1.63)	(1.91)	(2.52)	(1.91)	(4.16)	(1.63)
-0.70 MPa	20 <sup>bc</sup>	1 <sup>e</sup>	25 <sup>a</sup>	11 <sup>d</sup>	2 <sup>e</sup>	2 <sup>e</sup>	23 <sup>ab</sup>	0 <sup>e</sup>	0 <sup>e</sup>	9 <sup>d</sup>	17 <sup>c</sup>	2 <sup>e</sup>
3 d	(1.63)	(1.00)	(2.52)	(1.00)	(1.15)	(1.15)	(1.00)	(0)	(0)	(1.00)	(1.91)	(1.15)
-0.70 MPa	42 <sup>bc</sup>	15 <sup>f</sup>	41 <sup>bc</sup>	33 <sup>d</sup>	24 <sup>e</sup>	51 <sup>a</sup>	54 <sup>a</sup>	38 <sup>cd</sup>	18 <sup>f</sup>	18 <sup>f</sup>	45 <sup>b</sup>	19 <sup>ef</sup>
7 d	(1.15)	(1.91)	(1.00)	(1.00)	(1.63)	(1.91)	(2.00)	(2.58)	(2.58)	(1.15)	(3.00)	(1.00)
-0.99 MPa	7 <sup>c</sup>	0 <sup>d</sup>	19 <sup>a</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	9 <sup>b</sup>	0 <sup>d</sup>
3 d	(1.00)	(0)	(1.00)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.00)	(0)
-0.99 MPa	24 <sup>b</sup>	0 <sup>e</sup>	34 <sup>a</sup>	22 <sup>bc</sup>	15 <sup>d</sup>	0 <sup>e</sup>	0 <sup>e</sup>	0 <sup>e</sup>	0 <sup>e</sup>	20 <sup>c</sup>	14 <sup>d</sup>	0 <sup>e</sup>
7 d	(1.63)	(0)	(1.15)	(2.00)	(1.91)	(0)	(0)	(0)	(0)	(1.63)	(1.15)	(0)

***Indica* weedy rice displayed higher germination rate than *japonica* weedy rice under different buried depth**

The seed germination rate of all populations decreased with increasing burial depth (Table 7). At burial depths of 7.5 and 10.0 cm, seed germination was very low by 7 d after treatment. The germination rate of weedy rice and cultivated rice under different burial depths followed a similar trend to that observed under different temperature, pH, and osmotic stress treatments. Specifically, the germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different burial depths. Nonsignificant difference in germination rate was detected between cultivated rice and weedy rice belonging to the same subspecies.

***Indica* weedy rice had a higher germination rate than *japonica* under different soil moisture levels**

The seed germination rate of all populations decreased with decreasing soil moisture content (Table 8). No seeds germinated under 10% soil moisture content by 3 d after treatment. The germination rate of weedy rice and cultivated rice under different soil moisture conditions followed a similar trend to that observed under different temperature, pH, osmotic stress, and burial depth treatments. Specifically, the germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different soil moisture conditions. Nonsignificant difference in germination rate was detected between cultivated rice and weedy rice belonging to the same subspecies.

**Table 7.** Seed germination rates of different types of weedy rice under different buried depth. Values are shown as means of three replicates and the standard error is shown in the brackets; the different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

Buried depth of seeds (cm)	Germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
0 cm	86 <sup>abc</sup>	62 <sup>f</sup>	91 <sup>a</sup>	83 <sup>abc</sup>	83 <sup>abc</sup>	74 <sup>de</sup>	80 <sup>cd</sup>	82 <sup>bcd</sup>	70 <sup>e</sup>	89 <sup>ab</sup>	80 <sup>cd</sup>	60 <sup>f</sup>
5 d	(2.58)	(2.00)	(2.52)	(1.91)	(1.91)	(4.76)	(4.32)	(2.58)	(1.15)	(1.91)	(1.63)	(1.63)
0 cm	92 <sup>abc</sup>	82 <sup>d</sup>	96 <sup>ab</sup>	90 <sup>bc</sup>	92 <sup>abc</sup>	89 <sup>bcd</sup>	91 <sup>abc</sup>	90 <sup>bc</sup>	92 <sup>abc</sup>	98 <sup>a</sup>	96 <sup>ab</sup>	86 <sup>cd</sup>
10 d	(3.27)	(1.15)	(1.63)	(2.58)	(2.31)	(3.00)	(1.91)	(2.58)	(3.65)	(2.00)	(1.63)	(2.00)
2.5 cm	30 <sup>de</sup>	23 <sup>g</sup>	44 <sup>b</sup>	35 <sup>c</sup>	34 <sup>cd</sup>	23 <sup>g</sup>	49 <sup>a</sup>	29 <sup>ef</sup>	25 <sup>fg</sup>	52 <sup>a</sup>	37 <sup>c</sup>	21 <sup>g</sup>
5 d	(1.15)	(1.91)	(1.63)	(1.91)	(1.15)	(1.00)	(1.00)	(1.91)	(3.00)	(1.63)	(1.00)	(1.00)
2.5 cm	59 <sup>d</sup>	51 <sup>f</sup>	69 <sup>c</sup>	76 <sup>b</sup>	67 <sup>c</sup>	55 <sup>def</sup>	82 <sup>a</sup>	53 <sup>ef</sup>	58 <sup>de</sup>	79 <sup>ab</sup>	53 <sup>ef</sup>	50 <sup>f</sup>
10 d	(1.91)	(1.00)	(1.00)	(1.63)	(1.00)	(1.00)	(2.58)	(1.91)	(2.58)	(1.91)	(1.91)	(2.00)
5 cm	6 <sup>c</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	7 <sup>c</sup>	31 <sup>a</sup>	7 <sup>c</sup>	0 <sup>d</sup>	8 <sup>c</sup>	21 <sup>b</sup>	0 <sup>d</sup>
5 d	(1.15)	(0)	(0)	(0)	(0)	(1.91)	(1.91)	(1.91)	(0)	(1.63)	(1.00)	(0)
5 cm	30 <sup>b</sup>	14 <sup>de</sup>	19 <sup>cd</sup>	21 <sup>c</sup>	22 <sup>c</sup>	19 <sup>cd</sup>	50 <sup>a</sup>	19 <sup>cd</sup>	8 <sup>ef</sup>	20 <sup>cd</sup>	32 <sup>b</sup>	7 <sup>f</sup>
10 d	(2.58)	(1.15)	(1.91)	(1.91)	(2.58)	(1.91)	(2.58)	(1.00)	(1.63)	(4.32)	(1.63)	(1.91)
7.5 cm	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
10 d	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
7.5 cm	6 <sup>b</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	9 <sup>a</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	3 <sup>c</sup>	0 <sup>d</sup>
10 d	(1.15)	(0)	(0)	(0)	(0)	(0)	(1.00)	(0)	(0)	(0)	(1.00)	(0)
10.0 cm	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
5 d	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
10.0 cm	5 <sup>a</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	6 <sup>a</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	3 <sup>b</sup>	0 <sup>c</sup>
10 d	(1.00)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0)	(0)	(0)	(1.00)	(0)

**Table 8.** Seed germination rates of different types of weedy rice under different soil moisture content. Values are shown as means of three replicates and the standard error is shown in the brackets; the different letters indicate significant differences at  $p < 0.05$  (Duncan's test).

Soil moisture content	Germination rate (%)											
	PDSY	FXYX	JSYH	JDCJ	QPAG	MHLX	BSSF	PDRY	CMCJ	FXZH	9311	Nipponbare
50%	88 <sup>abc</sup>	69 <sup>f</sup>	93 <sup>a</sup>	81 <sup>cde</sup>	74 <sup>ef</sup>	80 <sup>de</sup>	78 <sup>de</sup>	82 <sup>bcd</sup>	89 <sup>ab</sup>	92 <sup>a</sup>	89 <sup>ab</sup>	78 <sup>de</sup>
5 d	(1.63)	(2.52)	(1.00)	(1.00)	(2.58)	(3.27)	(3.46)	(2.58)	(2.52)	(1.63)	(1.91)	(2.00)
50%	100 <sup>a</sup>	82 <sup>e</sup>	95 <sup>abc</sup>	88 <sup>d</sup>	91 <sup>cd</sup>	94 <sup>bc</sup>	95 <sup>abc</sup>	100 <sup>a</sup>	95 <sup>abc</sup>	98 <sup>ab</sup>	96 <sup>abc</sup>	87 <sup>d</sup>
10 d	(0)	(2.58)	(1.00)	(1.63)	(1.91)	(1.15)	(1.91)	(0)	(2.52)	(2.00)	(1.63)	(1.00)
40%	84 <sup>bc</sup>	43 <sup>e</sup>	89 <sup>ab</sup>	77 <sup>d</sup>	75 <sup>d</sup>	75 <sup>d</sup>	75 <sup>d</sup>	83 <sup>c</sup>	84 <sup>bc</sup>	91 <sup>a</sup>	83 <sup>c</sup>	43 <sup>e</sup>
5 d	(1.63)	(1.91)	(1.91)	(2.52)	(1.00)	(1.00)	(1.00)	(1.91)	(1.63)	(2.52)	(1.91)	(1.91)
40%	93 <sup>ab</sup>	58 <sup>f</sup>	91 <sup>abc</sup>	79 <sup>e</sup>	84 <sup>de</sup>	91 <sup>abc</sup>	84 <sup>de</sup>	86 <sup>cd</sup>	87 <sup>bcd</sup>	94 <sup>a</sup>	89 <sup>abcd</sup>	57 <sup>f</sup>
10 d	(1.91)	(2.58)	(1.91)	(1.91)	(1.63)	(1.91)	(1.63)	(2.58)	(1.00)	(1.15)	(1.00)	(3.45)
30%	45 <sup>cd</sup>	41 <sup>d</sup>	44 <sup>cd</sup>	54 <sup>a</sup>	46 <sup>bcd</sup>	43 <sup>d</sup>	44 <sup>cd</sup>	52 <sup>ab</sup>	42 <sup>d</sup>	52 <sup>ab</sup>	50 <sup>abc</sup>	40 <sup>d</sup>
5 d	(1.91)	(3.42)	(2.31)	(2.00)	(1.15)	(1.91)	(0)	(2.31)	(2.00)	(1.63)	(1.15)	(2.83)
30%	74 <sup>a</sup>	61 <sup>c</sup>	62 <sup>c</sup>	78 <sup>a</sup>	66 <sup>bc</sup>	62 <sup>c</sup>	72 <sup>ab</sup>	75 <sup>a</sup>	46 <sup>d</sup>	76 <sup>a</sup>	72 <sup>ab</sup>	47 <sup>d</sup>
10 d	(2.58)	(1.91)	(2.58)	(3.46)	(1.15)	(2.00)	(1.63)	(2.52)	(1.15)	(1.63)	(3.65)	(1.00)
20%	22 <sup>ef</sup>	0 <sup>h</sup>	38 <sup>bc</sup>	48 <sup>a</sup>	37 <sup>c</sup>	28 <sup>de</sup>	44 <sup>ab</sup>	16 <sup>fg</sup>	32 <sup>cd</sup>	44 <sup>ab</sup>	48 <sup>a</sup>	22 <sup>ef</sup>
5 d	(1.15)	(0)	(2.58)	(2.82)	(1.91)	(2.31)	(1.63)	(2.31)	(2.31)	(2.31)	(1.63)	(1.15)
20%	27 <sup>fg</sup>	9 <sup>h</sup>	53 <sup>c</sup>	69 <sup>a</sup>	63 <sup>ab</sup>	41 <sup>d</sup>	68 <sup>a</sup>	22 <sup>g</sup>	36 <sup>de</sup>	49 <sup>c</sup>	61 <sup>b</sup>	30 <sup>ef</sup>
10 d	(1.00)	(1.00)	(1.91)	(3.42)	(2.52)	(1.00)	(1.63)	(2.00)	(2.31)	(2.52)	(3.42)	(2.00)
10 %	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
5 d	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
10%	12 <sup>a</sup>	0 <sup>c</sup>	5 <sup>bc</sup>	13 <sup>a</sup>	12 <sup>a</sup>	11 <sup>a</sup>	12 <sup>a</sup>	5 <sup>bc</sup>	0 <sup>c</sup>	12 <sup>a</sup>	7 <sup>ab</sup>	3 <sup>bc</sup>
10 d	(2.83)	(0)	(1.91)	(1.91)	(1.63)	(1.91)	(2.31)	(1.00)	(0)	(2.31)	(1.91)	(1.91)

## DISCUSSION

### Morphological traits and molecular-methods identification of weedy rice subspecies

Morphological traits are often the first line of identification and can provide useful clues for distinguishing *indica* and *japonica* types. However, while morphological and physiological traits offer initial insights, molecular markers provide the most precise identification. To accurately identify *indica* and *japonica* types of weedy rice, a combination of morphological, physiological, and molecular approaches is recommended, especially when morphological traits yield ambiguous or conflicting results (Figure 1).

The seed color of weedy rice was consistent with expectations, as the *japonica* weedy rice exhibited a darker color compared to the *indica* weedy rice. However, the seed shape of the *japonica* weedy rice (FXYX) resembled that of *indica* rice '9311', while the seed shape of the *indica* weedy rice (FXZH) closely resembled that of *japonica* rice 'Nipponbare'. These observations highlight the limitations of relying solely on morphological traits for identification. Definitive conclusions could only be drawn with the assistance of molecular methods (Figure 2).

Given that rice production in Shanghai City has long been dominated by *japonica* rice, the results of morphological and molecular identification are consistent with expectations. To survive and expand in agricultural environments, weedy rice mimics local cultivars. This adaptation allows it to evade human intervention. This evolutionary process is complex and may involve multiple mechanisms, including geneflow from cultivated rice to its weedy and wild relatives (Chen, 2004), adaptation of wild rice varieties to cultivation, the creation of hybrids by *indica* and *japonica* rice (Londo and Schaal, 2007), de-domestication of cultivated rice to a wild form (Qiu et al., 2017) and hybrid rice evolving into weedy rice (Zhang et al., 2015) might drive this evolution. For example, Bhutan weedy rice, which shares characteristics with both *indica* and *japonica* rice, is thought to have originated from hybridization between *indica* and *japonica* rice, as evidenced by specific nuclear and cytoplasmic genome markers (Ishikawa et al., 2005). Similarly, studies on samples from Liaoning and Guangdong provinces in China support the de-domestication hypothesis, suggesting that weedy rice most likely originated from local cultivated rice (Zhang et al., 2012).

Weedy rice with a distinct appearance from *japonica* rice is gradually being eliminated in the rice fields of Shanghai, resulting in the remaining weedy rice populations closely resembling *japonica* rice. This is consistent with the observation that only one population, FXYX, did not exhibit a similar appearance to *japonica* rice. This phenomenon highlights the evolutionary adaptability of weedy rice.

Based on the *japonica* and *indica* indices presented in Table 2, FXYX is a typical *japonica* weedy rice with a 95.12% *japonica* index, while the *indica* indices of other *indica* weedy rice populations range from 78.05% to 95.12%. These findings suggest that *indica* weedy rice populations have undergone a complex evolutionary process, whereas the *japonica* weedy rice population (FXYX) appears to have limited genetic exchange with external gene pools. On one hand, populations like FXYX are rare and fortunate, as they have survived extensive human intervention. In contrast, *indica* weedy rice populations must undergo significant genetic changes to adapt to their environment and ensure survival. On the other hand, populations like FXYX may inevitably disappear over time due to their limited genetic diversity and adaptability. This may partially explain why *japonica* weedy rice is relatively rare. A similar pattern of weedy rice occurrence has been observed in Jiangsu Province, China, which shares a similar rice cultivation culture with Shanghai (Zhang et al., 2016).

### Germination behavior difference between *indica* and *japonica* weedy rice

In addition to differences in morphological traits, variations in seed germination characteristics are also key factors contributing to the gradual decline of *japonica* weedy rice. Understanding these differences is crucial for effective weed management and for studying the evolutionary dynamics of weedy rice populations. However, the germination characteristics of weedy rice populations are highly diverse (Schwanke et al., 2008; Zhang et al., 2012; Yang et al., 2022; Fukuda et al., 2023), leading to complex results in comparative studies. Rice production in Shanghai is dominated by *japonica* rice. Therefore, if *indica* weedy rice exhibits stronger germination abilities than *japonica* rice, this difference can be leveraged for non-chemical control strategies in agricultural practice.

**Temperature.** *Japonica* weedy rice performed significantly worse than *indica* weedy rice at 7 d after treatment across a wide temperature range (Table 3). Both *indica* weedy rice and cultivated rice demonstrated greater adaptability than their *japonica* counterparts. Previous studies have also shown that seed germination occurs within the temperature range of 15-35 °C (Fukuda et al., 2023; Zhang et al., 2024), indicating that weedy rice seeds can germinate throughout the rice-growing season. This is particularly true for *indica* weedy rice, which can cause earlier and more severe infestations in *japonica* rice fields, as nearly all weedy rice in Shanghai is *indica* weedy rice. Fortunately, this characteristic can be utilized in the false-seedbed technique (Merfield, 2013; Gazoulis et al., 2023). This technique involves inducing weedy rice to germinate before rice planting, allowing the seedlings to be eliminated by chemical or mechanical methods in advance (Lonsbary et al., 2003; Schutte et al., 2021). This reduces the pressure of weedy rice control during the rice growth period.

**NaCl stress.** The tolerance to NaCl stress followed the order: *indica* rice and weedy rice > *japonica* weedy rice > *japonica* rice (Table 4). NaCl tolerance was the only index where weedy rice outperformed cultivated rice (specifically, *japonica* weedy rice compared to *japonica* cultivated rice). The higher germination rate under NaCl stress endows weedy rice, particularly *indica* weedy rice, with greater adaptability to saline conditions. However, *indica* weedy rice displayed comparable NaCl tolerance to *indica* cultivated rice, as germination responses to NaCl stress were strain-specific. This indicates variability in NaCl tolerance among *indica* weedy rice populations. NaCl tolerance in weedy rice has been extensively studied and is recognized as one of its most important adaptive traits (Zhang et al., 2018; Fogliatto et al., 2020).

**pH conditions.** All weedy rice populations were capable of germinating across a wide pH range (3.50 to 9.50), demonstrating strong pH adaptability (Table 5). This ability facilitates the expansion of weedy rice in diverse environments. The germination rate of the *japonica* subspecies was consistently lower than that of the *indica* subspecies under varying pH conditions, aligning with trends observed in other treatments, such as temperature, NaCl stress, water stress, and burial depth. Limited information is available on the effects of pH on the germination of different weedy rice types.

**Water stress (osmotic stress and soil water content).** The germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different water availability treatments (Tables 6 and 8). Similarly, germination responses to water stress were strain-specific, consistent with previous reports (Fukuda et al., 2023). No germination was observed at -1.0 MPa in prior studies (Zhang et al., 2024), and similar results were obtained in this study, with few seeds germinating at -0.99 MPa (Table 6). The overall trend indicates that *indica* weedy rice exhibited higher germination rate than *japonica* weedy rice and cultivated rice under various water stress conditions. This aligns with previous findings that weedy rice performs better than coexisting cultivated rice in hyperosmotic solutions (Wang et al., 2019). These results suggest that weedy rice populations have a stronger ability to acquire water during the germination period, partially explaining why weedy rice causes greater yield losses in dry direct-seeded rice systems (Wang et al., 2019).

**Seed buried depth.** The germination rate of the *japonica* subspecies was lower than that of the *indica* subspecies in both cultivated and weedy rice under different burial depth treatments (Table 7). Germination responses to burial depth were also strain-specific, particularly for *indica* weedy rice. A noticeable decline in germination rates was observed when seeds were buried deeper than 5 cm, and no seedlings emerged from a depth of 7.5 cm, consistent with previous findings (Zhang et al., 2024). Based on these results, shallow tillage has little effect on weedy rice seedling emergence, while deep tillage may help bury seeds below the minimum emergence zone. Deep tillage practices and the false-seedbed technique can reduce weedy rice occurrence before rice planting by limiting seedling emergence (Fang et al., 2025).

In summary, *japonica* weedy rice exhibited lower germination rate compared to *indica* weedy rice across all treatments. Germination responses to environmental factors were population-specific, particularly for *indica* weedy rice. The complex genetic background of weedy rice is likely the primary reason for these observed differences. However, future research should include more *japonica* weedy rice populations to enrich the data on seed morphology and germination characteristics, providing a more comprehensive understanding of its behavior and adaptability.

## CONCLUSIONS

The combination of seed morphology comparison and PCR-based methods enabled accurate identification of the two subspecies of weedy rice. *Indica* weedy rice exhibited higher germination rates and adaptability under a wider range of experimental conditions, demonstrating its ability to thrive in adverse environments. In contrast, *japonica* weedy rice required more specific conditions for optimal germination. Furthermore, *indica* weedy rice was often associated with *indica* cultivated rice, while *japonica* weedy rice was frequently linked to *japonica* cultivated rice. These findings suggest that it is both effective and reasonable to develop targeted management strategies based on the primary threat posed by *indica* weedy rice. Integrated practices such as the false-seedbed technique and deep tillage could serve as effective measures for managing weedy rice populations.

### Author contribution

Conceptualization: Z.T. Methodology: J.F., G.Y., Y.G., L.Y., J.S. Validation: J.F. Investigation: G.Y., Y.G., L.Y., J.S. Resources: G.S., Z.T. Data curation: J.F. Writing-original draft: J.F. Writing-review & editing: G.S. Supervision: B.L., Z.T. All co-authors reviewed the final version and approved the manuscript before submission.

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### Reference

- Busconi, M., Rossi, D., Lorenzoni, C., Baldi, G., Fogher, C. 2012. Spread of herbicide-resistant weedy rice (red rice, *Oryza sativa* L.) after 5 years of Clearfield rice cultivation in Italy. *Plant Biology* 14(5):751-759. doi:10.1111/j.1438-8677.2012.00570.x.
- Busi, R., Nguyen, N.K., Chauhan, B.S., Vidotto, F., Tabacchi, M., Powles, S.B. 2017. Can herbicide safeners allow selective control of weedy rice infesting rice crops? *Pest Management Science* 73(1):71-77. doi:10.1002/ps.4411.
- Chachalis, D., Reddy, K.N. 2000. Factors affecting *Campsis radicans* seed germination and seedling emergence. *Weed Science* 48(2):212-216. doi:10.1614/0043-1745(2000)048[0212:FACRSG]2.0.CO;2.
- Chen, L.J. 2004. Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Annals of Botany* 93(1):67-73. doi:10.1093/aob/mch006.
- Chen, W., Shao, B., Lin, Y., Miao, T., Peng, J., Guo, J., et al. 2020. Identification method of Hsien and Keng rice by SYBR-Green fluorescent-PCR detection for chloroplast DNA. *China Port Science and Technology* 10:17-25 (In Chinese with English abstract).
- Dauer, J., Hulting, A., Carlson, D., Mankin, L., Harden, J., Mallory-Smith, C. 2017. Gene flow from single and stacked herbicide-resistant rice (*Oryza sativa*): Modeling occurrence of multiple herbicide-resistant weedy rice. *Pest Management Science* 74(2):348-355. doi:10.1002/ps.4711.
- Fang, J., Yuan, G., Gao, Y., Shen, G., Tian, Z. 2025. Effect of false-seedbed technique on weed management and soil quality in rice fields. *Chilean Journal of Agricultural Research* 85:796-810. doi:10.4067/S0718-58392025000600796.
- Fartyal, D., Agarwal, A., James, D., Borphukan, B., Ram, B., Sheri, V., et al. 2018. Developing dual herbicide tolerant transgenic rice plants for sustainable weed management. *Scientific Reports* 8:12. doi:10.1038/s41598-018-29554-9.
- Fogliatto, S., Patrucco, L., Milan, M., Vidotto, F. 2020. Sensitivity to salinity at the emergence and seedling stages of barnyardgrass (*Echinochloa crus-galli*), weedy rice (*Oryza sativa*), and rice with different tolerances to ALS-inhibiting herbicides. *Weed Science* 69(1):39-51. doi:10.1017/wsc.2020.72.
- Fukuda, M., Imaizumi, T., Koarai, A. 2023. Seed germination responses to temperature and water availability in weedy rice. *Pest Management Science* 79(2):870-880. doi:10.1002/ps.7256.
- Gazoulis, I., Kanatas, P., Antonopoulos, N., Tataridas, A., Travlos, I. 2023. False seedbed for agroecological weed management in forage cereal-legume intercrops and monocultures in Greece. *Agronomy* 13(1):1-21. doi:10.3390/agronomy13010123.
- Hakim, M.A., Juraimi, A.S., Hanafi, M.M., Selamat, A., Ismail, M.R., Karim, S.M.R. 2011. Studies on seed germination and growth in weed species of rice field under salinity stress. *Journal of Environmental Biology* 32(5):529-536.
- Ishikawa, R., Toki, N., Imai, K., Sato, Y.I., Yamagishi, H., Shimamoto, Y., et al. 2005. Origin of weedy rice grown in Bhutan and the force of genetic diversity. *Genetic Resources & Crop Evolution* 52(4):395-403. doi:10.1007/s10722-005-2257-x.
- Londo, J.P., Schaal, B.A. 2007. Origins and population genetics of weedy red rice in the USA. *Molecular Ecology* 16(21):4523-4535. doi:10.1111/j.1365-294X.2007.03489.x.

- Lonsbary, S.K., O'Sullivan, J., Swanton, C.J. 2003. Stale-seedbed as a weed management alternative for machine-harvested cucumbers (*Cucumis sativus*). *Weed Technology* 17(4):724-730. doi:10.1614/Wt02-123.
- Merfield, C. 2013. False and stale seedbeds: The most effective non-chemical weed management tools for cropping and pasture establishment. The BHU Future Farming Centre, Lincoln, New Zealand. Available at <https://www.bhu.org.nz/future-farming-centre> (accessed 15 February 2025).
- Merotto, A., Goulart, I., Nunes, A.L., Kalsing, A., Markus, C., Menezes, V.G., et al. 2016. Evolutionary and social consequences of introgression of nontransgenic herbicide resistance from rice to weedy rice in Brazil. *Evolutionary Applications* 9(7):837-846. doi:10.1111/eva.12387.
- Michel, B.E., Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiology* 51(5):914-916. doi:10.1104/pp.51.5.914.
- Nadir, S., Xiong, H.B., Zhu, Q., Zhang, X.L., Xu, H.Y., Li, J., et al. 2017. Weedy rice in sustainable rice production. A review. *Agronomy for Sustainable Development* 37(5):1-14. doi:10.1007/s13593-017-0456-4.
- Qiu, J., Zhou, Y., Mao, L., Ye, C., Wang, W., Zhang, J., et al. 2017. Genomic variation associated with local adaptation of weedy rice during de-domestication. *Nature Communications* 8(1):15323. doi:10.1038/ncomms15323.
- Schutte, B.J., Sanchez, A.D., Beck, L.L., Idowu, O.J. 2021. False seedbeds reduce labor requirements for weeding in chile pepper. *HortTechnology* 31(1):64-73. doi:10.21273/horttech04732-20.
- Schwanke, A.M.L., Andres, A., Noldin, J.A., Concenco, G., Procopio, S.O. 2008. Seed germination and dormancy of red rice ecotypes. *Planta Daninha* 26(3):497-505. doi:10.1590/s0100-83582008000300004.
- Shen, Y.J., Jiang, H., Jin, J.P., Zhang, Z.B., Xi, B., He, Y.Y., et al. 2004. Development of genome-wide DNA polymorphism database for map-based cloning of rice genes. *Plant Physiology* 135(3):1198-1205. doi:10.1104/pp.103.038463.
- Shivrain, V.K., Burgos, N.R., Gealy, D.R., Sales, M.A., Smith, K.L. 2009. Gene flow from weedy red rice (*Oryza sativa* L.) to cultivated rice and fitness of hybrids. *Pest Management Science* 65(10):1124-1129. doi:10.1002/ps.1802.
- Sudianto, E., Beng-Kah, S., Ting-Xiang, N., Saldain, N.E., Scott, R.C., Burgos, N.R. 2013. Clearfield® rice: Its development, success, and key challenges on a global perspective. *Crop Protection* 49:40-51. doi:10.1016/j.cropro.2013.02.013.
- Sun, J., Ma, D., Tang, L., Zhao, M., Zhang, G., Wang, W., et al. 2019. Population genomic analysis and De Novo assembly reveal the origin of weedy rice as an evolutionary game. *Molecular Plant* 12(5):632-647. doi:10.1016/j.molp.2019.01.019.
- Svizzero, S. 2021. Agronomic practices and biotechnological methods dealing with the occurrence, dispersion, proliferation and adaptation of weedy rice (*Oryza sativa* f. *spontanea*). *International Journal of Pest Management* 70(1):62-75. doi:10.1080/09670874.2021.1944697.
- Ushiki, J., Ishii, T., Ishikawa, R. 2005. Morpho-physiological characters and geographical distribution of japonica and indica weedy rice (*Oryza sativa*) in Okayama Prefecture, Japan. *Breeding Research* 7(4):179-187. doi:10.1270/jsbbr.07.179.
- Wang, Y., Mo, S.D., Kong, M.Y., Chao, J., Chen, X.F., Yang, J.L., et al. 2019. Better performance of germination in hyperosmotic solutions in conspecific weedy rice than cultivated rice. *Journal of Systematics and Evolution* 57(5):519-529. doi:10.1111/jse.12495.
- Xu, Q., Xu, H.Y., Wei, X.H., Tang, S.X., Chen, L.J. 2012. SSILP, InDel and SSR Markers-based indica-japonica classification for weedy rice. *Chinese Journal of Rice Science* 26(6):686-692 (In Chinese with English abstract)
- Yang, B., Zeng, J., Chen, S., Li, S., Wu, L., Wan, X. 2022. Genome-wide association study reveals the genetic basis of seed germination in japonica rice. *Agriculture* 13(1):118. doi:10.3390/agriculture13010118.
- Yean, R.A., Dilipkumar, M., Rahman, S., Song, B.K. 2021. A Two-in-One strategy: Target and nontarget site mechanisms both play important role in IMI-resistant weedy rice. *International Journal of Molecular Science* 22(3):982. doi:10.3390/ijms22030982.
- Zhang, L., Dai, W., Wu, C., Song, X., Qiang, S. 2012. Genetic diversity and origin of Japonica- and Indica-like rice biotypes of weedy rice in the Guangdong and Liaoning provinces of China. *Genetic Resources & Crop Evolution* 59(3):399-410. doi:10.1007/s10722-011-9690-9.
- Zhang, B.H., Dong, E.J., Zhang, H., Shi, Z.H., Song, X.L., Qiang, S., et al. 2016. Use of hybrid rice and the proliferation of weedy rice: A case in Jiangsu, China. *Crop Science* 56(2):673-681. doi:10.2135/cropsci2015.04.0210.
- Zhang, Y., Fang, J., Wu, X., Dong, L. 2018. Na<sup>+</sup>/K<sup>+</sup> balance and transport regulatory mechanisms in weedy and cultivated rice (*Oryza sativa* L.) under salt stress. *BMC Plant Biology* 18(1):375. doi:10.1186/s12870-018-1586-9.
- Zhang, Z., Gao, P.L., Dai, W.M., Song, X.L., Hu, F., Qiang, S. 2019. Effect of tillage and burial depth and density of seed on viability and seedling emergence of weedy rice. *Journal of Integrative Agriculture* 18(8):1914-1923. doi:10.1016/s2095-3119(19)62583-9.
- Zhang, J.X., Lu, Z.M., Dai, W.M., Song, X.L., Peng, Y.F., Valverde, B.E., et al. 2015. Cytoplasmic-genetic male sterility gene provides direct evidence for some hybrid rice recently evolving into weedy rice. *Scientific Reports* 5:10591. doi:10.1038/srep10591.
- Zhang, Z., Xue, J., Gu, T., Wang, H., Chauhan, B.S. 2024. Effect of environmental factors on seed germination and seedling emergence of weedy rice (*Oryza sativa* f. *spontanea*) in China. *Weed Science* 72(6):754-760. doi:10.1017/wsc.2024.59.
- Zhang, P., Yuan, X., Wang, X., Wang, H., Li, S., Zhou, L. 2025. Effects of environmental factors on seed germination and seedling emergence of Tausch's goatgrass (*Aegilops tauschii* Coss.) *Chilean Journal of Agricultural Research* 85:206-214. doi:10.4067/S0718-58392025000200206.