

FLOODING TREATMENT RESTRAINS VOLUNTEER RICE GERMINATION AND SEEDLING GROWTH

LUO, H. W.^{1,2#} – DU, B.^{1,2#} – ZHENG, A. X.^{1#} – LAI, R. F.¹ – YOU, Z. S.¹ – WANG, M.¹ –
WANG, Z. M.³ – HE, L. X.^{1,2} – ZHANG, T. T.^{1,2} – TANG, X. R.^{1,2*}

¹*Department of Crop Science and Technology, College of Agriculture, South China Agricultural University, Guangzhou 510642, PR China*

²*Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture, Guangzhou 510642, PR China*

³*Key Laboratory of Key Technology for South Agricultural Machine and Equipment, Ministry of Education, Guangzhou 510642, PR China*

[#]*These authors have contributed equally to this work.*

**Corresponding author*

e-mail: luohaowen@stu.scau.edu.cn

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Abstract. Volunteer rice is a global problem in rice production, especially conservation tillage. Here volunteer rice from four rice cultivars viz. *Xiangyaxiangzhan*, *Meixiangzhan*, *Changnongjing 1* and *Yliangyou1173* was treated with different flooding treatments. Two weeks of water flooding were applied at four depths: 2.0-2.5 cm (F1), 3.0-3.5 cm (F2), 4.5-5 cm (F3) and 6.0-6.5 cm (F4). A control was set by maintaining the soil wet as normal farmer practice of this region. It was found volunteer rice germination was more reduced with increased submergence levels, while treatments F3 and F4 both decreased the germination index and germination energy greatly for all rice varieties. Flooding treatments also reduced the seedling rate and inhibited the growth of volunteer seedling. Treatments F3 and F4 decreased seedling rates of *Changnongjing 1* and *F2 generation of Yliangyou1173* to zero, while the flooding treatment induced the malonaldehyde accumulation and altered the activities of peroxidase, superoxide and catalase in seedlings for all rice varieties. Hence, we suggest 2-week flooding at soil depth 6 cm after the harvest of early-season rice in South China in order to prevent the harvest loss in late-season rice caused by volunteer rice.

Keywords: *rice, flood, seed germination, rice seedling, anti-oxidative enzyme*

Introduction

Oryza sativa L. (Rice), one of the main crops and a staple food, contributes to 35%-75% of caloric intake by about 3 billion people worldwide. During the rice production, the primary considerations are the yield and grain quality, which can be affected by many factors, especially volunteer rice. As a common problem in paddy field, volunteer rice emerges from the previous crop (Gealy and Gressel, 2005; Warwick et al., 2005; Street et al., 1995) because of shattered seeds or harvest loss. The severity of this global problem in rice production differs depending mostly on the region, cultivating measures and cultivars planted. Volunteer rice in temperate regions germinates either before winter (October-November) or in spring (April-May) (Sudianto et al., 2013). Seeds of volunteer plants could be harvested with the cultivated rice. The grains of volunteer rice from a different variety or plant type that matures earlier or later would reduce the overall milling quality (Singh et al., 2016a).

In tropics and subtropics, since volunteer rice germinates after the harvest, farmers take mechanical or chemical control measures to eliminate or lower the interference with the succeeding or ratooned rice. Volunteer rice is regarded as a weed phenologically and morphologically different from the planted cultivar and has lower yield and grain quality. Furthermore, volunteer rice from hybrid rice is a segregating plant that competes with crop and reduces grain yield and rice quality, thus decreasing the final productivity of paddy field and the overall level of grain quality. Thus, volunteer rice from conventional varieties is considered less influential than that from hybrid or weedy rice. Moreover, volunteer seeds from herbicide-resistant rice such as Clearfield riceTM could even become the agent for resistant gene flow between rice cultivars and natural weedy rice by outcrossing (Shivrain et al., 2007; Gressel and Valverde, 2010; Gealy et al., 2003). Normally, less than 1% cross pollination happens between cultivars and weedy populations (Cao et al., 2006), but introgressed crop genes are able to persist in weedy population indefinitely (Ellstrand et al., 1999). Therefore, the outcrosses can promote the rise to weedy type population, which then competes with the rice cultivar and contaminates the rice, inducing economic losses.

In recent years, conservation tillage is increasingly recognized and accepted since traditional tillage is faced by many limitations, such as poor soil structure and negative effect on soil organism. Organic farmers in Europe have great interest in conservation tillage (Peigné et al., 2018). There are many practices, including direct seeding without any tillage, or tillage at depth down to 15-20 cm. The rice root system and soil nitrogen can accumulate in the surface soil layer in zero-tillage compared with conventional tillage (Triplett and Dick, 2008; Huang et al., 2016). During rice production in China, zero-tillage has become increasingly popular for its benefits of saving fuel, labor and equipment and conserving soil (Huang et al., 2011). However, conservation tillage is still limited by many urgent problems, including volunteer rice. In South China, rice normally grows twice each year, in March (early season) and in July (late season) (Kong et al., 2017). In conservation tillage, the volunteer rice from early season easily germinates and seedlings in late season because the paddy fields are not ploughed. As a result, the volunteer plants would compete with the rice cultivar for nutrients and sunshine. Meanwhile, because of the commensalism between volunteer plants and the rice cultivar, the rice population becomes denser and prone to inducing various diseases and insect pests, which will complicate rice management and reduce the yield and grain quality of late season rice.

As reported, winter-flood reduced the germination rate of volunteer rice (Singh et al., 2016a), indicating flood treatment could be an effective means to deal with volunteer rice in double-cropping rice in South China. Under the hypothesis that flood treatment can reduce the germination of volunteer rice and the growth of volunteer seedlings, we conducted experiments in artificial weather boxes.

Materials and methods

Seed materials

Seeds from two fragrant rice cultivars (*Meixiangzhan*, *Xiangyaxiangzhan*), one japonica rice cultivar (*Changnongjing 1*, Yangtze University, Hubei, China) and the F₂ generation of hybrid rice (*Yliangyou1173*) were used as the experimental

materials (Those cultivars were well-known and widely grown in South China). Seeds of early season rice were collected from College of Agriculture, South China Agricultural University, Guangzhou, China. All seeds were obtained from the upper parts of panicle.

Flood treatment and growth conditions

Normally, there was about two weeks between the harvest of early season and transplantation of late season rice in South China. Hence, after sowing seeds into soil-containing plastic pots (100× 80 × 20 cm³; 500 seeds in each pot), we began the 2-week flood treatments as follows:

CK: soil was contained wet without any water layer at soil surface.

F1, F2, F3, F4: 2.0-2.5, 3-3.5, 4.5-5, 6-6.5 cm water layer were maintained at soil surface, respectively.

After the treatments F1, F2, F3 and F4, the water layers were drained off and all the pots contained wet soil without any water layer for a week in order to determinate the seedling rate and some biochemical indicators. The temperature for germination and growth was 33 °C in the day and 27 °C at night under 1200X yellow light. The experimental soil was sandy loam containing 1.443% total nitrogen, 24.56% organic matter, 0.927% total phosphorous and 18.220% total potassium at pH 6.54.

Seed bioassay

After sowing, the germination of each treatment was observed daily for 7 days according to the Association of Official Seed Analysis (Wiesner, 1990). The number of days to reach 50% germination (T_{50}) was calculated by a devised method with modification (Farooq et al., 2013):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right) (t_j - t_i)}{n_j - n_i}$$

where N is the final number of emerged seeds; n_i and n_j are the cumulative numbers of emerged seeds at time t_i and t_j respectively when $n_i < N/2 < n_j$.

Mean germination time (MGT) was calculated as follows (Ellis, 1981):

$$MGT = \Sigma Dn / \Sigma n$$

where n is the number of emerged seeds on day D , which is counted from the beginning of emergence.

Germination index (GI) was calculated according to the equation of Association of Official Seed Analysis:

$$GI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

The energy of germination (*GE*) was observed on the 3rd day after sowing. This percentage of germinating seeds 3 d after sowing is relative to the total number of seeds tested (Ruan et al., 2002).

The radicle and plumule lengths of twenty seedlings randomly selected from each experimental unit were measured 7 d after sowing. Three weeks after sowing, seedling rate was recorded while 30 seedlings randomly selected from each experimental unit were measured for fresh weight and then oven-dried at 80 °C for 48 h to determine the dry biomass.

Biochemical analyses

After malonaldehyde (MDA) reacted with thiobarbital acid, absorbance of solutions was recorded at 532, 600, and 450 nm. The MDA content ($\mu\text{mol/L}$) was determined as $= 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56\text{OD}_{450}$ (Pan, 2013), and final result was expressed as $\mu\text{mol/g FW}$.

The superoxide (SOD, EC 1.15.1.1) activity was detected according to Zhang (Zhang et al., 2008). The reaction mixture contained 1.75 ml of sodium phosphate buffer (SPB, pH 7.8), 0.3 ml of $130 \text{ mM}\cdot\text{l}^{-1}$ methionine buffer, 0.3 ml of $750 \mu\text{mol}\cdot\text{l}^{-1}$ nitroblue tetrazolium (NBT) buffer, 0.3 ml of $100 \mu\text{mol}\cdot\text{l}^{-1}$ ethylene diamine tetraacetic acid disodium (EDTA-Na_2) buffer, 0.3 ml of $20 \mu\text{mol}\cdot\text{l}^{-1}$ lactoflavin and 0.05 ml of enzyme extract. The color change was measured at 560 nm after the reaction. One unit of activity was equal to the volume of extract needed to inhibit 50% of the color reaction. The peroxidase (POD EC1.11.1.7) activity was examined according to Yu (Xia et al., 2009). Enzyme extract (50 μl) was added to the reaction solution which contained 1 ml of 0.3% H_2O_2 , 0.95 ml of 0.2% guaiacol, and 1 ml of $50 \text{ mM}\cdot\text{l}^{-1}$ SPB (pH 7.0). The absorbance was read at 470 nm. One unit of POD activity was expressed as the absorbance increase because of guaiacol oxidation by 0.01 (U/g FW). Catalase (CAT, EC 1.11.1.6) activity was measured according to Aebi (1984). An aliquot of enzyme extract (50 μl) was added to the reaction solution containing 1 ml of 0.3% H_2O_2 and 1.95 ml of SPB and, the absorbance was recorded at 240 nm. One unit of CAT activity was expressed as the absorbance decrease by 0.01 (U/g FW).

Statistical analyses

Data were analyzed using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) while differences among means were separated by using least significant difference (LSD) test at the 5% probability level. Graphical representation was conducted via Sigma Plot 14.0 (Systat Software Inc., California, USA).

Results

Germination rate

Flooding treatment significantly affected the emergence of volunteer rice. The germination of seeds started after 3 days in both treatments F3 and F4 for all rice varieties (Fig. 1). For *Xiangyaxiangzhan*, the speeds of germination were not significantly different among treatments CK, F1 and F2, but were significantly slowed down after treatments F3 and F4. Similar trend was found in *Meixiangzhan*.

For both *Changnongjing 1* and F2 generation of *Yliangyou1173*, the trend of speed germination was recorded as: CK > F1 > F2 > F3 > F4.

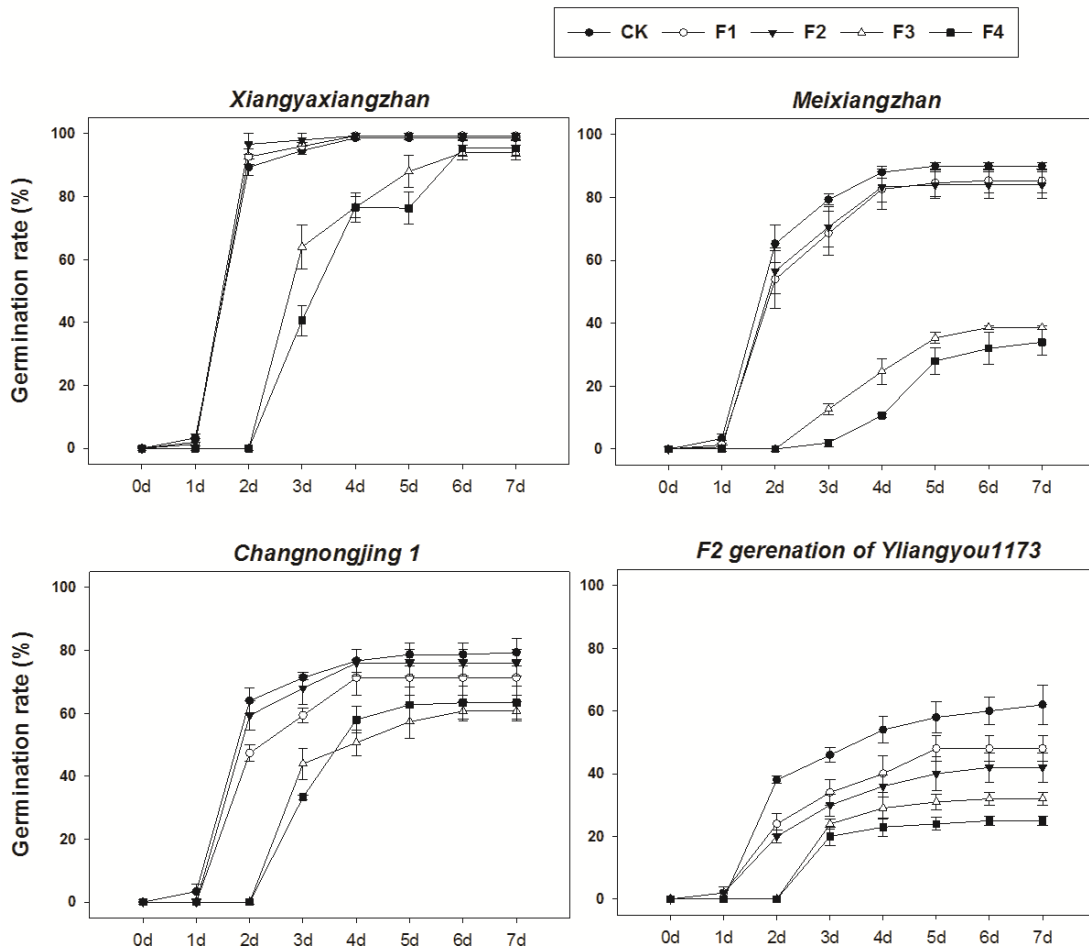


Figure 1. Effects of flood treatments on germination rate of volunteer rice

Emergence attributes

Flooding treatments influenced germination vigor significantly (Table 1). Treatments F3 and F4 both decreased the GI, GE, lengths of radicle and plumule, but increased the MGT and T₅₀ significantly for all rice varieties. For *Xiangyaxiangzhan*, GIs in F3 and F4 declined by 43.56% and 47.72%, respectively, and GEs in F3 and F4 decreased by 22.29% and 22.37%, respectively, compared with CK. For *Meixiangzhan*, the lowest GI, GE and FGP were found in F4; Compared with CK, GIs in F3 and F4 decreased by 77.42% and 85.32%, respectively, while 71.97% and 87.88% lower were recorded in F3 and F4, respectively. For *Changnongjing 1*, no significant difference between treatments F3 and F4 was found in GI, GE or FGP, while GI, GE and FGP significantly all declined in F3 and F4. For F2 generation of *Yliangyou1173*, the changing trend of GI, GE and FGP was recorded as: CK > F1 > F2 > F3 > F4, while F3 and F4 prolonged MGT and T₅₀ significantly. Furthermore, F1 and F2 treatments did not significantly affect FGP for *Xiangyaxiangzhan*, *Meixiangzhan* or *Changnongjing 1* whilst both F3 and F4 decreased the lengths of radicle and plumule for all 4 varieties compared with CK.

Table 1. Effects of flooding treatments on the germination vigor of volunteer rice

Treatments		MGT (d)	T ₅₀ (d)	GI	GE (%)	FGP (%)	Radicle length (cm)	Plumule length (cm)
<i>Xiangyaxiangzhan</i>	CK	4.53±0.02c	1.50±0.03c	77.25±1.57a	98.67±0.67a	98.67±0.67ab	5.35±1.06bc	4.27±0.16b
	F1	4.53±0.02c	1.52±0.03c	77.56±0.96a	99.33±0.67a	99.33±0.67a	7.68±0.59a	5.72±0.71a
	F2	4.50±0.02c	1.48±0.02c	79.22±0.96a	99.33±0.67a	99.33±0.67a	5.98±0.25b	5.32±0.42a
	F3	5.19±0.05b	2.75±0.07b	43.60±2.23b	76.67±4.67b	94.00±2.31b	4.37±0.64cd	3.09±0.44c
	F4	5.32±0.02a	3.28±0.04a	40.38±1.74b	76.59±3.33b	95.33±2.40ab	3.58±0.41d	3.70±0.49bc
<i>Meixiangzhan</i>	CK	4.63±0.04c	1.63±0.08c	65.15±2.83a	88.00±2.00a	90.00±1.15a	6.12±1.06a	4.68±0.40a
	F1	4.72±0.08c	1.80±0.13c	57.62±5.34a	82.67±6.36a	85.33±3.71a	2.92±0.35b	3.20±0.73bc
	F2	4.69±0.04c	1.75±0.08c	58.09±4.73a	83.33±4.81a	84.00±4.16a	4.42±0.71b	4.30±0.49ab
	F3	5.45±0.08b	3.63±0.27	14.71±0.83b	24.67±4.06b	38.67±0.67b	4.38±0.43b	2.41±0.44c
	F4	5.80±0.04a	4.38±0.03	9.56±1.24b	10.67±0.67c	34.00±4.00b	4.51±0.92ab	3.22±0.74bc
<i>Changnongjing 1</i>	CK	4.58±0.04c	1.55±0.02d	59.23±1.50a	76.67±3.71a	79.33±4.37a	7.48±0.73a	3.72±0.18a
	F1	4.69±0.06b	1.76±0.09c	48.81±2.36b	71.33±5.70a	71.33±5.70abc	5.80±0.56b	2.22±0.13b
	F2	4.63±0.02bc	1.64±0.02cd	55.03±3.56ab	76.00±4.16a	76.00±4.16ab	5.50±1.03b	2.28±0.32b
	F3	5.16±0.03a	2.70±0.05b	28.79±2.31c	50.67±4.06b	60.67±2.96c	2.37±0.21c	1.68±0.33c
	F4	5.23±0.02a	2.95±0.06a	28.87±2.01c	58.00±4.16b	63.33±5.21bc	2.12±0.04c	1.05±0.17d
<i>F2 generation of Yliangyou1173</i>	CK	4.76±0.03b	1.81±0.06c	39.15±2.48a	3.20±0.40ab	62.00±6.24a	4.33±0.85a	3.20±0.40ab
	F1	4.83±0.06b	2.14±0.12b	29.90±3.54b	3.98±0.46a	48.00±4.04b	4.32±0.75a	3.98±0.46a
	F2	4.81±0.06b	2.08±0.10bc	26.00±1.92b	3.03±0.61b	42.00±4.62bc	3.33±0.77ab	3.03±0.61b
	F3	5.13±0.02a	2.67±0.02a	15.68±1.25c	3.15±0.33ab	32.00±2.08cd	3.10±0.29ab	3.15±0.33ab
	F4	5.11±0.06a	2.65±0.08a	12.48±1.24c	2.73±0.15b	25.00±1.53d	2.40±0.21b	2.73±0.15b

Figures not sharing the same letters differ significantly at P = 0.05 (the same below). FGP: final germination percentage

Seedling quality

Flooding treatments reduced the seedling quality of volunteer rice significantly. The trend of seedling rate was recorded as: CK > F1 > F2 > F3 > F4 for both *Xiangyaxiangzhan* and *Meixiangzhan* (Table 2). In treatments F1 and F2 compared with CK, seedling rate was 81.51% and 81.94% lower in *Changnongjing 1*, respectively, while 43.55 and 57.98 lower in *F2 generation of Yliangyou1173*, respectively. No seed could grow or develop into a whole seedling in F3 or F4 for either *Changnongjing 1* or *F2 generation of Yliangyou1173*. Moreover, dry weights were 5.2%, 10.09%, 45.15% and 39.58% lower in F1, F2, F3 and F4 respectively for *Xiangyaxiangzhan*, respectively. For *Meixiangzhan*, the trend of dry weight was recorded as: CK > F1 > F2 > F3 > F4. Both F1 and F2 decreased the dry weight in *Changnongjing 1* and *F2 generation of Yliangyou1173*.

MDA contents and anti-oxidant responses of seedlings

Flooding treatments influenced the anti-oxidative activities in terms of POD, SOD and CAT, and caused oxidative damage by increasing lipid per-oxidation (MDA content). For *Xiangyaxiangzhan*, MDA contents in F1, F2, F3 and F4 were 19.39%, 10.20%, 36.73% and 64.29% higher, respectively. POD and CAT activities in F1 and F2 all were higher than CK while lower in F3 and F4. Similar discussion was found in *Meixiangzhan*. For *Meixiangzhan*, MDA contents were 12.33, 11.53, 37.09 and 85.11% higher in F1, F2, F3 and F4, respectively. Highest and lowest SOD activity were recorded in F2 and F4, respectively. For *Changnongjing 1* and *F2 generation of Yliangyou1173*, both F1 and F2 treatments reduced the activities of POD, SOD and CAT while promoting the production of MDA (Table 3).

Table 2. Effect of flood treatments on seedling quality of volunteer rice

Treatments		Seedling rate (%)	Fresh weight (mg)	Dry weight (mg)
<i>Xiangyaxiangzhan</i>	CK	98.67±0.67a	63.43±1.26a	9.22±0.21a
	F1	88.67±1.77b	57.87±1.41ab	8.75±0.13a
	F2	65.67±3.48c	64.50±0.79a	8.29±0.29a
	F3	64.33±3.48c	45.80±1.01c	5.06±0.35b
	F4	48.67±3.71d	53.28±4.89bc	5.57±0.90b
<i>Meixiangzhan</i>	CK	90.00±1.15a	97.92±1.81a	15.62±0.61a
	F1	74.00±6.11b	83.81±2.34ab	11.37±0.28b
	F2	68.00±4.00b	78.55±3.96b	10.30±0.12b
	F3	26.00±1.56c	66.88±5.21b	7.81±0.58c
	F4	18.67±2.52c	72.12±10.69b	6.86±0.54c
<i>Changnongjing 1</i>	CK	79.33±4.37a	105.62±2.13a	14.58±1.34a
	F1	14.67±0.67b	62.29±1.72b	8.48±0.38b
	F2	14.33±2.33b	57.45±2.01c	7.86±0.12b
	F3	0	-	-
	F4	0	-	-
<i>F2 generation of Yliangyou1173</i>	CK	62.00±6.24a	85.24±1.88a	8.80±0.31a
	F1	35.00±2.65b	32.31±1.45b	3.48±0.28b
	F2	26.05±2.10b	34.19±1.10b	3.35±0.24b
	F3	0	-	-
	F4	0	-	-

Table 3. Effects of flood treatments on MDA contents and anti-oxidant responses of seedlings

Treatments		POD (U·g ⁻¹ ·min ⁻¹)	SOD (U·g ⁻¹)	CAT (U·g ⁻¹ ·min ⁻¹)	MDA (umol·g ⁻¹)
<i>Xiangyaxiangzhan</i>	CK	124.33±2.60c	178.67±2.91b	160.59±2.91c	3.27±0.12c
	F1	133.67±0.89b	185.67±2.02ab	169.25±1.54b	3.90±0.17bc
	F2	145.02±4.04a	193.33±2.34a	179.32±3.13a	3.60±0.06c
	F3	105.33±2.73d	164.79±3.18c	143.10±2.39d	4.47±0.26b
	F4	89.33±1.45e	147.15±4.09d	125.61±1.70e	5.38±0.24a
<i>Meixiangzhan</i>	CK	113.33±2.71c	201.74±4.09b	153.59±3.80c	3.76±0.14c
	F1	122.09±1.08b	213.81±3.78a	162.25±1.64b	4.22±0.20bc
	F2	134.15±3.06a	216.51±2.81a	172.32±4.37a	4.19±0.13bc
	F3	94.33±2.13d	187.17±2.17c	136.10±2.80d	5.15±0.30b
	F4	78.33±0.96e	174.00±1.53d	118.61±2.86e	6.96±0.28a
<i>Changnongjingone</i>	CK	154.33±2.19a	238.46±3.11a	190.59±4.05a	2.11±0.03c
	F1	131.25±1.45b	164.12±5.17b	137.00±4.16b	6.40±0.29b
	F2	104.67±1.86c	128.00±5.19c	118.76±4.36c	7.77±0.19a
	F3	-	-	-	-
	F4	-	-	-	-
<i>F2 generation of Yliangyou1173</i>	CK	107.67±1.91a	143.00±2.08a	132.85±2.08a	5.13±0.15c
	F1	84.33±2.91b	115.84±2.09b	106.00±1.33b	8.45±0.19b
	F2	67.61±1.79c	97.55±4.10c	87.63±3.14c	10.08±0.38a
	F3	-	-	-	-
	F4	-	-	-	-

Correlation analysis

Seedling rate was significantly and positively correlated with dry weight, POD, SOD, CAT, GI, GE, and FGP (Table 4). Significant positive correlations were also observed among GI, GE and FGP, but negative correlations were found between MDA and seedling rate and between T₅₀ and FGP.

Table 4. Relationship between seedling rate, anti-oxidant responses and seed bioassay

Index	Seedling rate	Dry weight	POD	SOD	CAT	MDA	GI	GE	T ₅₀	FGP
Dry weight	0.600*									
POD	0.585*	0.679**								
SOD	0.585*	0.805**	0.749**							
CAT	0.742**	0.731**	0.919**	0.904**						
MDA	-0.778**	-0.715**	-0.754**	-0.878**	-0.920**					
GI	0.759**	0.526*	0.797**	0.452	0.735**	-0.597*				
GE	0.735**	0.466	0.749**	0.423	0.699**	-0.605*	0.962**			
T ₅₀	-0.522*	-0.424	-0.650**	-0.155	-0.449	0.212	-0.807**	-0.732**		
FGP	0.739**	0.394	0.666**	0.424	0.676**	-0.659**	0.884**	0.967**	-0.554*	
Plumule length	0.716**	0.263	0.413	0.357	0.543*	-0.482	0.644**	0.560*	-0.382	0.555*

Significant correlations as *P < 0.05 and **P < 0.01

Discussion

Volunteer rice is a global problem of rice production, especially in conservation tillage. In the United States, areas of rice growing normally begin to be flooded from early autumn after the rice harvest until the tillage operations in spring (Fogliatto et al., 2010) because anaerobic environment could limit the germination and growth of abundant weeds such as weedy rice (Bird et al., 2002). The present study confirms this view by showing the decline of emergence properties of volunteer rice in flooding treatments. Specifically, the germination speed slowed as the water layer increased, especially F3 and F4. Once the water layer was over 4.5 cm, the time to start germination was extended and the quantity of eventually germinated volunteer rice was weakened. Flooding treatment significantly altered the germination characteristics of volunteer rice. Since the seed germination and seedling establishment phase is highly vulnerable to injury, disease and stress, we believe this phase is a key time period to prevent harvest loss caused by volunteer rice. Germination vigor is driven by the ability of plant embryos, embedded in seeds, to resume the metabolic activity in a coordinated and sequential manner (Rajjou et al., 2012). The germination vigor can be evaluated using many indexes. We observed that as water layer rose in flooding treatments, MGT and T₅₀ were prolonged for most rice varieties while the GI and GE reduced. Both F4 and F3 significantly influenced volunteer rice germination by minimizing the germination rate, and drastically declined both radicle and plumule lengths for all rice varieties. These results agreed with Prakash (Rajjou et al., 2012) who found rice germination was further reduced with the rise of submergence levels. In addition, the volunteer rice from *Xiangyaxiangzhan* was strongly tolerant against submergence. The FGPs in F1, F2, F3 and F4 were all not significantly different from those in CK. Thus,

Xiangyaxiangzhan might be one of the tolerant genotypes against flooding stress. The flooding tolerance during germination is a complicated process involving many mechanisms. Ismail already identified several genotypes with higher flooding tolerance during germination (Ismail et al., 2009). However, the submergence tolerance during germination is associated with the flooding tolerance during the vegetative stage, as reported in the sensitivity of FR13A, a landrace that is most tolerant of complete inundation during this stage (Ella et al., 2003; Jackson and Ram, 2003).

We also noticed flooding treatment greatly reduced seedling rate and dry matter weight. As reported similarly, flooding treatments decreased root, shoot and total dry matter production in all rice cultivars and with more reduction in higher than lower flooding levels (5 vs. 1 cm) (Prakash et al., 2016a). These results indicate the seeds of volunteer rice even under the flooding environment could germinate successfully, but the chance of developing into whole normal seedlings was very low, which was greatly embodied by the zero seedling rates of both *Changnongjing 1* and *F2 generation of Yliangyou1173* in treatments F3 and F4. After 3 weeks of treatment, MDA production in volunteer seedlings was enhanced with the increasing submergence level. The MDA over-accumulation might be attributed to oxidation of polyunsaturated fatty acids (e.g., linoleic acids, galacto- and phospho-lipid components), which are critical in the functional and structural characteristics of thylakoid membranes and the arrangement of reaction centers of photosystems (Elshintinawy, 2000). MDA also reacts with free amino acids to even form ethylene in cellular membranes (Rakwal et al., 2003; Jiang and Zhang, 2002). Thus, MDA accumulation is a key indicator of oxidative stress, which is characteristic of intercellular and intracellular membranes and hence intensifies the ion leakage through cell membranes (Dash and Mohanty, 2002). In this study, the improvement of MDA content meant flooding treatment caused submergence stress and some oxidative damage to volunteer seedlings. Furthermore, the anti-oxidative enzymatic activities all differed among the flooding conditions. Anti-oxidant plays an important role in quenching reactive oxygen species. For example, when superoxide radicals accumulate in organisms, SOD is responsible for breakdown and then POD and CAT are involved in scavenging H₂O₂ (Sairam et al., 2000). In the present study, treatments F1 and F2 both enhanced the activities of POD, SOD and CAT for both *Xiangyaxiangzhan* and *Meixiangzhan*, whilst F3 and F4 greatly decreased the anti-oxidative enzymatic activities. The main reason might be that lower flooding level (1.5-3.5 cm) already caused oxidative stress to rice seedlings, but the damage could be mitigated by the anti-oxidant regulation of seedlings. Thus volunteer seedlings under lower submergence level would activate the anti-oxidation mechanism in order to prevent the oxidant damage caused by flooding stress. However, under higher flooding level (4.5-6.5 cm), the submergence stress during germination and seedling development already caused irreversible damage and even destroyed the anti-oxidative system, so that the POD, SOD and CAT activities decreased while the MDA content increased greatly. Moreover, *Changnongjing 1* and *F2 generation of Yliangyou1173* were sensitive to flooding stress so that the anti-oxidative enzymatic activities declined in F1 and F2.

Conclusion

In conclusion, long-term (2 weeks) flooding treatment significantly limited or even prevented the germination and growth of volunteer rice, while treatment F4 (6.0-6.5 cm)

was the most effective because of the lowest seedling rate. In order to prevent the harvest loss in late season rice caused by volunteer rice and further investigation should be at the field trials, the possible effects of flooding on the late-season rice production should be studied before the real application.

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APPENDIX

