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Full Length Research Paper

Germination rates of hybrid seeds of rice (*Oryza sativa* L) Zhuliangyou 02 with different treatments of dehydration, storage and soaking

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Zhuliangyou 02 is a hybrid rice from Zhu 1S, as the female, and ZR02, as the male. We studied the germination rates of Zhuliangyou 02 under different treatments including dehydration, storage variation, and seed soaking, and used Lu 18S/ZR02, H155S/Hua 284, Zhu 1S, and Nipponbare as controls. The results obtained show that the rates of dehydration of air-drying and cold-drying were the quickest and slowest. The germination rate of fresh seeds of Zhuliangyou 02 was higher than the stored seeds and it decreased after aging treatment. Taken together, Zhuliangyou 02 seeds were not resistant to high temperature, high moisture, or long storage. The harvested seeds would exhibit longer lives and improve germination rates if they were dehydrated by quick air-drying at 35°C, stored in air-proof container and intermission soaking performed.

Key words: Zhuliangyou 02, dehydration, storage, seed soaking, germination rates.

INTRODUCTION

Germination rate is an important index of the quality of rice seeds and the yield of rice. The hybrid rice seeds with high quality have stronger metabolism superiority and higher vigor index than the conventional rice seeds in the germination stage (Chen, 1993). However, hybrid rice seeds generally are less tolerant to storage and have lower germination rates than the conventional rice seeds (Chen, 1994; Jiang et al., 1996; Zhang et al., 1998; Lu, 2000). It has been demonstrated that soaking time and temperature of soaking water affect different types of rice seeds and their germination rates (Zhang and Zhu, 2002) and that the low vigor seeds are especially sensitive to the soaking methods (Yan and Zhang, 2000). The optimal soaking time for the hybrid rice seeds are shorter than that of the conventional rice (Zhou and Ou, 2004). Different treatments of dehydration and storage also affect germination rates (Xia et al., 2007). As a result, the same seeds might have different germination rates under different treatments of dehydration, storage and soaking.

Zhu 1S is the most genetically stable male sterile rice line in China and has desired ability of combination currently. Two and eight combinations have passed the national certification and the provincial approval, respectively. Due to the obvious advantages, the application of the combinations have been generalized and used for many years. One drawback of using Zhu 1S as the female parent in combinations is the reduced germination rate after dehydration and storage. Thus, it is important to address the lower seed germination rate of Zhu 1S combinations in order to reduce the economic loss and fully exploit the advantage of its ability of combination. The study explored the methods to improve the quality and germination rates of the seeds of Zhuliangyou 02, hybrid of Zhu 1S and ZR02, in order to provide important clues to improve germination rates and increase yield of hybrid rice with Zhu 1S combinations.

MATERIALS AND METHODS

The rice seeds of Zhuliangyou 02, Lu 18S/Z R02, H 155S/Hua 284, Zhu 1S, and Nipponbare were provided by the Academy of Seed Science of Hunan Yahua. Dehydration of seeds according to Huang et al. (2010): Seeds were harvested and dehydrated at the same

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Table 1. Dehydration time (h) under different dehydrating conditions.

Treatment	Air- drying	Oven-drying	Sun- drying	Shade-drying (d)	Cold -drying
Time	6 ± 0.24 E	8 ± 0.42 D	9 ± 0.57 C	120 ± 12.45B	216 ± 24.12A

day with different methods as subsequently indicated. The seeds were fast air-dried in blast drying oven under 35.0°C (air-drying), fast dried in drying oven under 35.0°C (oven-drying), fast dried under the sun (the temperature ranged from 28.6 to 32.3°C) with seeds monolayer (sun-drying), dried in shade in a room (the temperature was 25.9 to 27.1°C, shade-drying) and dried in the desiccators with silica gel followed by drying at 4.0°C in the refrigerator (cold-drying), respectively. The water content of the seeds was lower than 12.5% when each processing was finished.

According to Huang et al. (2010): as regards seed storage, sun-dried seeds were used for the storage experiment. Seeds were placed in sealed plastic bags and kept on a shelf at room temperature (conventional storage), placed in sealed bags and kept above silica gel in a desiccators that were on a shelf at room temperature (dry storage) and placed in sealed bags that were kept in a refrigerator at 4°C (cold storage), respectively.

Seed soaking according to Ou et al. (2007): Sun-dried seeds without storage were used for the soaking experiment. The seeds were wrapped with two layers of gauze, soaked in water, and took out at different time points to accelerate germination (directly seed soaking), or wrapped with two layers of gauze, soaked in water for 3 h, took out for air dry with the gauze cauda still in the water to keep absorbing water, and soaked again after 3 h. Different circulation was performed according to request (intermittent seed soaking).

Seed vitality: Sun dried seeds were used for the vitality experiment according to the TCC method (Li, 2001).

Germination rates according to Zhou and Ou (2004): One hundred seeds were soaked 24 h using intermittent seed soaking method at 30 ±1°C for 7 d. Germination rates were determined based on data from the averages of three repeats.

Aging according to Zhou and Ou (2004): Aging was determined by measuring the germination rates of seeds at 40°C and 100% relative humidity for 4d and 7d.

Activity of dehydrogenase according to Ou et al. (2007): Sun-dried seeds were used to measure the activity of dehydrogenase. Half gram of seed embryo was treated with 5 ml 1% TTC for 3 h at 37°C followed with an addition of 5 ml acetone for 3 h at 37°C. The extraction was colorimetric analyzed under 480 nm wave. Every other 50 d for 5 times and optical density of embryo per gram expressed enzyme activity.

RESULTS

Dehydration rates with different drying methods

The rates of dehydration as measured by the descent of seed water, differed with dehydrated methods (Table 1), with the quickest and slowest to be air-drying and cold-drying, respectively, and oven-drying to be in the middle.

Germination rates with different dehydrated methods

The germination rates of different seeds varied according to the methods of dehydration after storing using the conventional method (Table 2). The germination rate of fresh seeds of Zhuliangyou 02 was higher than the stored seeds, indicative of the lack of dormancy. However, the other seeds had the characteristics of dormancy evident by the highest germination rate of seeds from Lu 18S/ ZR02 after 100 d storage.

The different methods of dehydrating seeds had obvious effects on storage tolerance and germination rates. The seeds dehydrated by air-drying with the shortest time had the strongest storage tolerance, followed by the seeds dehydrated by oven-drying or sun-drying; the shade-drying seeds had little storage tolerance and low germination rates. The methods of dehydrating most notably affected germination of the seeds for Zhuliangyou 02 which had no characteristics of dormancy, but had little effect on seeds with characteristics of dormancy. All seeds with cold-drying had low germination rates.

Change of germination rate under different storage methods

The germination rates of seeds of Zhuliangyou 02 always decreased after storage, whereas germination rates of other seeds increased first and then decreased after storage using different storage methods (Table 3). The seeds had different germination rates under different storage conditions, besides, the seeds germination rates under dry storage and cold storage, which were notably higher than those stored under conventional conditions.

Seeds vitality of Zhuliangyou 02 under sun-drying

The seed vitality changed little during the storage in the first 50 d, suggestive of the best storage time. The quantities of seeds with strong and weak vitality reduced and increased, respectively, after 50 d of storage (Figure 1). The percentage of germinated seeds was always smaller than that of seeds with vitality, implicating some seeds with weak vitality had only the trend of germination.

Effects of aging seeds on germination using the conventional storage time

Germination rates of different seeds were affected differently after aging treatment (Table 4). The germination rates of the fresh seeds of Zhuliangyou 02 decreased 21.4 and 25.9% after aging 4 and 7 d, respectively. The vitality of seeds was also reduced. The

Table 2. Germination rates (%) of conventional storage under different dehydrating conditions.

Material	Dehydrating method	Storage time /d				
		0	50	100	150	200
Zhuliangyou 02	Air -drying	88.2 ± 3.2	85.9 ± 5.2	83.1 ± 5.0	82.2 ± 3.6	79.8 ± 4.5
	Oven-drying	87.6 ± 3.6	85.2 ± 3.1	81.8 ± 3.0	79.5 ± 5.1	76.3 ± 2.1
	Sun-drying	86.5 ± 3.5	84.4 ± 2.6	79.4 ± 3.4	76.2 ± 4.4	72.3 ± 2.6
	Shade-drying	68.8 ± 2.8	64.6 ± 2.9	58.4 ± 2.9	56.5 ± 2.3	50.4 ± 3.2
	Cold- drying	4.2 ± 2.3	4.7 ± 3.8	6.1 ± 3.6	4.2 ± 3.2	2.3 ± 1.5
Lu18S/ZR02	Air -drying	75.5 ± 3.6	87.9 ± 6.4	86.5 ± 4.1	82.1 ± 5.1	81.6 ± 6.9
	Oven-drying	74.8 ± 4.8	85.4 ± 5.2	84.8 ± 7.6	80.8 ± 5.2	79.9 ± 2.6
	Sun-drying	76.3 ± 4.1	86.5 ± 4.4	84.5 ± 5.4	79.4 ± 4.6	76.9 ± 5.1
	Shade-drying	72.3 ± 3.2	73.4 ± 3.6	65.8 ± 4.2	62.3 ± 3.2	58.2 ± 2.5
	Cold- drying	2.3 ± 2.1	12.7 ± 1.8	8.6 ± 3.8	5.7 ± 3.6	2.0 ± 0.6
H155S/Hua284	Air -drying	53.1 ± 3.6	95.4 ± 6.8	97.8 ± 4.8	94.3 ± 5.9	87.6 ± 4.8
	Oven-drying	51.6 ± 5.4	92.3 ± 7.4	95.3 ± 5.5	93.9 ± 8.5	85.7 ± 7.6
	Sun-drying	48.1 ± 2.7	92.4 ± 5.2	92.6 ± 7.4	87.5 ± 5.7	82.1 ± 4.8
	Shade-drying	50.4 ± 2.8	81.7 ± 5.9	85.8 ± 5.4	74.4 ± 3.2	71.2 ± 3.6
	Cold- drying	2.3.0 ± 2.4	12.9 ± 2.0	12.3 ± 1.2	4.2 ± 0.9	3.2 ± 0.7
Zhu 1S	Air -drying	61.4 ± 4.2	97.9 ± 8.4	97.5 ± 5.7	95.4 ± 8.7	94.5 ± 7.8
	Oven-drying	61.9 ± 2.5	94.3 ± 7.6	93.8 ± 6.3	90.6 ± 2.5	90.1 ± 6.5
	Sun-drying	59.5 ± 3.6	87.4 ± 4.2	89.8 ± 7.4	85.6 ± 5.6	83.9 ± 4.2
	Shade-drying	58.3 ± 3.6	82.1 ± 5.9	81.7 ± 4.1	76.5 ± 3.6	68.5 ± 4.3
	Cold- drying	8.6 ± 1.4	25.7 ± 2.3	28.6 ± 1.5	22.3 ± 2.4	15.9 ± 2.1
Nipponbare	Air -drying	43.2 ± 3.2	91.7 ± 7.5	95.6 ± 7.5	91.9 ± 7.6	89.5 ± 7.6
	Oven-drying	42.8 ± 4.6	90.6 ± 4.7	94.4 ± 6.4	88.9 ± 5.8	86.6 ± 5.3
	Sun-drying	42.6 ± 2.5	89.7 ± 5.1	92.6 ± 7.6	86.6 ± 6.2	81.4 ± 4.2
	Shade-drying	44.6 ± 2.5	81.5 ± 3.6	78.7 ± 4.1	76.8 ± 3.2	70.6 ± 3.6
	Cold- drying	21.0 ± 2.3	21.8 ± 2.1	7.6 ± 2.5	7.4 ± 1.2	3.8 ± 2.1

biggest decrease of germination rates was more than 40%. The germination rates of fresh seeds of Lu 18S/ZR02 had little difference from the control, suggesting that the former was resistant to aging. The germination rates of other seeds did not decline but rather increased after aging, suggesting that the aging treatment broke dormancy and promoted physiological maturation. When seeds were stored for 100 d, the dormancy was finished accompanied with high temperature and humidity and decreased germination rates, though with smaller changes than Zhuliangyou 02.

Effects of soaking methods on germination rates using different soaking time

The germination rates of seeds using the method of intermittent soaking were always higher than using the approach of direct soaking in each time point of soaking, with the average and highest values to be 2.11 and 6.71%, respectively (Figure 2). The best soaking time was 24 h in which the seeds showed quick water absorption and high efficiency to germinate promptly.

Dehydrogenase activity of seeds of Zhuliangyou 02

The dehydrogenase activity reflects the reducing power of seeds and is an important physiological index for seed metabolism. The dehydrogenase activity of seeds treated with intermittent soaking was higher than that with direct soaking under different storage time, with the average and the highest values to be 0.07 and 0.104, respectively. Collectively, these data indicated that the seeds with intermittent soaking had high reducing power, which directly reflected higher germination rates (Figure 3).

DISCUSSION

Except for the recalcitrant seeds, decreasing water

Table 3. Germination rates (%) of sun-drying seeds under different storage conditions.

Material	Storage method	Storage time /d				
		0	50	100	150	200
Zhuliangyou 02	Conventional storage	86.5 ± 3.5	84.4 ± 2.6	79.4 ± 3.4	76.2 ± 4.4	72.3 ± 2.6
	Dry storage	86.5 ± 4.3	85.4 ± 3.5	83.4 ± 5.4	80.5 ± 5.1	78.3 ± 3.5
	Cold storage	86.5 ± 3.9	85.3 ± 4.6	82.8 ± 6.3	81.2 ± 4.5	78.1 ± 4.3
Lu 18S/ZR02	Conventional storage	76.3 ± 4.1	86.5 ± 4.4	84.5 ± 5.4	79.4 ± 4.6	76.9 ± 5.1
	Dry storage	76.3 ± 4.3	87.8 ± 3.5	88.4 ± 8.4	85.6 ± 6.2	82.3 ± 5.2
	Cold storage	76.3 ± 2.9	88.6 ± 5.1	88.7 ± 7.6	84.6 ± 5.4	83.5 ± 4.6
H155S/Hua 284	Conventional storage	48.1 ± 2.7	92.4 ± 5.2	92.6 ±7.4	87.5 ± 5.7	82.1 ± 4.8
	Dry storage	48.1 ± 3.0	92.9 ± 4.9	93.2 ± 8.1	91.4 ± 7.4	87.1 ± 6.4
	Cold storage	48.1 ± 3.4	92.6 ± 5.3	93.2 ± 6.3	90.4 ± 7.0	87.8 ± 5.1
Zhu 1S	Conventional storage	59.5 ± 3.6	87.4 ± 4.2	89.8 ± 7.4	85.6 ± 5.6	83.9 ± 4.2
	Dry storage	59.5 ± 3.8	92.8 ± 5.4	92.9 ± 5.2	89.6 ± 6.5	87.6 ± 3.1
	Cold storage	59.5 ± 2.9	92.2 ± 4.7	93.5 ± 8.4	88.7 ± 3.4	88.3 ± 5.4
Nipponbare	Conventional storage	42.6 ± 2.5	89.7 ± 5.1	92.6 ± 7.6	86.6 ± 6.2	81.4 ± 4.2
	Dry storage	42.6 ± 1.9	92.9 ± 5.3	91.8 ± 7.4	90.7 ± 7.5	86.6 ± 6.1
	Cold storage	42.6 ± 2.7	92.6 ± 4.7	92.7 ± 4.6	89.9 ± 4.9	87.3 ± 5.8

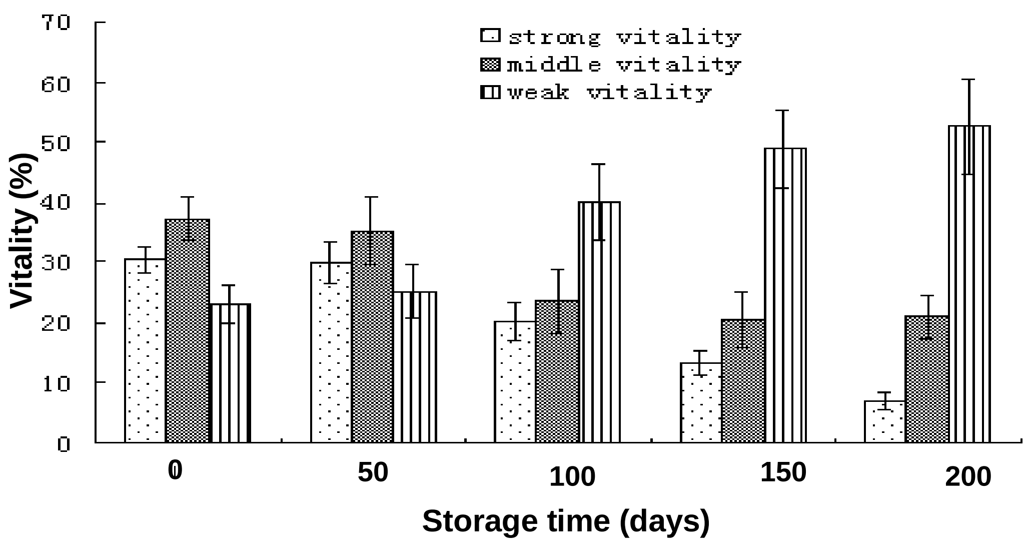

Figure 1. Vitality of Zhuliangyou 02 at different storage time.

content by dehydration is the most effective measure to improve the storability of rice seeds. The current study showed that different dehydrating methods had significant effects on storability of hybrid rice seeds. The negative effects of seeds using dehydration in 4°C were most significant, possibly caused by the injury of chilling and destruction of cell structure. Zhuliangyou 02, which were less tolerant of storage, would easily be deteriorated metabolically which can lead to decreased activity if they had high water content too long during the process of dehydration in room temperature. The air blast drying in 35°C was the quickest dehydration method and beneficial for inhibition of metabolism. However, it was not realistic to air dry at 35°C in production. Thus, sun drying was still the best dehydration method for the seeds that were unable to tolerate storage.

It has been shown that rice seeds with the characteristics of dormancy are slightly more advantageous in

Table 4. Germination rate of speedy sunning seeds with aging treatment under different storage times.

Material	Aging time/d	Germination rate (%)				
		0	50	100	150	200
Zhuliangyou 02	0	86.5 ± 5.3	84.4 ± 7.4	79.4 ± 4.0	76.2 ± 5.8	72.3 ± 5.1
	4	65.1 ± 3.8	59.7 ± 6.3	52.8 ± 2.8	46.6 ± 5.4	44.3 ± 1.5
	7	60.6 ± 5.1	51.5 ± 4.8	42.1 ± 3.6	35.9 ± 4.3	31.6 ± 2.6
Liu 18S/ZR02	0	76.3 ± 3.0	86.5 ± 6.7	84.5 ± 4.8	79.4 ± 8.7	76.9 ± 5.4
	4	76.9 ± 4.2	80.1 ± 4.7	72.8 ± 1.7	66.0 ± 3.4	63.6 ± 3.8
	7	70.4 ± 5.7	72.8 ± 5.8	60.6 ± 3.9	50.5 ± 2.9	46.4 ± 3.8
H155S/Hua 284	0	48.1 ± 2.3	92.4 ± 6.9	92.6 ± 6.8	87.5 ± 7.0	82.1 ± 2.9
	4	64.2 ± 4.9	83.4 ± 5.7	79.2 ± 3.4	72.7 ± 4.8	65.6 ± 3.6
	7	62.3 ± 6.1	78.2 ± 4.8	65.8 ± 6.8	58.7 ± 2.8	52.3 ± 2.7
Zhu 1S	0	59.5 ± 2.3	87.4 ± 7.8	89.8 ± 5.9	83.6 ± 5.9	81.9 ± 4.8
	4	82.3 ± 7.3	82.8 ± 4.6	78.6 ± 1.2	71.3 ± 12.3	65.0 ± 5.7
	7	82.5 ± 4.0	79.0 ± 6.4	73.5 ± 4.9	65.8 ± 7.8	60.5 ± 2.4
Nipponbare	0	42.6 ± 3.7	89.7 ± 8.7	92.6 ± 5.7	86.6 ± 5.7	81.4 ± 4.9
	4	64.7 ± 2.1	82.4 ± 6.9	81.6 ± 1.0	73.5 ± 3.1	65.3 ± 6.1
	7	62.9 ± 5.1	77.3 ± 4.2	72.8 ± 3.5	64.4 ± 2.2	53.2 ± 2.3

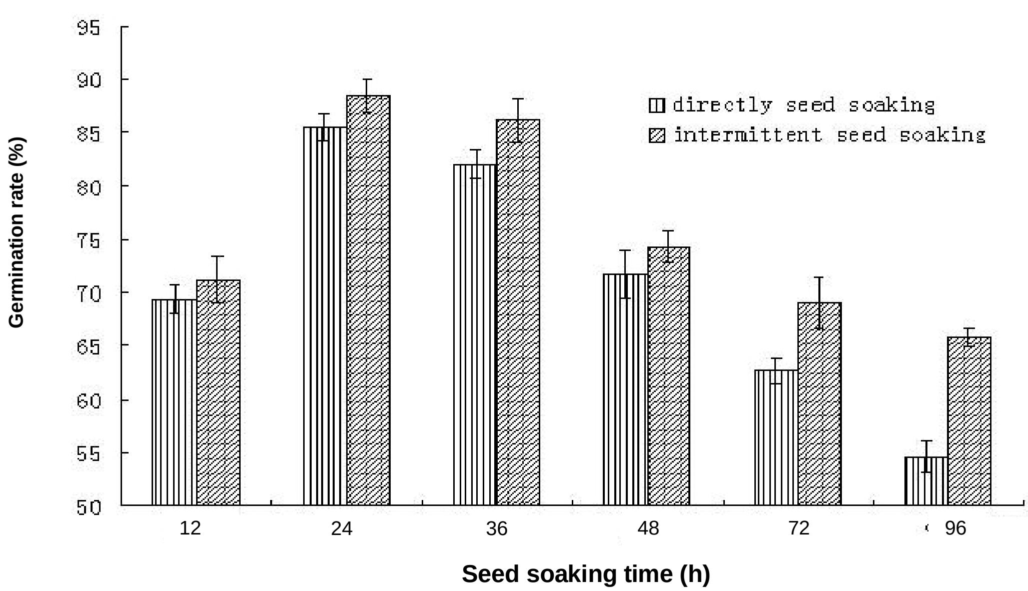

Figure 2. Germination rate of Zhuliangyou 02 at different soaking conditions.

storability than those with weak or no characteristics of dormancy and are also more resistant to aging (Dong et al., 2004). The aging treatments have already been widely used in studying the storage physiology and deterioration mechanism (Zeng et al., 2003). The aging treatment of seeds in high temperature of 40°C and relative humidity of 100% leads to abnormal metabolic rate of seeds and accelerates deterioration of seeds. Our results showed that fresh seeds of Zhuliangyou 02 were sensitive to aging treatment with dramatic decrease of germination rate. Thus, it is advisable to harvest seeds of Zhuliangyou 02 properly after maturation and avoid high temperature and high humidity since stack to thermal generation is the main cause of decreased germination

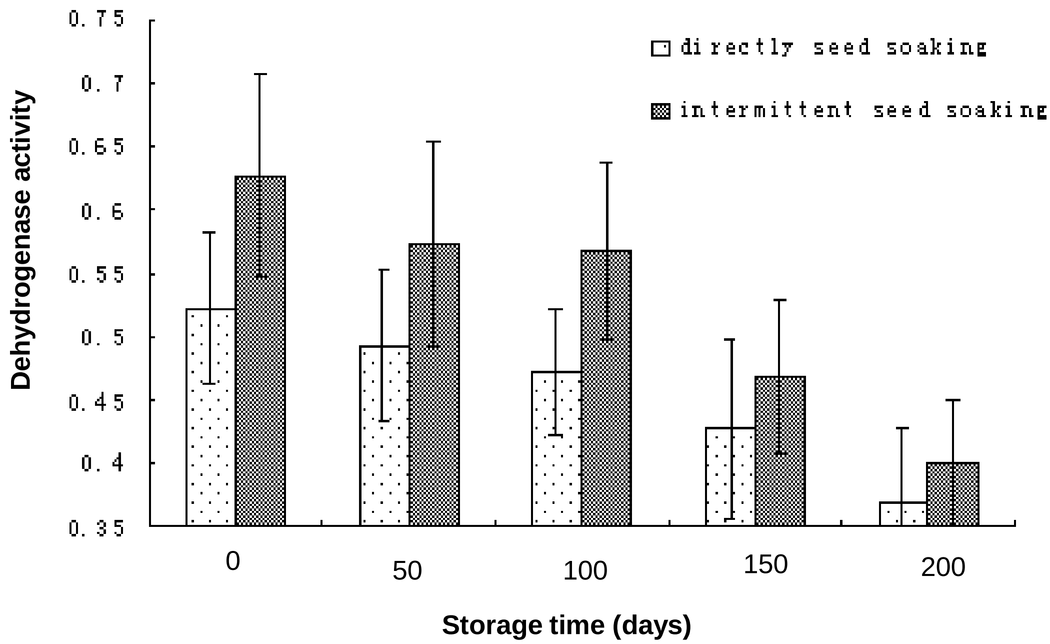

Figure 3. Dehydrogenase activity of Zhuliangyou 02 at different storage time.

rates and moisture content of seeds should not be higher than the safe moisture level.

Application of soaking methods improves germination rates. Our results suggest that intermittent soaking is a simple and desirable method for agricultural production, especially, for the hybrid seeds that are tolerant to storage and aging. Intermittent seed soaking could avoid the lengthy soaking of seeds directly in water, which lead to anaerobic respiration and consumption of excessive energy thus, affecting the supply of nutrition from sprouting to photosynthesis. Additionally, intermittent seed soaking could avoid excessive production of alcohol that affects seed germination.

In conclusion, it is advisable to take the appropriate methods of fast sun-drying, dry storage or cold storage, intermittent soaking and as short as possible of storage time to improve germination rates for rice seeds such as Zhuliangyou 02 that have no characteristics of dormancy and easy panicle sprouting.

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