Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Drying temperature affects rice seed vigor via gibberellin, abscisic acid, and antioxidant enzyme metabolism*#

Yu-tao HUANG[1], Wei WU[2], Wen-xiong ZOU[2], Hua-ping WU[3], Dong-dong CAO[†‡1,3]
[1]*Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*
[2]*Seed Management Station of Zhejiang Province, Hangzhou 310020, China*
[3]*Huzhou Keao Seed Co., Ltd., Huzhou 313000, China*
[†]E-mail: jellycao@163.com

Received June 3, 2020; Revision accepted July 12, 2020; Crosschecked Sept. 9, 2020

Abstract: Seed vigor is a key factor affecting seed quality. The mechanical drying process exerts a significant influence on rice seed vigor. The initial moisture content (IMC) and drying temperature are considered the main factors affecting rice seed vigor through mechanical drying. This study aimed to determine the optimum drying temperature for rice seeds according to the IMC, and elucidate the mechanisms mediating the effects of drying temperature and IMC on seed vigor. Rice seeds with three different IMCs (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. The results showed that the drying temperature and IMC had significant effects on the drying performance and vigor of the rice seeds. The upper limits of drying temperature for rice seeds with 20%, 25%, and 30% IMCs were 45, 42, and 38 °C, respectively. The drying rate and seed temperature increased significantly with increasing drying temperature. The drying temperature, drying rate, and seed temperature showed extremely significant negative correlations with germination energy (GE), germination rate, germination index (GI), and vigor index (VI). A high IMC and drying temperature probably induced a massive accumulation of hydrogen peroxide (H_2O_2) and superoxide anions in the seeds, enhanced superoxide dismutase (SOD) and catalase (CAT) activity, and increased the abscisic acid (ABA) content. In the early stage of seed germination, the IMC and drying temperature regulated seed germination through the metabolism of H_2O_2, gibberellin acid (GA), ABA, and α-amylase. These results indicate that the metabolism of reactive oxygen species (ROS), antioxidant enzymes, GA, ABA, and α-amylase might be involved in the mediation of the effects of drying temperature on seed vigor. The results of this study provide a theoretical basis and technical guidance for the mechanical drying of rice seeds.

Key words: Drying temperature; Rice; Seed vigor; Gibberellin acid (GA); Abscisic acid (ABA); Antioxidant enzyme
https://doi.org/10.1631/jzus.B2000297 **CLC number:** S351.1

1 Introduction

Rice is a key food crop, consumed by 80% of the global population. In China, it also serves as the primary cereal crop. In the field of agricultural production, seed quality represents an important factor that covers two aspects, namely, sowing quality and variety quality (Holdsworth et al., 2008). Sowing quality refers to aspects of seed quality related to field emergence, such as moisture, vigor, and cleanliness, whereas variety quality refers to aspects related to genetic features as reflected in purity and authenticity.

[‡] Corresponding author

[*] Project supported by the Key Research and Development Program of Zhejiang Province (No. 2019C02011) and the "San Nong Liu Fang" Science and Technology Cooperation Project of Zhejiang Province (No. 2018SNLF004), China

[#] Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B2000297) contains supplementary materials, which are available to authorized users

ORCID: Dong-dong CAO, https://orcid.org/0000-0002-2691-6143

In addition, seed vigor represents an integrated seed characteristic determining its capacity for rapid and neat emergence in the complicated field environment (Rajjou et al., 2012). Seed longevity is an important element of seed quality, dependent on genetic and physiological protection potential together with the conditions encountered in the process of storage (Dang et al., 2014). Seeds with high vigor are closely associated with high field productivity and emergence, whereas those with low vigor usually have reduced yields.

Moisture content is a vital factor influencing seed vigor, and should be maintained within a suitable range during long-term storage (Tangney et al., 2019; Zhang et al., 2019). Generally, harvested rice seeds have a high initial moisture content (IMC) ranging from 20% to 35% (He et al., 2015). This is because rice seeds are usually harvested during the rainy season (Bie et al., 2007; Hu et al., 2016). The high IMC might result in microorganism growth and premature germination, leading to deterioration in seed vigor. The optimal moisture content of rice seeds should be around 12%–14% (Jittanit et al., 2010a, 2010b). Nonetheless, a low IMC can induce cracks in seeds due to unwanted consumption of energy in the process of drying (Endoh et al., 2018). Therefore, it is important to investigate the optimum conditions for drying, so as to produce rice seeds with high vigor.

Seed drying is a non-linear process due to the long timeframe involved and its considerable complexity. Notably, the IMC, dryer design, drying temperature, and drying rate are vital parameters affecting seed vigor in response to hot air drying (Souza et al., 2015). Among them, drying temperature plays a key role in maintaining seed vigor, and significantly affects the drying rate and seed temperature (Uddin et al., 2016). Drying seed at a high temperature may induce damage, including stress cracks, which lowers germination and destroys specific enzymes (Igathinathane et al., 2008; Gawrysiak-Witulska et al., 2019). Drying at high temperature also decreases seed vigor through suppressing catabolism in the endosperm. Aquerreta et al. (2007) reported that drying rice seeds from an IMC of 18% to a moisture content below 13% with a 40 °C drying temperature by a heat pump dryer produced high-vigor rice seeds. Hasan et al. (2014) indicated that a low drying rate and drying temperature resulted in high seed vigor. In wheat seeds, a drying temperature at 40 °C gives rise to the highest germination rate (Jittanit et al., 2010a). The normal metabolic, subcellular, and turnover mechanisms of seeds become inactive when a seed is dried. Nonetheless, such mechanisms are reactivated in hydrated seeds, but the efficiency of reactivation is dependent on the accumulated damage (Zhou et al., 2018).

There have been a few studies on the mechanical drying of rice seeds, but research on the optimum drying conditions for rice seeds with different IMCs has not been reported. Moreover, the effects of drying conditions on rice seed vigor have not been examined. Therefore, this study aimed to determine the optimum drying temperature for rice seeds according to the IMC, and to elucidate the mechanism mediating the effects of drying temperature and IMC on seed vigor of rice. Rice seeds with three different IMCs (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. The effects of seed IMC and drying temperature on drying performance and seed vigor were tested in terms of germination capacity. The effects of IMC and drying temperature on reactive oxygen species (ROS), antioxidant enzymes, plant hormone, and amylase metabolism during drying and germination were also tested. The results of this study have theoretical and practical values for the design of drying technologies for rice seeds.

2 Materials and methods

2.1 Materials

Seeds of rice (*Oryza sativa* L.) “Zhehugeng 25” were used as the test materials in this study. This cultivar has been widely planted in Zhejiang Province, China, due to its high yield, better flavor, and wide adapt ability. Seeds with three different IMCs (20%, 25%, and 30%) were harvested on 5th, 18th, and 24th November, 2018, respectively. The IMC was measured using a corn moisture apparatus (GAC-2100AGRI, Tuopu, Hangzhou, China), with ten biological replications. The weather conditions during harvest time are shown in Table 1.

2.2 Drying experiment

Rice seeds with three different IMCs (20%, 25%, and 30%) were dried to the target moisture content

Table 1 Weather conditions during harvest time in Huzhou, Zhejiang Province, China

Date (year-month-day)	Air temperature (°C) (maximum/minimum)	Weather condition
2018-11-03	23/12	Sunny
2018-11-04	21/12	Sunny
2018-11-05	**18/11**	**Sunny**
2018-11-16	24/13	Overcast
2018-11-17	27/9	Overcast
2018-11-18	**19/5**	**Overcast**
2018-11-22	21/11	Light rain
2018-11-23	24/14	Overcast
2018-11-24	**23/8**	**Light rain**

Rice seeds with three different IMCs (20%, 25%, and 30%) were harvested on 5th, 18th, and 24th November, 2019, respectively. Harvest dates are shown in bold type. IMC: initial moisture content

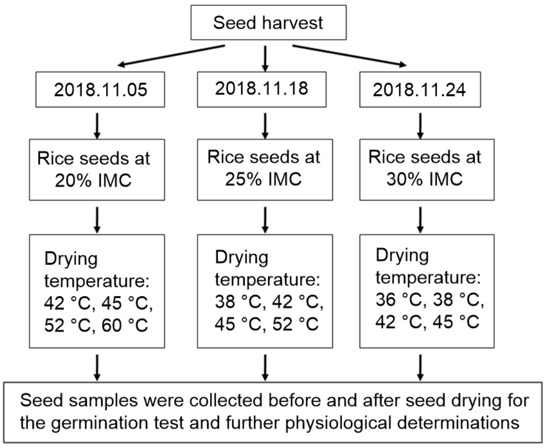

Fig. 1 Schematic diagram of the drying experiments
IMC: initial moisture content

(14%) at four different drying temperatures (Fig. 1). The seeds were dried using a Sanjiu low-temperature dryer (NEW PRO-120 H, Sanjiu, Shanghai, China). Each drying experiment used a sample size of 8 t. Seeds samples used for germination testing and further physiological determinations were collected before and after seed drying. The drying experiment at each drying temperature was performed with three biological replications. A temperature-humidity recorder (RS-WS-GPRS, Renke, Shandong, China) was installed for real-time detection of the drying air temperature and seed temperature during the drying process. The air temperature and humidity in the seed drying workshop were recorded every 6 h using a temperature and humidity instrument (TH602F, Anymetre, Shanghai, China). The drying rate was estimated based on the formula: drying rate=(IMC−14%)/drying time. Rice seeds from each treatment were collected from the head, middle, and tail of the Sanjiu low-temperature dryer, and uniformly mixed as a bulk seed sample following drying. The samples were stored in a closed container at room temperature.

2.3 Determination of seed germination and seedling characteristics

Rice seeds stored for 1 or 10 months were used for the standard germination test. One hundred seeds of each treatment were adopted for germination within the germination boxes (12 cm×12 cm×6 cm). Seeds were germinated at 25 °C, under a 12-h light/dark cycle for 14 d, with four replicates of each treatment. Seeds with a radicle length of 2 mm or more were considered germinated. The number of geminated seeds was calculated every day. The germination energy (GE) is the seed germination rate in the initial stage of the germination test. The germination percentage (GP) is the seed germination rate in the end stage of the germination test. The GE and GP were measured on Days 4 and 14, respectively. After germination for 14 d, vernier calipers were used to measure seedling height. Then, rice seedlings were dried for 24 h at 80 °C, to determine their dry weight (Pieruzzi et al., 2011). The formula $GI=\sum(N_t/T_t)$ was adopted to calculate the germination index (GI), where N_t is the number of germinated seeds on Day t, and T_t is the time related to N_t (d). The seed vigor index (VI) was determined according to the formula: VI=GI×seedling dry weight (Li et al., 2014). The loss of germination rate (LGR) was determined based on the formula: $LGR=GP_n-GP_m$, where GP_n corresponds to the GP of naturally dried rice seeds, and GP_m is the GP of mechanically dried rice seeds.

2.4 H_2O_2, $O_2^{\bullet-}$, and MDA analysis

The H_2O_2 level was measured according to the method of Huang et al. (2011). Seed samples were homogenized in 6.0 mL of 0.15% (1.5 g/L) trichloroacetic acid at 4 °C, and then centrifuged at 15000*g* for 20 min. Afterwards, 0.5 mL of 10 mmol/L potassium phosphate-buffered saline (PBS; pH 7.0), together with 1 mL of 1 mmol/L KI, was mixed with 0.5 mL of supernatant, and absorbance at 390 nm was determined to measure the H_2O_2 level.

The superoxide radicals ($O_2^{\bullet-}$) level was measured according to the method of Jiang and Zhang (2001). Seed samples were homogenized in 5 mL of

60 mmol/L potassium PBS (pH 7.8) and centrifuged at 12000*g* for 15 min. Later, 0.1 mL of supernatant was collected and added to 0.9 mL of 60 mmol/L potassium PBS (pH 7.8) and 0.2 mL of 10 mmol/L hydroxylamine hydrochloride. The resultant mixture was subjected to 18 h of incubation at 25 °C, followed by the addition of 1 mL of 7 mmol/L α-naphthylamine and 1 mL of 17 mmol/L sulphanilamide to the above mixed solution, and then 15 min of incubation at 35 °C. Finally, the $O_2^{\bullet-}$ level was detected by absorbance of the supernatant at 530 nm.

The malonaldehyde (MDA) level was measured according to the method of Gao et al. (2009) with slight modification. Seed samples were homogenized in 3 mL of 60 mmol/L PBS (pH 7.8) at 4 °C, and centrifuged at 10000*g* for 15 min. Then, the supernatant was collected, mixed with 2.5 mL of 5% (0.05 g/mL) trichloroacetic acid, and boiled to 100 °C for 15 min. The resultant mixture was subjected to 15 min of centrifugation at 10000*g*. Afterwards, the MDA level in the supernatant was determined by absorbance of the supernatant at the wavelengths of 532 and 600 nm.

2.5 Antioxidant enzymes

Potassium PBS (50 mmol/L, 8 mL, pH 7.8) was added to homogenize the seed samples, and the mixture was centrifuged at 12000*g* for 20 min. The activity of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) in the supernatant was determined according to the method of Qiu et al. (2005).

2.6 Amylase activity

Amylase activity was measured according to the method of Li et al. (2014) with slight modification. Seed samples were ground to a fine powder under liquid nitrogen, followed by homogenization with 10 mL purified water and 20 min of centrifugation at 10000*g*. The supernatants were transferred and used for the color reactions. Amylase activity was measured according to the formula: enzyme activity=$M\times T/(R\times W\times t)$, where M is the maltose content, T is the total extraction volume, R is the reaction extraction volume, t is the reaction time, and W is the seed sample weight.

2.7 GA and ABA content analyses

The contents of gibberellin acid (GA) and abscisic acid (ABA) were determined according to the method of Huang et al. (2017) using high-performance liquid chromatography (HPLC; Shimadzu Essentia LC-15C, SIL-10AF automatic sampler, Japan). The mobile phase comprised 64% methanol and 36% pure water. The HPLC system was equipped with a 5-mm particle size reverse-phase (C18) column. The GA and ABA contents within the homogenate extracted from each seed sample were determined using a fluorescence detector (SPD-15C, Shimadzu, Guangzhou, China). The mobile phase flow rate was set at 1.0 mL/min.

2.8 Statistical analysis

Statistical Analysis System (SAS) software was used for statistical data analysis by one-way analysis of variance (ANOVA). The least significant difference at $P<0.05$ ($LSD_{0.05}$) was used for multiple comparisons. The percentage data were subjected to arcsin-transformation before statistical comparison, according to $\hat{y}=\arcsin[\mathrm{sqrt}(x/100)]$.

3 Results

3.1 Effect of drying temperature on the drying process

With the increase in drying temperature, the drying time was remarkably reduced, and the drying rate and seed temperatures were obviously increased in rice seeds with the same IMC. At the drying temperatures of 42 and 45 °C, the drying time of rice seeds evidently increased with increasing IMC, while the drying rate dramatically declined. However, the seed temperature showed no differences with different IMCs at the drying temperatures of 42 and 45 °C (Table 2).

3.2 Effects of drying temperature on seed germination

For rice seeds with an IMC of 20%, the seed GP at the drying temperatures of 42 and 45 °C were higher than 90%, and the LGR was lower than 2.5%. The GE, GI, and VI were maintained at high levels. With increasing drying temperature, the seed VIs decreased markedly. The GPs at the drying temperatures of 52 and 60 °C were only 80.50% and 61.25%, while the LGR reached 11.25% and 32.25%, respectively. In addition, the GE, GI, and VI in the 52 and 60 °C treatments were also significantly lower than those in the 42 and 45 °C treatments (Table 3).

Table 2 Effects of drying temperature on drying time, drying rate, and seed temperature of rice seeds

Seed lot	IMC (%)	Drying temperature (°C)	Drying time (h)[*]	Drying rate (%/h)[*]	Seed temperature (°C)[*]
1	19.7	42	24.41 ± 0.53^{e}	0.32 ± 0.02^{f}	38.31 ± 0.83^{d}
2	19.8	45	21.52 ± 0.47^{f}	0.36 ± 0.03^{e}	41.46 ± 1.02^{c}
3	19.3	52	15.27 ± 0.86^{g}	0.48 ± 0.02^{c}	47.55 ± 1.56^{b}
4	19.9	60	12.68 ± 0.58^{h}	0.62 ± 0.03^{a}	53.41 ± 1.72^{a}
5	24.9	38	42.32 ± 1.28^{b}	0.32 ± 0.01^{f}	36.82 ± 0.77^{f}
6	25.0	42	33.13 ± 1.72^{c}	0.39 ± 0.02^{e}	38.48 ± 0.85^{d}
7	25.0	45	30.80 ± 1.59^{b}	0.41 ± 0.03^{d}	40.88 ± 1.23^{c}
8	25.0	52	21.73 ± 1.05^{f}	0.60 ± 0.04^{a}	45.63 ± 1.54^{b}
9	29.8	36	48.57 ± 2.34^{a}	0.37 ± 0.02^{e}	35.71 ± 0.39^{g}
10	29.9	38	45.22 ± 2.75^{ab}	0.40 ± 0.02^{d}	37.07 ± 0.46^{e}
11	30.2	42	35.03 ± 1.83^{c}	0.52 ± 0.03^{b}	38.66 ± 0.83^{d}
12	30.0	45	33.15 ± 1.41^{d}	0.54 ± 0.03^{b}	41.82 ± 1.12^{c}

[*] Values are presented as mean±standard error (SE) from three replicates. Values followed by a different superscript letter within a column are significantly different at the 0.05 probability level. IMC, initial moisture content

Table 3 Effects of drying temperature on seed germination energy, germination percentage, germination index, vigor index, and loss of germination rate of rice

Seed lot	IMC (%)	Drying temperature (°C)	GP (%)[*]	LGR (%)[*]	GE (%)[*]	GI[*]	VI[*]
1	19.7	42	91.75 ± 3.75^{a}	0.75 ± 0.11^{fg}	79.25 ± 5.13^{ab}	21.35 ± 1.72^{a}	4.38 ± 0.31^{b}
2	19.8	45	90.75 ± 4.52^{a}	2.50 ± 0.23^{f}	75.75 ± 4.31^{c}	20.11 ± 1.53^{b}	3.92 ± 0.22^{c}
3	19.3	52	80.50 ± 2.51^{b}	11.25 ± 1.23^{d}	42.50 ± 2.75^{f}	17.53 ± 0.89^{d}	3.24 ± 0.26^{d}
4	19.9	60	61.25 ± 4.26^{d}	32.25 ± 2.59^{a}	30.00 ± 1.56^{g}	12.53 ± 1.76^{f}	1.69 ± 0.11^{g}
5	24.9	38	91.25 ± 3.31^{a}	0.50 ± 0.07^{g}	76.75 ± 3.85^{c}	21.89 ± 2.12^{a}	4.49 ± 0.52^{a}
6	25.0	42	91.00 ± 2.57^{a}	1.00 ± 0.17^{fg}	75.50 ± 5.15^{c}	20.23 ± 1.52^{b}	3.90 ± 0.29^{c}
7	25.0	45	78.25 ± 4.59^{b}	9.25 ± 0.97^{e}	67.25 ± 3.76^{d}	18.86 ± 1.43^{c}	3.30 ± 0.31^{d}
8	25.0	52	61.50 ± 5.77^{d}	28.75 ± 1.75^{b}	34.50 ± 2.54^{g}	13.42 ± 0.83^{e}	2.08 ± 0.15^{f}
9	29.8	36	90.50 ± 2.83^{a}	1.50 ± 0.21^{fg}	82.00 ± 4.31^{a}	21.57 ± 1.38^{a}	4.51 ± 0.37^{a}
10	29.9	38	91.00 ± 1.73^{a}	0.75 ± 0.08^{fg}	78.75 ± 3.68^{b}	20.73 ± 1.77^{b}	4.37 ± 0.26^{b}
11	30.2	42	81.50 ± 2.00^{b}	8.00 ± 0.59^{e}	64.00 ± 5.54^{d}	19.12 ± 1.22^{c}	3.31 ± 0.19^{d}
12	30.0	45	70.25 ± 3.31^{c}	20.75 ± 2.53^{c}	52.50 ± 3.57^{e}	16.09 ± 1.83^{d}	2.45 ± 0.17^{e}

[*] Values are presented as mean±standard error (SE) from three replicates. Values followed by a different superscript letter within a column are significantly different at the 0.05 probability level. IMC, initial moisture content; GP, germination percentage; LGR, loss of germination rate; GE, germination energy; GI, germination index; VI, vigor index

At the drying temperatures of 38 and 42 °C, rice seeds with an IMC of 25% maintained high seed vigor, with a GP of over 90% and an LGR of less than 1.0%. At the drying temperatures of 45 and 52 °C, the GPs were only 78.25% and 61.50%, while the LGRs were as high as 9.25% and 28.75%, respectively. The GE, GI, and VI of seeds with an IMC of 25% also decreased significantly with increasing drying temperature. Rice seeds with an IMC of 30% showed a high GP (>90%) and a low LGR (<1.50%) at drying temperatures of 36 and 38 °C, with significantly higher GE, GI, and VI than those in the 42 and 45 °C treatments. At the drying temperatures of 42 and 45 °C, the GP, GE, GI, and VI decreased significantly with increasing IMC, while the LGR showed the opposite trend (Table 3).

To detect the effect of drying temperature on germination after storage, the standard germination test of rice seeds stored for 10 months was carried out. Similarly, negative effects of high IMC and drying temperature on seed germination were observed after storage for 10 months (Table S1).

3.3 Correlations between drying parameters and seed GIs

Drying temperature showed extremely significant positive correlations with seed temperature and drying rate, but an extremely significant negative correlation with drying time. Drying temperature, seed temperature, and drying rate showed extremely significant negative correlations with the GP, GE, GI, and VI, while they showed extremely significant positive correlations with the LGR. However, drying time was not significantly correlated with various seed VIs (Table 4).

3.4 Effects of drying temperature on activity of antioxidant enzymes and ROS content during drying and early germination

The CAT activity in dried rice seeds increased significantly with increasing drying temperature. By contrast, CAT activity decreased significantly with increasing drying temperature on Days 1 and 3 of germination (Fig. 2a). On Days 1 and 3 of germination, the POD activity in seeds with an IMC of 20% at 42 or 45 °C was markedly lower than that in those grown at 52 or 60 °C. Similar results were observed in rice seeds with an IMC of 25% or 30% (Fig. 2b). Moreover, the SOD activity in dried seeds increased with increasing drying temperature. However, drying temperature and IMC had no consistent effect on SOD activity on Day 1 or 3 of germination (Fig. 2c).

Drying temperature also had an effect on the accumulation of ROS in rice seeds during drying and early germination (Fig. 3). The H_2O_2 and $O_2^{\bullet-}$ contents were significantly elevated with increasing drying temperature in the dried seeds. On Days 1 and 3 of germination, the H_2O_2 content decreased with increasing drying temperature (Fig. 3a), while the $O_2^{\bullet-}$ content showed no significant trend under the influence of drying temperature (Fig. 3b). However, neither the IMC nor the drying temperature had a consistent effect on the change of MDA content in rice seeds during drying and early germination (Fig. 3c).

Correlation analysis showed that the CAT activity in dried seeds was significantly and negatively correlated with GP, GE, GI, and VI, and significantly and positively correlated with LGR. By contrast, POD activity showed no significant correlation with seed VIs in the dried rice seeds or on Day 1 or 3 of germination. SOD activity in dried seeds showed significant negative correlations with GP, GE, GI, and VI, and a significant positive correlation with LGR (Table 5).

The H_2O_2 content was more closely correlated with seed vigor than the ROS or MDA content. The H_2O_2 content in dried seeds showed extremely significant negative correlations with GP, GE, GI, and VI. However, on Day 3 of germination, these correlations were opposite to those in dried seeds. Neither $O_2^{\bullet-}$ nor MDA showed significant correlation with GP, GE, GI, VI, or LGR during drying or early germination (Table 5).

3.5 Effects of drying temperature on GA and ABA contents during drying and early germination

GA content remained at a low level in dried rice seeds, and increased on Days 1 and 3 of germination. Drying temperature and IMC had no effect on GA content in dried rice seeds. On Days 1 and 3 of germination, the GA content significantly decreased with increasing drying temperature. At the drying temperatures of 42 and 45 °C, the GA content significantly decreased with increasing IMC on Days 1 and 3 of germination (Fig. 4a).

Table 4 Correlations between drying temperature, seed temperature, drying time, drying rate and GP, LGR, GE, GI, VI of rice seeds

Parameter	Drying temperature	Seed temperature	Drying time	Drying rate
Drying temperature	1.000			
Seed temperature	0.981^*	1.000		
Drying time	-0.895^*	-0.847^*	1.000	
Drying rate	0.807^*	0.783	−0.499	1.000
GP	-0.838^*	-0.840^*	0.584	-0.915^*
LGR	0.848^*	0.857^*	−0.602	0.931^*
GE	-0.917^*	-0.923^*	0.722	-0.914^*
GI	-0.896^*	-0.898^*	0.663	-0.940^*
VI	-0.868^*	-0.869^*	0.641	-0.945^*

* Statistically significant difference at the 0.001 probability level. GP, germination percentage; LGR, loss of germination rate; GE, germination energy; GI, germination index; VI, vigor index

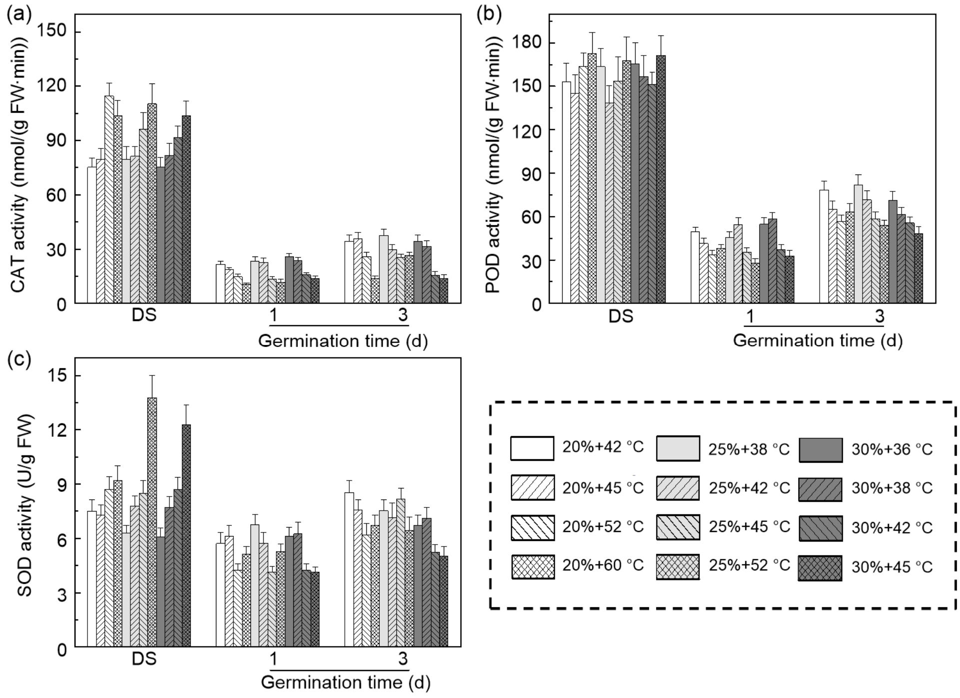

Fig. 2 Effects of drying temperature and seed IMC on the CAT (a), POD (b), and SOD (c) activity of rice seeds
Rice seeds with three different initial moisture contents (IMCs) (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. FW, fresh weight; DS, dried seed; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase. Results are representative of four independent experiments. Error bar denotes standard error (SE) of biological replicates within an experiment

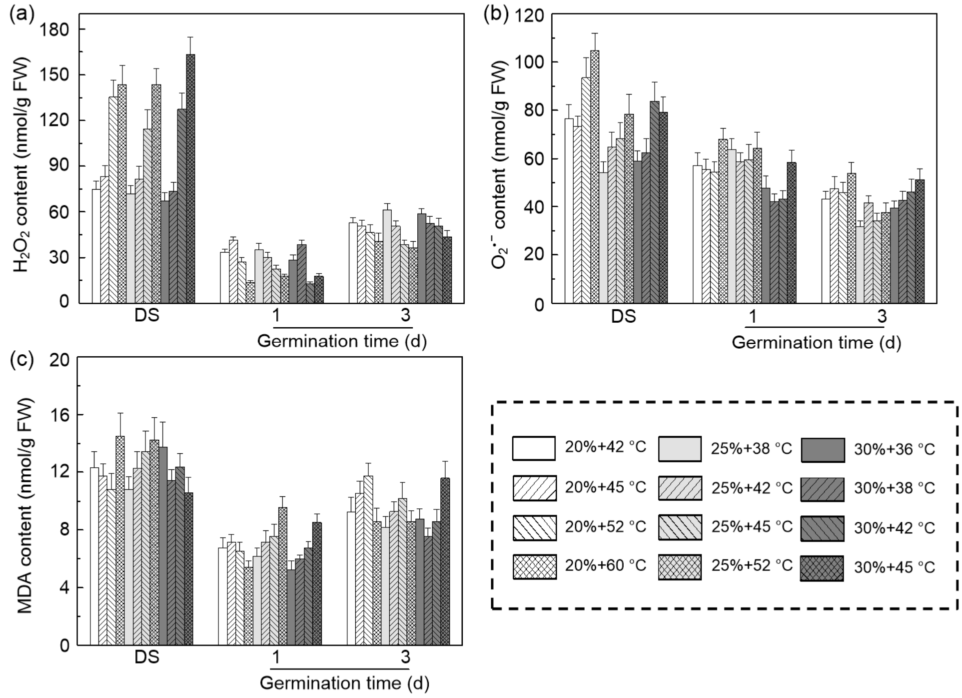

Fig. 3 Effects of drying temperature and seed IMC on H_2O_2 (a), $O_2^{\bullet -}$ (b), and MDA (c) contents of rice seeds
Rice seeds with three different initial moisture contents (IMCs) (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. FW, fresh weight; DS, dried seed; H_2O_2, hydrogen peroxide; $O_2^{\bullet -}$, superoxide radical; MDA, malondialdehyde. Results are representative of four independent experiments. Error bar denotes standard error (SE) of biological replicates within an experiment

Table 5 Correlations between CAT, POD, and SOD activity, H_2O_2, $O_2^{\bullet -}$, and MDA contents, and the GP, LGR, GE, GI, and VI of rice seeds

Parameter	Stage	GP	LGR	GE	GI	VI
CAT	DS	−0.832*	0.818*	−0.918*	−0.831*	−0.845*
	Day 1	0.856*	−0.866*	0.862*	0.868*	0.919*
	Day 3	0.739	−0.770	0.750	0.778	0.796
POD	DS	−0.659	0.669	−0.613	−0.575	−0.521
	Day 1	0.748	−0.749	0.777	0.728	0.786
	Day 3	0.613	−0.617	0.612	0.643	0.691
SOD	DS	−0.814*	0.809*	−0.743	−0.805*	−0.814*
	Day 1	0.513	−0.519	0.558	0.519	0.627
	Day 3	0.403	−0.435	0.482	0.450	0.501
H_2O_2	DS	−0.855*	0.867*	−0.879*	−0.869*	−0.922*
	Day 1	0.759	−0.771	0.713	0.733	0.806*
	Day 3	0.833*	−0.810*	0.763	0.834*	0.853*
$O_2^{\bullet -}$	DS	−0.684	0.717	−0.826*	−0.759	−0.755
	Day 1	−0.593	0.569	−0.526	−0.537	−0.524
	Day 2	−0.327	0.401	−0.420	−0.466	−0.482
MDA	DS	−0.489	0.471	−0.319	−0.440	−0.400
	Day 1	−0.432	0.394	−0.339	−0.406	−0.451
	Day 2	−0.131	0.141	−0.241	−0.169	−0.241

* Statistically significant difference at the 0.001 probability level. DS, dried seed; GP, germination percentage; LGR, loss of germination rate; GE, germination energy; GI, germination index; VI, vigor index; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; $O_2^{\bullet -}$, superoxide radical; MDA, malondialdehyde

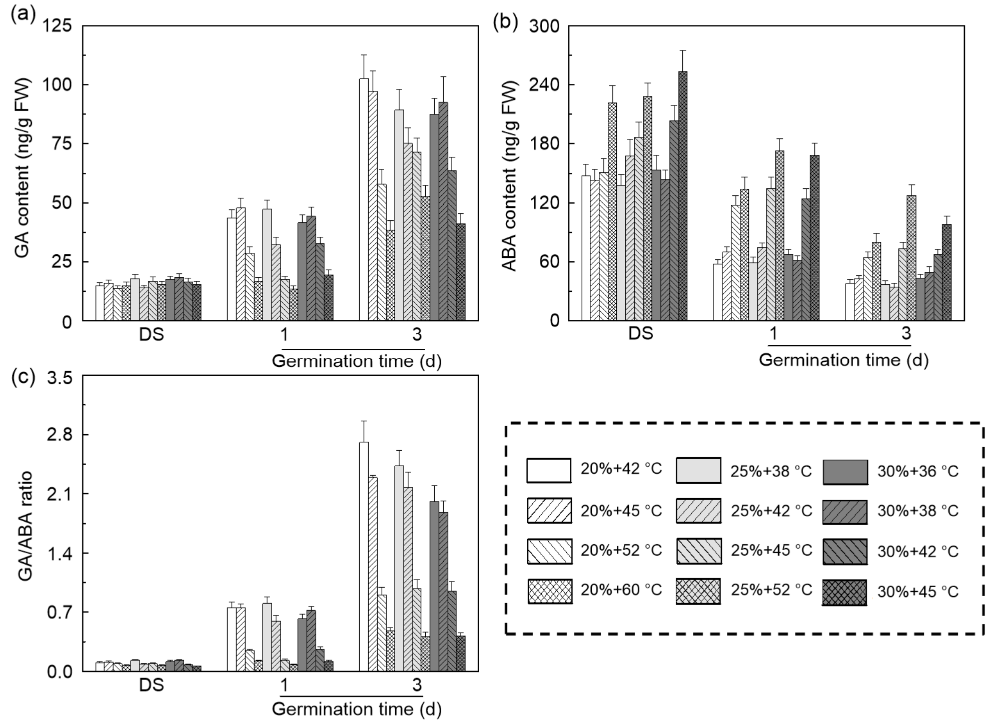

Fig. 4 Effects of drying temperature and seed IMC on GA (a) and ABA (b) contents, and the GA/ABA ratio (c) of rice seeds
Rice seeds with three different initial moisture contents (IMCs) (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. FW, fresh weight; DS, dried seed; GA, gibberellin acid; ABA, abscisic acid. Results are representative of four independent experiments. Error bar denotes standard error (SE) (n=4) of biological replicates within an experiment

For rice seeds with an IMC of 20%, no significant difference in ABA content was observed among the 42, 45, and 52 °C treatments. However, the 60 °C treatment significantly enhanced the ABA content after seed drying. For rice seeds with an IMC of 25%, the ABA content significantly increased with increasing drying temperature after seed drying. For rice seeds with an IMC of 30%, the ABA content in dried seeds at 42 or 45 °C was significantly higher than that at 36 or 38 °C. The ABA content in dried rice seeds increased significantly with increasing IMC in the 42 and 45 °C treatments. On Days 1 and 3 of germination, the ABA content increased with increasing drying temperature. At the drying temperatures of 42 and 45 °C, the ABA content significantly increased with increasing IMC in rice seeds at the early germination stage (Fig. 4b).

The GA/ABA ratio remained at an extremely low level in dried rice seeds, but increased rapidly on Days 1 and 3 of germination. On Days 1 and 3 of germination, the GA/ABA ratio in seeds with an IMC of 20% that were dried at 42 or 45 °C was significantly higher than that in the 52 or 61 °C treatment. Similarly, the higher drying temperature also significantly decreased the GA/ABA ratio in rice seeds with an IMC of 25% or 30% during early germination. In the 42 and 45 °C treatments, the GA/ABA ratio significantly decreased with increasing IMC on Days 1 and 3 of germination (Fig. 4c).

The GA content in dried seeds showed no significant correlation with the seed VI. On Days 1 and 3 of germination, the GA content and GA/ABA ratio were significantly and positively correlated with GP, GE, GI, VI, and LGR. Conversely, the ABA content in dried rice seeds on Days 1 and 3 of germination showed significant negative correlations with GP, GE, GI, and VI (Table 6).

3.6 Effects of drying temperature on α-amylase and β-amylase activity during drying and early germination

Neither the IMC nor drying temperatures had a consistent effect on α-amylase activity in dried rice seeds. On Days 1 and 3 of germination, α-amylase activity decreased significantly with increasing drying temperature. The α-amylase activity in rice seeds with an IMC of 30% was significantly lower than that of rice seeds with an IMC of 20% or 25% at a drying temperature of 42 or 45 °C (Fig. 5a). Drying temperatures had no consistent effect on β-amylase activity in rice seeds during drying and early germination (Fig. 5b).

Correlation analysis indicated that the α-amylase activity in rice seeds at Day 1 of germination was significantly and negatively correlated with GP, GI, and VI, and significantly and positively correlated with LGR. The α-amylase activity and β-amylase activity in dried seeds were not significantly correlated with the seed GI (Table 7).

4 Discussion

Seed represents the basic agricultural capital goods, and seed vigor plays a critical role in seed germination, seedling growth, and yield formation. During the production of rice seeds, mechanical drying is an important link in whole-process seed quality control. High temperature drying will result in seed damage, including stress cracks, lowering the

Table 6 Correlations between GA and ABA contents, the GA/ABA ratio and GP, LGR, GE, GI, and VI of rice seeds

Parameter	Stage	GP	LGR	GE	GI	VI
GA	DS	0.377	−0.381	0.538	0.481	0.485
	Day 1	0.894^*	-0.870^*	0.816^*	0.862^*	0.898^*
	Day 3	0.869^*	-0.877^*	0.885^*	0.883^*	0.922^*
ABA	DS	-0.827^*	0.838^*	−0.698	-0.807^*	-0.873^*
	Day 1	-0.878^*	0.872^*	-0.817^*	-0.838^*	-0.887^*
	Day 3	-0.898^*	0.881^*	-0.805^*	-0.856^*	-0.860^*
GA/ABA ratio	DS	0.793	-0.801^*	0.762	0.821^*	0.884^*
	Day 1	0.860^*	-0.852^*	0.831^*	0.838^*	0.893^*
	Day 3	0.878^*	-0.878^*	0.862^*	0.869^*	0.904^*

* Statistically significant difference at the 0.001 probability level. DS, dried seed; GP, germination percentage; LGR, loss of germination rate; GE, germination energy; GI, germination index; VI, vigor index; GA, gibberellin acid; ABA, abscisic acid

Table 7 Correlations between α-amylase and β-amylase activity and GP, LGR, GE, GI, and VI of rice seeds

Parameter	Stage	GP	LGR	GE	GI	VI
α-Amylase	DS	0.217	−0.150	0.158	0.211	0.210
	Day 1	0.840*	−0.828*	0.785	0.808*	0.855*
	Day 3	0.730	−0.731	0.769	0.740	0.811*
β-Amylase	DS	−0.229	0.202	−0.124	−0.197	−0.227
	Day 1	0.543	−0.517	0.574	0.591	0.494
	Day 3	0.591	−0.629	0.584	0.601	0.645

* Statistically significant difference at the 0.001 probability level. DS, dried seed; GP, germination percentage; LGR, loss of germination rate; GE, germination energy; GI, germination index; VI, vigor index

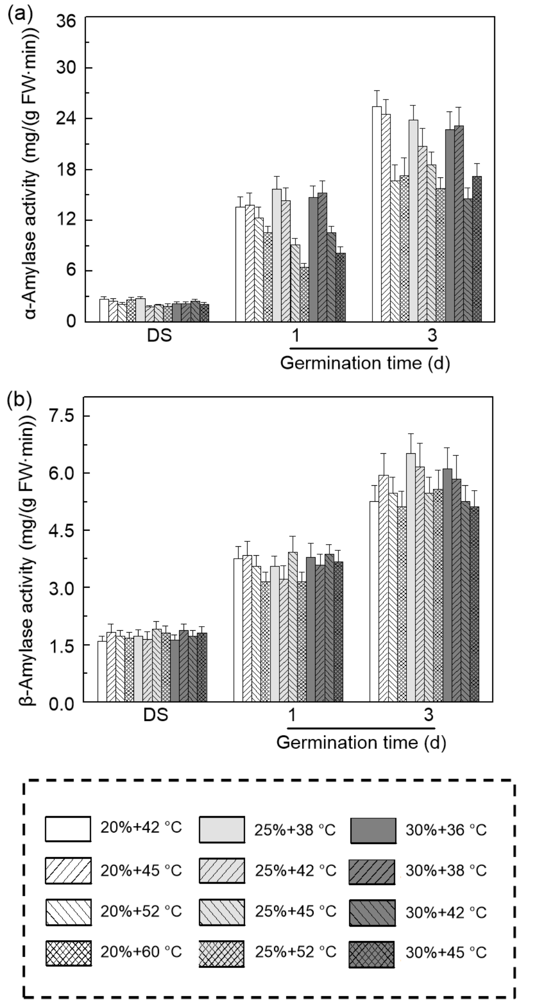

Fig. 5 Effects of drying temperature and seed IMC on α-amylase (a) and β-amylase (b) activity of rice seeds
Rice seeds with three initial moisture contents (IMCs) (20%, 25%, and 30%) were dried to the target moisture content (14%) at four different drying temperatures. DS, dried seed. Results are representative of four independent experiments. Error bar denotes standard error (SE) of biological replicates within an experiment

seed germination, or destroying enzymes (Musielak, 2000; Igathinathane et al., 2008). High-temperature drying also reduces seed vigor by inhibiting the catabolism of storage substances in seed endosperm (Thakur and Gupta, 2006). Our previous study showed that 60% of seed enterprises in Zhejiang Province suffered accidents in the mechanical drying process of rice seed within the past five years, resulting in a significant decrease of seed vigor (Huang et al., 2020). It is crucial to investigate the optimum drying temperature for rice seeds with different IMCs, and to elucidate the mechanisms mediating the effect of drying temperature on rice seed vigor. In this study, we found that the optimum drying temperature of rice seeds decreased with increasing seed IMC. The drying temperature upper limits for rice seeds with 20%, 25%, and 30% IMC were 45, 42, and 38 °C, respectively. A high drying temperature and seed IMC had adverse effects on seed vigor during the seed drying process. Correlation analysis also showed that drying temperature was significantly and negatively correlated with seed GIs. Consistent with these results, Aquerreta et al. (2007) pointed out that a lower temperature and moderate duration of heat treatment generally resulted in higher seed viability. Madamba and Yabes (2005) found that the GP of dried seeds tended to increase when lower air-drying temperatures were used, with the highest germination rate of 92% achieved with a drying temperature of 35 °C. The above results suggest that high moisture rice seeds should be dried at a low drying temperature. Drying at a high temperature might significantly reduce seed vigor and inhibit germination.

The mechanical drying of seeds is a non-linear process affected by a series of factors, such as seed properties, drying temperature, seed temperature, drying rate, and environmental factors (Ye et al., 2003). The seed IMC and drying temperature are two important drying parameters for the mechanical drying of seeds. In our study, drying temperature showed significant positive correlations with seed temperature and drying rate, and a significant negative correlation with drying time. Similar results were observed by Hasan et al. (2014), who found that higher temperatures increased the drying rate by increasing the transfer and internal diffusion rate. This consistent effect of drying temperature on seed temperature and drying rate was also observed in wheat (Ueno, 2003), strawberry (Doymaz, 2008), and tomato (Doymaz, 2007). It was proposed that the drying rate, drying time, and seed temperature might play important roles in the mediation of drying temperature on rice seed vigor during the mechanical drying process.

Heat stress can easily induce the excessive accumulation of ROS, including H_2O_2, $O_2^{\bullet-}$, and hydroxyl radicals (OH), thereby aggravating lipid peroxidation and causing damage to cellular membranes (Ratajczak et al., 2015). MDA is one of the products of cell membrane peroxidation and reflects the degree of membrane peroxidation (Su et al., 2017). The roles of ROS and MDA in the response of higher plants to heat stress have been extensively studied (Nguyen et al., 2014). However, whether ROS and MDA participate in the mediation of the effects of drying temperature on rice seed vigor has not been reported. Our data showed that the H_2O_2 and $O_2^{\bullet-}$ contents in dried rice seeds increased significantly with increasing drying temperature. The H_2O_2 content in dry seeds showed significant negative correlations with GP, GE, GI, and VI. Similarly, Kalemba and Pukacka (2014) suggested that the loss of seed vigor was tightly associated with the accumulation of H_2O_2 and superoxide anions during the seed drying and storage processes, especially in high temperature and high-humidity environments. It was suggested that high temperature drying led to a massive accumulation of H_2O_2 and $O_2^{\bullet-}$, resulting in the decrease of germination capacity. On the other hand, a certain amount of H_2O_2 during the early imbibition stage of seeds is of crucial importance to seed germination. H_2O_2 can boost metabolic enzyme activity, and activate catabolism of reserve substances. Moreover, H_2O_2 weakens embryo tissues, enhancing the penetration of the seed radicle into the seed coat, thereby facilitating the seed germination process (Barba-Espín et al., 2011). In our study, the H_2O_2 content at the early stage of germination (Days 1 and 3) was substantially lower than that of dried seeds, and was significantly and positively correlated with GI. Thus, the response of H_2O_2 metabolism to drying temperature during the drying and germination processes might be closely correlated with changes in seed vigor.

Under abiotic stress, plants can activate antioxidant enzyme activity, including SOD, POD, and CAT, to scavenge the oxidative stress and protect the membrane system (Soliman et al., 2011; Liu et al., 2018). Our data showed that the CAT and SOD activity in dry seeds increased significantly as the drying temperature increased, revealing significant negative correlations with GI. Drying temperature had no consistent effect on POD activity in dried rice seeds. CAT catalyzes H_2O_2 decomposition to water and oxygen in plant tissues, while SOD is responsible for catalyzing the decomposition of superoxide anions into H_2O_2 and oxygen. The remarkably increased activity of SOD and CAT may reflect the response of rice seed to excessive ROS accumulation at higher drying temperatures. SOD and CAT might be the critical antioxidizes in response to high temperature drying. In addition, the activity of CAT within seeds in early germination was positively correlated with the GI, suggesting the potential involvement of CAT metabolism in the mediation of the effect of drying temperature on rice seed germination.

GA and ABA play important roles in seed germination and stress response. Generally, a high ABA level induces seed dormancy and inhibits seed germination (Boccaccini et al., 2016). An elevated ABA content induced by abiotic stress can amplify ABA signal transduction and increase the expression of stress-response genes (Ji et al., 2011; Vishwakarma et al., 2017). In contrast, a relatively low ABA content under abiotic stress tends to enhance the abiotic stress resistance of plants (Du et al., 2013; Huang et al., 2016; Cao et al., 2019). However, the relationship between seed vigor and the ABA and GA pathways during the seed drying stage has rarely been investigated. In this study, the ABA content increased significantly with increasing drying temperature and seed IMC during drying and early germination. ABA content showed significant negative correlation with the GI. High-temperature drying of high-IMC seeds was more likely to induce massive ABA accumulation in the seeds, thereby suppressing germination. Similarly, Wang et al. (2006) found that high temperature treatment decreased the contents of GA, zeatin riboside (ZR), and indole-3-acetic acid (IAA) in rice seeds during the late filling stage, but evidently elevated the level of ABA.

GA can activate hydrolase activity and weaken barrier tissues, thus contributing to breaking seed dormancy and promoting seed germination (Yamauchi et al., 2004; Rajjou et al., 2012). In our study, the drying temperature and IMC had no significant effect on GA content in dried rice seeds. However, at the early germination stage, the GA content and GA/ABA ratio decreased significantly with increasing drying temperature, showing extremely significant positive correlations with seed GP, GE, GI, and VI. Moreover, under the same drying temperatures (42 and 45 °C), the GA content and GA/ABA ratio during the early germination period decreased significantly as the IMC increased. The above results suggest that drying temperature might regulate the metabolism of endogenous GA and ABA in rice seeds during the drying processes, thereby affecting rice seed germination.

Starch is a form of storage polysaccharide that is synthesized and reserved in the form of starch granules (Hussain et al., 2015). The products from starch degradation by amylase are the main material and energy source in the process of seed germination. The activity of amylase is tightly correlated with rice seed vigor and seed germination (Kim et al., 2006). Unlike β-amylase, α-amylase has an important role in starch hydrolysis in rice seed germination (Zhao and Wang, 2001). In this study, the α-amylase activity of rice seeds decreased significantly with increasing drying temperature and IMC during early germination. Moreover, a significant negative correlation was observed between seed vigor and α-amylase activity on Day 1 of germination. However, drying temperature had no consistent effect on β-amylase activity in rice seeds during drying and early germination. Wang et al. (2017) also reported that high temperature treatment of barley seeds dramatically decreased the activity of α-amylase in the process of early germination, suppressed starch hydrolysis, and depressed germination. This suggests that α-amylase metabolism participates in the mediation of the effect of drying temperature on rice seed vigor.

5 Conclusions

According to our experimental results, drying temperature and IMC had significant effects on rice seed vigor in terms of germination performance. The upper limits of drying temperature for rice seeds with 20%, 25%, and 30% IMCs were 45, 42, and 38 °C, respectively. Higher drying temperatures remarkably reduced seed vigor. The metabolisms of ROS, antioxidant enzymes, GA, ABA, and α-amylase might be involved in the mediation of the effect of drying temperature on seed vigor.

Contributors

Yu-tao HUANG performed the experiments, analyzed the data, and drafted the manuscript. Wei WU and Wen-xiong ZOU contributed to the seed germination test. Hua-ping WU performed the experiments of HPLC analysis. Dong-dong CAO conceived and designed the whole experiment and revised the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yu-tao HUANG, Wei WU, Wen-xiong ZOU, Hua-ping WU, and Dong-dong CAO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Aquerreta J, Iguaz A, Arroqui C, et al., 2007. Effect of high temperature intermittent drying and tempering on rough rice quality. *J Food Eng*, 80(2):611-618. https://doi.org/10.1016/j.jfoodeng.2006.06.012

Barba-Espín G, Diaz-Vivancos P, Job D, et al., 2011. Understanding the role of H_2O_2 during pea seed germination: a combined proteomic and hormone profiling approach. *Plant Cell Environ*, 34(11):1907-1919. https://doi.org/10.1111/j.1365-3040.2011.02386.x

Bie WB, Srzednick G, Driscoll RH, 2007. Study of temperature and moisture distribution in paddy in a triangular spouted bed dryer. *Dry Technol*, 25(1):177-183. https://doi.org/10.1080/07373930601161062

Boccaccini A, Lorrai R, Ruta V, et al., 2016. The DAG1 transcription factor negatively regulates the seed-to-seedling transition in Arabidopsis acting on ABA and GA levels. *BMC Plant Biol*, 16:198. https://doi.org/10.1186/s12870-016-0890-5

Cao DD, Wu W, Chen SY, et al., 2019. Seed vigor testing by low temperature germination in early rice. *J Zhejiang Univ (Agric Life Sci)*, 45(6):657-666 (in Chinese).

https://doi.org/10.3785/j.issn.1008-9209.2019.02.142

Dang XJ, Thi TGT, Dong GS, et al., 2014. Genetic diversity and association mapping of seed vigor in rice (*Oryza sativa* L.). *Planta*, 239(6):1309-1319. https://doi.org/10.1007/s00425-014-2060-z

Doymaz I, 2007. Air-drying characteristics of tomatoes. *J Food Eng*, 78(4):1291-1297. https://doi.org/10.1016/j.jfoodeng.2005.12.047

Doymaz I, 2008. Convective drying kinetics of strawberry. *Chem Eng Process Process Intensificat*, 47(5):914-919. https://doi.org/10.1016/j.cep.2007.02.003

Du H, Wu N, Chang Y, et al., 2013. Carotenoid deficiency impairs ABA and IAA biosynthesis and differentially affects drought and cold tolerance in rice. *Plant Mol Biol*, 83(4-5):475-488. https://doi.org/10.1007/s11103-013-0103-7

Endoh K, Matsushita M, Kimura MK, et al., 2018. Cryopreservation of *Fagus crenata* seeds: estimation of optimum moisture content for maintenance of seed viability by Bayesian modeling. *Can J For Res*, 48(2):192-196. https://doi.org/10.1139/cjfr-2017-0286

Gao CH, Hu J, Zhang S, et al., 2009. Association of polyamines in governing the chilling sensitivity of maize genotypes. *Plant Growth Regul*, 57(1):31-38. https://doi.org/10.1007/s10725-008-9315-2

Gawrysiak-Witulska M, Siger A, Rudzińska M, et al., 2019. The effect of drying on the native tocopherol and phytosterol content of *Sinapis alba* L. seeds. *J Sci Food Agric*, 100(1):354-361. https://doi.org/10.1002/jsfa.10047

Hasan AAM, Bala BK, Rowshon MK, 2014. Thin layer drying of hybrid rice seed. *Eng Agric Environ Food*, 7(4):169-175. https://doi.org/10.1016/j.eaef.2014.06.002

He YQ, Cheng JP, Liu LF, et al., 2015. Effects of pre-harvest chemical application on rice desiccation and seed quality. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 16(10): 813-823. https://doi.org/10.1631/jzus.B1500032

Holdsworth MJ, Finch-Savage WE, Grappin P, et al., 2008. Post-genomics dissection of seed dormancy and germination. *Trends Plant Sci*, 13(1):7-13. https://doi.org/10.1016/j.tplants.2007.11.002

Hu QJ, Fu YY, Guan YJ, et al., 2016. Inhibitory effect of chemical combinations on seed germination and pre-harvest sprouting in hybrid rice. *Plant Growth Regul*, 80(3):281-289. https://doi.org/10.1007/s10725-016-0165-z

Huang AX, She XP, Cao BH, et al., 2011. Distribution of hydrogen peroxide during adventitious roots initiation and development in mung bean hypocotyls cuttings. *Plant Growth Regul*, 64(2):109-118. https://doi.org/10.1007/s10725-010-9545-y

Huang L, Hong YB, Zhang HJ, et al., 2016. Rice NAC transcription factor ONAC095 plays opposite roles in drought and cold stress tolerance. *BMC Plant Biol*, 16:203. https://doi.org/10.1186/s12870-016-0897-y

Huang YT, Lin C, He F, et al., 2017. Exogenous spermidine improves seed germination of sweet corn via involvement in phytohormone interactions, H_2O_2 and relevant gene expression. *BMC Plant Biol*, 17:1. https://doi.org/10.1186/s12870-016-0951-9

Huang YT, Cao DD, Zou WX, et al., 2020. Study on issue and solution of rice seed mechanical drying in Zhejiang Province. *China Rice*, 26(3):91-95 (in Chinese). https://doi.org/10.3969/j.issn.1006-8082.2020.03.023

Hussain S, Zheng MM, Khan F, et al., 2015. Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. *Sci Rep*, 5:8101. https://doi.org/10.1038/srep08101

Igathinathane C, Chattopadhyay PK, Pordesimo LO, 2008. Moisture diffusion modeling of parboiled paddy accelerated tempering process with extended application to multi-pass drying simulation. *J Food Eng*, 88(2):239-253. https://doi.org/10.1016/j.jfoodeng.2008.02.014

Ji XM, Dong BD, Shiran B, et al., 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant Physiol*, 156(2):647-662. https://doi.org/10.1104/pp.111.176164

Jiang MY, Zhang JH, 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol*, 42(11):1265-1273. https://doi.org/10.1093/pcp/pce162

Jittanit W, Srzednicki G, Driscoll R, 2010a. Corn, rice, and wheat seed drying by two-stage concept. *Dry Technol*, 28(6):807-815. https://doi.org/10.1080/07373937.2010.485081

Jittanit W, Srzednicki G, Driscoll R, 2010b. Seed drying in fluidized and spouted bed dryers. *Dry Technol*, 28(10): 1213-1219. https://doi.org/10.1080/07373937.2010.483048

Kalemba EM, Pukacka S, 2014. Carbonylated proteins accumulated as vitality decreases during long-term storage of beech (*Fagus sylvatica* L.) seeds. *Trees*, 28(2):503-515. https://doi.org/10.1007/s00468-013-0967-9

Kim SK, Son TK, Park SY, et al., 2006. Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *J Environ Biol*, 27(2):181-186.

Li Z, Peng Y, Zhang XQ, et al., 2014. Exogenous spermidine improves seed germination of white clover under water stress via involvement in starch metabolism, antioxidant defenses and relevant gene expression. *Molecules*, 19(11): 18003-18024. https://doi.org/10.3390/molecules191118003

Liu JK, Xie M, Li XZ, et al., 2018. Main allelochemicals from the rhizosphere soil of *Saussurea lappa* (Decne.) Sch. Bip. and their effects on plants' antioxidase systems. *Molecules*, 23(10):2506. https://doi.org/10.3390/molecules23102506

Madamba PS, Yabes RP, 2005. Determination of the optimum intermittent drying conditions for rough rice (*Oryza sativa*, L.). *Food Sci Technol*, 38(2):157-165. https://doi.org/10.1016/j.lwt.2004.04.018

Musielak G, 2000. Influence of the drying medium parameters on drying induced stresses. *Dry Technol*, 18(3):561-581. https://doi.org/10.1080/07373930008917726

Nguyen DN, Lee KJ, Kim DL, et al., 2014. Modeling and validation of high-temperature induced spikelet sterility in rice. *Field Crop Res*, 156:293-302. https://doi.org/10.1016/j.fcr.2013.11.009

Pieruzzi FP, Dias LLC, Balbuena TS, et al., 2011. Polyamines, IAA and ABA during germination in two recalcitrant seeds: *Araucaria angustifolia* (Gymnosperm) and *Ocotea odorifera* (Angiosperm). *Ann Bot*, 108(2):337-345. https://doi.org/10.1093/aob/mcr133

Qiu J, Wang RM, Yan JZ, et al., 2005. Seed film coating with uniconazole improves rape seedling growth in relation to physiological changes under waterlogging stress. *Plant Growth Regul*, 47(1):75-81. https://doi.org/10.1007/s10725-005-2451-z

Rajjou L, Duval M, Gallardo K, et al., 2012. Seed germination and vigor. *Ann Rev Plant Biol*, 63:507-533. https://doi.org/10.1146/annurev-arplant-042811-105550

Ratajczak E, Małecka A, Bagniewska-Zadworna A, et al., 2015. The production, localization and spreading of reactive oxygen species contributes to the low vitality of long-term stored common beech (*Fagus sylvatica* L.) seeds. *J Plant Physiol*, 174:147-156. https://doi.org/10.1016/j.jplph.2014.08.021

Soliman WS, Fujimori M, Tase K, et al., 2011. Oxidative stress and physiological damage under prolonged heat stress in C_3 grass *Lolium perenne*. *Grassl Sci*, 57(2):101-106. https://doi.org/10.1111/j.1744-697X.2011.00214.x

Souza GFMV, Miranda RF, Barrozo MAS, 2015. Soybean (*Glycine max* L. Merrill) seed drying in fixed bed: process heterogeneity and seed quality. *Dry Technol*, 33(14):1779-1787. https://doi.org/10.1080/07373937.2015.1039542

Su LQ, Li JG, Xue H, et al., 2017. Super absorbent polymer seed coatings promote seed germination and seedling growth of *Caragana korshinskii* in drought. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 18(8):696-706. https://doi.org/10.1631/jzus.B1600350

Tangney R, Merritt DJ, Fontaine JB, et al., 2019. Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds. *J Ecol*, 107(3):1093-1105. https://doi.org/10.1111/1365-2745.13095

Thakur AK, Gupta AK, 2006. Two stage drying of high moisture paddy with intervening rest period. *Energ Convers Manage*, 47(18-19):3069-3083. https://doi.org/10.1016/j.enconman.2006.03.008

Uddin Z, Suppakul P, Boonsupthip W, 2016. Effect of air temperature and velocity on moisture diffusivity in relation to physical and sensory quality of dried pumpkin seeds. *Dry Technol*, 34(12):1423-1433. https://doi.org/10.1080/07373937.2015.1119840

Ueno K, 2003. Effects of temperature during drying of immature wheat seed on germination. *Seed Sci Technol*, 31(3):587-595. https://doi.org/10.15258/sst.2003.31.3.08

Vishwakarma K, Upadhyay N, Kumar N, et al., 2017. Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Front Plant Sci*, 20(8):161. https://doi.org/10.3389/fpls.2017.00161

Wang F, Cheng FM, Liu Y, et al., 2006. Dynamic changes of plant hormones in developing grains at rice filling stage under different temperatures. *Acta Agron Sin*, 32(1):25-29 (in Chinese). https://doi.org/10.3321/j.issn:0496-3490.2006.01.005

Wang F, Qi JZ, Zheng XG, et al., 2017. Effects of artificial aging on seed vigor, starch decomposition and endogenous hormones of barley in the early germination. *Xinjiang Agric Sci*, 54(1):33-42 (in Chinese). https://doi.org/10.6048/j.issn.1001-4330.2017.01.005

Yamauchi Y, Ogawa M, Kuwahara A, et al., 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell*, 16(2):367-378. https://doi.org/10.1105/tpc.018143

Ye YY, Liu YM, Ji FL, et al., 2003. The present situation and development of crop seed dryers. *J Chin Agric Mech*, 41(5):22-24 (in Chinese). https://doi.org/10.3969/j.issn.1006-7205.2003.05.008

Zhang JP, Wang HY, Liao SX, et al., 2019. Appropriate ultra-low seed moisture content stabilizes the seed longevity of *Calocedrus macrolepis*, associated with changes in endogenous hormones, antioxidant enzymes, soluble sugars and unsaturated fatty acids. *New For*, 50(3):455-468. https://doi.org/10.1007/s11056-018-9670-4

Zhao YJ, Wang T, 2001. Analysis of the relationship between α-amylase and germinating rate of rice seeds during the process of seed germination. *Chin Bull Bot*, 18(2):226-230 (in Chinese). https://doi.org/10.3969/j.issn.1674-3466.2001.02.016

Zhou XX, Liu L, Fu PC, et al., 2018. Effects of infrared radiation drying and heat pump drying combined with tempering on the quality of long-grain paddy rice. *Int J Food Sci Tech*, 53(11):2448-2456. https://doi.org/10.1111/ijfs.13834

List of electronic supplementary materials

Table S1 Effects of drying temperature on seed germination energy (GE), germination percentage (GP), germination index (GI) and vigor index (VI) of rice stored for 10 months

中文概要

题　目：干燥温度通过赤霉素、脱落酸和抗氧化酶代谢影响水稻种子活力

目　的：本研究旨在根据种子初始含水量确定水稻种子的最佳干燥温度，并阐明干燥温度和种子初始水分影响水稻种子干燥后种子活力的机制。

创新点：根据不同初始水分的水稻种子，设置了不同梯度的干燥温度，系统地研究了干燥温度和种子初始水分对种子机械干燥过程与种子活力的影响，并得到了不同初始水分的适宜干燥温度。

方　法：在不同天气，分别收获初始含水量为20%、25%与30%的水稻种子样品，并分别设置4个温度梯度的干燥温度。采用三九远红外线干燥机（NP-120e）干燥水稻种子，对干燥后的水稻种子进行标准发芽试验，并测定相关生理生化指标，并进行相关性分析。

结　论：初始含水量为20%、25%与30%的水稻种子的适宜干燥温度分别为45、42与38 °C。较高的干燥温度显著降低了水稻种子的活力。种子干燥过程与萌发初期水稻种子内部活性氧、抗氧化酶、赤霉素、脱落酸和α-淀粉酶的代谢可能与干燥温度调控水稻种子活力的机制密切相关。

关键词：干燥温度；水稻；种子活力；赤霉素；脱落酸；抗氧化酶