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# High-Vigor Seeds Associated with Seed Hardness and Water Absorption Rate in Rice (*Oryza sativa* L.)

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**Abstract:** Seed physical properties are stable and visualized, and believed to be reference indicators for screening high-vigor seeds. However, the detailed relationship between seed vigor and its physical properties is not fully understood in rice. To elucidate the relationship mentioned above, seed physical properties such as seed size, hardness, and water absorption rate, and seed vigor indicators including germination rate, salt-stressed germination rate, and drought-stressed germination rate were determined among different rice cultivars. Significant differences in seed vigor indicators and seed physical properties were recorded among different rice cultivars. Germination rate, salt-stressed germination rate, drought-stressed germination rate, seed hardness, and water absorption rate ranged from 32.0 ± 1.7% to 99.7 ± 0.3%, 14.4 ± 2.4% to 99.7 ± 0.3%, 3.3 ± 2.6% to 95.7 ± 2.1%, 69.15 ± 0.15 N to 74.56 ± 0.14 N, and 0.09 ± 0.00 g/h to 0.12 ± 0.00 g/h, respectively. Additionally, correlation analysis showed that seed hardness and water absorption rate were significantly positively related to seed vigor ($r$ = 0.33 **−0.41 **, from 2014 to 2016; $r$ = 0.45 **−0.65 **, in 2021). Moreover, principal component analysis determined that the first principal component explained 91.4%, 90.1%, and 89.9% of the variance of seed physical properties, respectively, and loaded on seed hardness and water absorption rate. These results indicate that seed hardness and water absorption rate can be recommended as efficient indicators for screening rice seeds with high vigor.

**Keywords:** physical properties; rice; seed hardness; seed vigor; water absorption rate

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## 1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, and more than 65% of the population in China lives on rice [1]. Recently, direct-seeded rice is gaining popularity in China, as it is less labor-intensive and more conducive for mechanized cultivation compared to transplanted rice, which requires a large amount of water and labor [1,2]. Dry direct-seeded rice often results in nonuniform seedling establishment due to high/low temperatures. Therefore, a direct-seeded rice system requires many agronomic characteristics such as high seed vigor, which is the sum of those properties that determine the activity and performance of seed lots of an acceptable germination rate in a wide range of environments [3,4]. Highly vigorous seeds have obvious growth advantages including high germination rate and potential under different adverse germination conditions and result in high grain yield. Conversely, low seed vigor of rice will result in poor seedling establishment, low grain yield, and economic losses [5–7]. Generally, standard germination test and a complex stressing vigor test are usually used to measure seed vigor, and germination rate, germination potential, germination index, vigor index, and field emergence rate are involved in evaluating seed vigor levels. Therefore, planting rice cultivars with high vigor is beneficial to the sustainable development of direct-seeded rice production.

For a long time, seed vigor has been ignored in the process of rice breeding programs in China. Additionally, there is a lack of effective parameters which help to select rice cultivars with high vigor during seed production and conditioning [8–10]. Previous studies have found that several seed physiological indicators were significantly related to the seed vigor of rice [11–13]. For instance, Fu et al. [11] and Wang et al. [7] found that starch content was significantly positively correlated with seed vigor. Interestingly, Fu et al. [11] also found that soluble sugar and protein of rice seed were significantly negatively related to seed vigor. However, physiological indicators were susceptible to the environment, and measurement of them are time-consuming [14,15]. Conversely, seed physical properties are relatively stable and visualized, and can be used as reference indicators for selecting rice seeds with high quality [16–18]. In this study, seed physical properties including seed length, width, thickness, projection area, hardness, and water absorption rate were measured among different rice cultivars in different years to determine the relationship between seed physical properties and seed vigor indicators. This study will provide valuable reference for screening rice seeds with high vigor.

## 2. Material and Methods

### *2.1. Seed Material*

From 2014 to 2016, all sixty rice cultivars (twenty rice cultivars in each year) were collected from major propagation locations (Table 1), which have been widely commercialized and planted over wide climatic areas in China. Seeds of these rice cultivars presented an average seed moisture content of 13.0%, and seed physical properties (e.g., seed length, width, thickness, projection area, hardness and water absorption rate) and seed vigor indicators (e.g., germination rate, salt-stressed germination rate and drought-stressed germination rate) were measured for each cultivar. To verify the results obtained from 2014 to 2016, twenty cultivars were collected again in 2021 and these cultivars belong to sixty rice cultivars mentioned above.

### *2.2. Seed Size Determination*

Thirty seeds of each rice cultivar were randomly selected with three replications and sequentially placed in a scanner (BenQ K500 color scanner, Taiwan, China). The images of rice seeds were imported into seed identification software V1.0 (http://www.microsoft.com/zh-cn/download, accessed on 5 March 2015) developed by China Agricultural University in Beijing, China, and seed length, width, and projection area were automatically determined. Additionally, the seed thickness of thirty seeds on the scanner was measured by a vernier caliper with the accuracy of 0.05 (EXPLOIT, Shanghai, China).

### *2.3. Seed Hardness Determination*

Thirty seeds of each rice cultivar were randomly selected for hardness determination with three replications. Each seed was determined by a hardness tester (GWJ-1, Zhejiang, China). Firstly, one seed was placed on the working table of the hardness tester by a tweezer, and then the handwheel was turned to make the top rod move forward slowly. At this time, the instrument started to indicate the pressure load, and the maximum pressure was recorded when the seed was crushed.

**Table 1.** Details of different rice cultivars collected from major propagation locations.

| Cultivar | Propagation Location in 2014 | Cultivar | Propagation Location in 2015 | Cultivar | Propagation Location in 2016 | Cultivar | Propagation Location in 2021 |
|---|---|---|---|---|---|---|---|
| Tianliangyou 616 | Hubei | Mingliangyou 829 | Yunnan | YLiangyou 3218 | Hunan | Y Liangyou 900 | Zhejiang |
| Guangliangxiangyou 66 | Jiangsu | Shenliangyou 5814 | Chongqing | Liangyou 688 | Fujian | Chunyou 84 | Zhejiang |
| Yixiang 725 | Sichuan | Liangyou 378 | Chongqing | NLiangyou 1 | Hunan | Yongyou 12 | Zhejiang |
| YLiangyou 5813 | Hunan | YLiangyou 5867 | Jiangsu | Teyou 922 | Guangxi | Yongyou 538 | Zhejiang |
| Wandao 153 | Zhejiang | Benliangyou 9 | Zhejiang | Heyou 3 | Sichuan | Fengliangyou 9 | Guangdong |
| YLiangyou 900 | Zhejiang | Hongxiangyou 68 | Chongqing | Gangyou 99-14 | Fujian | Xiushui 519 | Zhejiang |
| Zhunliangyou 608 | Hunan | Yongyou 9 | Zhejiang | Guliduo | Sichuan | II You 7954 | Shanghai |
| Chunyou 84 | Zhejiang | YLiangyou 2 | Jiangsu | Chuanxiangyou 2 | Sichuan | Shenliangyou 5814 | Chongqing |
| Quanxiangyou 512 | Fujian | Neixiangyou 1 | Sichuan | Mian Liangyou 838 | Sichuan | Fengliangyou 4 | Jiangshu |
| Fengliangyou 9 | Guangdong | Liangyou 1528 | Hubei | Wuyouhuazhan | Hunan | Zhongjiazao 17 | Zhejiang |
| Fengliangyou 6 | Jiangsu | Fengliangyou 4 | Jiangsu | YLiangyou 9918 | Hunan | Y Liangyou 1 | Hunan |
| Fengliangyouxiang 1 | Jiangsu | Guangliangyou 4 | Jiangsu | Zhongyou 465 | Hainan | Mingliangyou 829 | Yunnan |
| Ning 88 | Zhejiang | Jiangza 1 | Jiangxi | Qianyou 0506 | Zhejiang | Yongyou 9 | Zhejiang |
| Shaonuo 9714 | Zhejiang | Zhongjiazao 17 | Zhejiang | Xinliangyou 223 | Jiangsu | Y Liangyou 2 | Jiangsu |
| Xiushui 519 | Zhejiang | Zhunliangyou 1141 | Zhejiang | Jinzao 47 | Zhejiang | Y Liangyou 3218 | Hunan |
| Yongyou 538 | Zhejiang | Yongyou 366 | Guangxi | Xianghu 13 | Zhejiang | Liangyou 688 | Fujian |
| Yongyou 12 | Zhejiang | Luliangyou 106 | Hunan | Xiushui 134 | Zhejiang | Y Liangyou 9918 | Zhejiang |
| IIYou 7954 | Shanghai | TYou 463 | Jiangxi | Jia 58 | Zhejiang | Xiushui 134 | Hunan |
| Jingliangyouhuazhan | Hainan | YLiangyou 1 | Hunan | Xiushui 09 | Zhejiang | Wuyouhuazhan | Hunan |
| Longliangyou 534 | Hainan | Gangyou 725 | Sichuan | Yangeng 68 | Shenyang | Jia 58 | Zhejiang |

### *2.4. Water Absorption Determination*

One hundred seeds of each rice cultivar were randomly selected and weighed separately with three replications. Then, the seeds were respectively poured into beakers with enough water, and the water was stirred to let the seeds absorb water for 1 h. The seeds were filtered out and spread on absorbent paper to absorb the water on the surface of the seeds. The rate of water absorption was equal to the ratio of the difference of seed weight before and after water absorption to that before water absorption.

### *2.5. Standard Germination Test*

The method was used by Wang et al. [7] and Fu et al. [11]. One hundred healthy seeds from each cultivar with three replications were surface sterilized with 6 g/L sodium hypochlorite solution for 15 min and then rinsed three times with sterile distilled water. Seeds were then placed in a plastic box (120 mm × 120 mm × 50 mm) with two sheets of filter paper (Anchor, Minneapolis, MN, USA), and 9 mL of distilled water was added. Seeds were germinated in a growth chamber at 20 °C for 16 h with the dark condition and at 30 °C for 8 h with the light condition of 12,000 Lx. Seeds were recognized as germinated when their root length reached the seed length and shoot length reached half of the seed length. The number of germinated seeds was counted at 14 days. Germination rate was calculated according to the following formulas: Germination rate (%) = The number of germinated seeds at 14th day/The total number of seeds × 100.

### 2.6. Salt-Stress Germination Test

The method referred to the description by Fu et al. [11]. The Salt-stress germination test was conducted on three replications according to the method mentioned in the standard germination test. However, 9 mL of distilled water was replaced by 12 mL of 1.5% NaCl solution. The salt-stress germination rate was calculated according to the formula mentioned in the standard germination test.

### 2.7. Drought-Stress Germination Test

The method referred to the description by Fu et al. [11]. A drought-stress germination test was conducted on three replications according to the method mentioned in the standard germination test. However, 9 mL of distilled water was replaced by 12 mL of 25% polyethylene glycol (PEG-6000) solution. The drought-stress germination rate was calculated according to the formula mentioned in the standard germination test.

### 2.8. Data Analysis

Data were analyzed using the multivariate analysis of variance (MANOVA) procedure in SPSS 24.0 (IBM, Chicago, IL, USA), and the multiple comparisons were explored using Duncan's test at 0.01 and 0.05 probability levels, respectively. Before MANOVA, the percentage value was determined by arcsine transformation. Correlation analysis was performed and Pearson's simple correlation coefficient ($r$) was used to establish the relationship between seed physical properties and seed vigor indicators. The principal component analysis was also performed among seed physical properties and seed vigor indicators based on covariance matrix and the threshold value of accumulative contribution rate of major factors to total variation was 85%.

## 3. Results

### 3.1. The Comparisons of Seed Vigor among Different Rice Cultivars in Different Years

Significant differences in seed vigor were recorded among sixty rice cultivars collected from 2014 to 2016 (Figure 1). Germination rate, salt-stressed germination rate, and drought-stressed germination rate ranged from 32.0 ± 1.7% to 99.7 ± 0.3%, 14.4 ± 2.4% to 99.7 ± 0.3%, and 3.3 ± 2.6% to 95.7 ± 2.1% with means of 88.2 ± 1.3%, 72.1 ± 2.5%, 44.8 ± 2.7%, respectively. To verify the results obtained from 2014 to 2016, twenty rice cultivars were collected in 2021. Significant differences in germination rate, salt-stressed germination rate and drought-stressed germination rate were also observed among twenty rice cultivars (Figure 1). Germination rate, salt-stress germination rate and drought-stress germination rate ranged from 77.5 ± 0.3% to 96.0 ± 2.0%, 38.0 ± 1.2% to 92.7 ± 0.7%, and 16.7 ± 2.3% to 63.3 ± 2.0% with means of 87.7 ± 1.3%, 65.7 ± 3.4%, and 45.8 ± 3.3%, respectively. Therefore, rice cultivars collected in this study are characterized by different seed vigor.

### 3.2. The Comparisons of Seed Physical Properties among Different Cultivars in Different Years

Significant differences in seed physical properties were obtained among different rice cultivars (Table 2). From 2014 to 2016, seed thickness, width, length, and projection area ranged from 1.95 ± 0.03 mm to 2.07 ± 0.0.04 mm, 2.90 ± 0.06 mm to 3.13 ± 0.09 mm, 8.33 ± 0.20 mm to 9.58 ± 0.15 mm, and 18.08 ± 0.26 $mm^2$ to 19.67 ± 0.40 $mm^2$, respectively. Moreover, seed hardness and water absorption rate of rice cultivars collected from different years ranged from 69.15 ± 0.15 N to 74.56 ± 0.14 N and 0.09 ± 0.00 g/h to 0.12 ± 0.00 g/h, respectively. Notably, seed physical properties of rice cultivars collected from different years were relatively stable, which indicated that the results obtained from this study are representative.

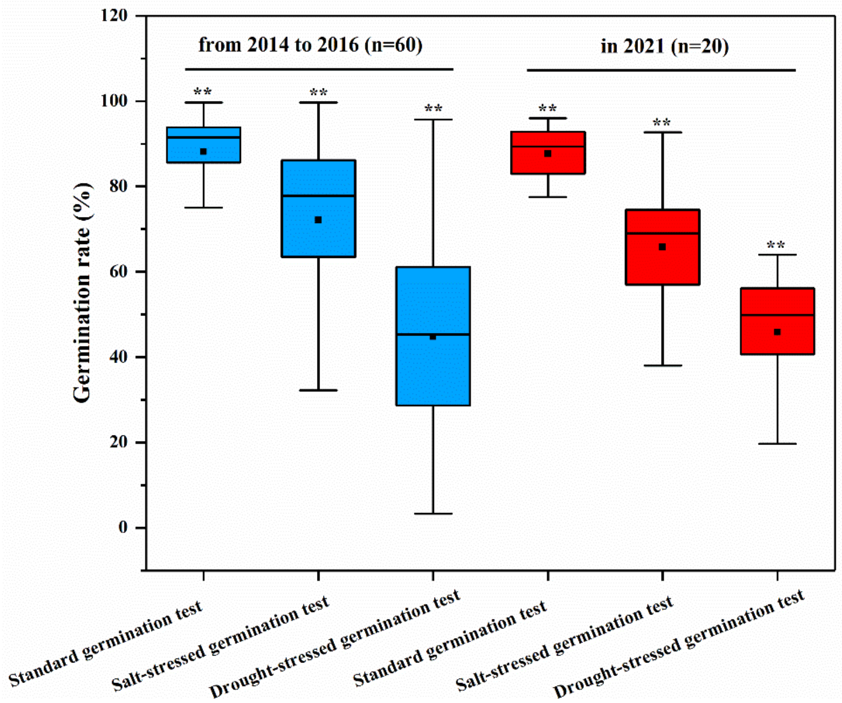


**Figure 1.** Seed vigor indicators of different rice cultivars. The box-whisker diagrams show the maximum (top of the vertical line), 75th percentile (top of the box), average (solid square within the box), 25th percentile (bottom of the box), median (horizontal line within the box), and minimum (bottom of the vertical line) values of the data. ** denotes significant differences in seed vigor indicators of different rice cultivars at the 0.01 probability level according to the Duncan's test.

**Table 2.** MANOVA analysis of seed physical properties of different rice cultivars in different years.

| Year | Seed Thickness (mm) | Seed Width (mm) | Seed Length (mm) | Projection Area (mm²) | Seed Hardness (N) | Water Absorption Rate (g/h) |
|---|---|---|---|---|---|---|
| 2014 | 2.04 ± 0.04 Aab | 3.13 ± 0.09 Aa | 9.11 ± 0.21 ABa | 19.67 ± 0.40 Aa | 74.56 ± 0.14 Aa | 0.12 ± 0.00 Aa |
| 2015 | 1.95 ± 0.03 Ab | 2.90 ± 0.06 Aa | 9.58 ± 0.15 Aa | 19.13 ± 0.41 ABab | 73.43 ± 0.16 Aa | 0.11 ± 0.01 ABa |
| 2016 | 2.07 ± 0.04 Aa | 3.00 ± 0.08 Aa | 8.33 ± 0.20 Bb | 18.08 ± 0.26 Bb | 70.33 ± 0.14 Aa | 0.09 ± 0.00 Bb |
| 2021 | - | - | - | - | 69.15 ± 0.15 Aa | 0.10 ± 0.01 ABab |
| Cultivar | ** | ** | ** | ** | ** | ** |

Data are mean ± SE ($n$ = 20). Different capital letters and lower-case denote significant differences in seed physical properties of different years at the 0.01 and 0.05 probability level according to the Duncan test, respectively. ** denote significant differences in seed physical properties of different rice cultivars at the 0.01 probability level according to the Duncan's test.

### *3.3. Correlation Analysis between Seed Vigor Indicators and Seed Physical Properties*

Significant correlations were recorded between seed vigor indicators and seed physical properties (Table 3). Seed hardness was significantly positively correlated with salt-stressed germination rate ($r$ = 0.38 **) and drought-stressed germination rate ($r$ = 0.41 **). Moreover, water absorption rate was significantly positively related to all seed vigor indicators, and the highest Pearson's simple correlation coefficient was recorded between water absorption rate and drought-stressed germination rate ($r$ = 0.37 **). However, non-significant correlations were recorded between seed thickness, width, projection area, and seed vigor indicators. To verify the relationship between seed hardness, water absorption and seed vigor indicators, twenty rice cultivars were collected in 2021. Notably, seed hardness and water absorption rate were significantly positively related to seed vigor indicators ($r$ = 0.50 *–0.67 **).

**Table 3.** Correlation analysis between seed vigor indicators and physical properties of rice cultivars collected from different years.

| Index | From 2014 to 2016 ($n$ = 60) | | | In 2021 ($n$ = 20) | | |
|---|---|---|---|---|---|---|
| | Germination Rate (%) | Salt-Stressed Germination Rate (%) | Drought-Stressed Germination Rate (%) | Germination Rate (%) | Salt-Stressed Germination Rate (%) | Drought-Stressed Germination Rate (%) |
| Seed hardness (N) | 0.23 | 0.38 ** | 0.41 ** | 0.52 * | 0.56 * | 0.41 |
| Water absorption rate (g/h) | 0.33 ** | 0.36 ** | 0.37 ** | 0.65 ** | 0.45 * | 0.55 * |
| Seed thickness (mm) | 0.13 | 0.14 | −0.16 | | | |
| Seed width (mm) | −0.20 | 0.09 | −0.20 | | | |
| Seed length (mm) | −0.21 | −0.06 | 0.27 * | | | |
| Projection area ($mm^2$) | −0.23 | 0.01 | 0.01 | | | |

Data are Pearson's simple correlation coefficient values. * and ** indicate significant correlation at the 0.05 and 0.01 probability levels, respectively.

### 3.4. Principal Component Analysis between Seed Vigor Indicators and Seed Physical Properties

The principal component analysis was used to eliminate redundancy in seed physical properties, and two principal components accounted for most of the variability among rice varieties with different seed vigor levels (Table 4). In seed vigor indicators, the first principal component explained 91.4%, 90.1%, and 89.9% of the variance of seed physical properties and loaded on seed hardness and water absorption rate, respectively. The second principal component accounted for 6.8%, 7.6%, and 8.2% of the variation, respectively. All of the first principal component were more than 85%, which indicated that seed vigor of rice cultivars were more closely associated with seed hardness and water absorption rate.

**Table 4.** Principal component analysis between seed vigor indicators and seed physical properties of rice cultivars collected from different years.

| Seed Physical Properties | Vectors of Germination Rate (%) | | Vectors of Salt-Stressed Germination Rate (%) | | Vectors of Drought-Stressed Germination Rate (%) | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| Seed hardness (N) | 0.56 | −0.04 | 0.58 | −0.04 | −0.41 | 0.05 |
| Water absorption rate (g/h) | 0.43 | 0.06 | 0.42 | 0.01 | −0.48 | 0.07 |
| Seed thickness (mm) | 0.01 | −0.01 | 0.01 | −0.01 | −0.02 | 0.01 |
| Seed width (mm) | −0.01 | 0.03 | −0.01 | 0.02 | 0.02 | −0.03 |
| Seed length (mm) | 0.02 | 0.50 | 0.03 | 0.38 | −0.04 | −0.30 |
| Projection area ($mm^2$) | 0.04 | 0.73 | 0.03 | 0.92 | −0.08 | −0.92 |
| Contribution (%) | 91.4 | 6.8 | 90.1 | 7.6 | 89.9 | 8.2 |

## 4. Discussion

Seed vigor is an important agronomic characteristic, and the standard germination test remains the principle and is widely used to estimate seed vigor of rice seeds in China [7,11]. However, under field conditions the standard germination test has overestimated the performance of seeds since it is performed under optimal conditions for each species [19,20]. In this study, seed vigor indicators were measured under different tests, which could improve the accuracy of results. However, we found that the order of seed vigor levels among rice cultivars was different under various seed vigor tests. This finding is consistent with previous studies [21–23]. These results indicate that it is more accurate to select high-vigor rice cultivars based on the results of different seed vigor tests. Seed vigor studies are in general conducted on seed lots that belong to the same cultivar and with the same genetic structure. For the present study, sixty seed lots were selected from sixty cultivars. This was an obligation in the study, in that each cultivar belongs to one company with its rights and has mother and father parent lines [24]. Moreover, some studies have been conducted for ranking cultivars [25,26].

Water absorbing characteristics are considered important indicators during seed germination. In this study, seed water absorption rate was significantly positively related to seed vigor (Table 3). This finding is consistent with Ma et al. [27]. In general, seed coat structure influences water permeability, which affects seed germination to a certain extent [28]. Ma et al. [29] found that seed coat permeability was stronger with the increase of the water absorption rate. These results indicate that higher water absorption rate increases seed coat permeability, and ultimately improves seed quality. Additionally, a higher water absorption rate will accelerate the degradation of starch, and provide energy for seed germination and growth [20]. Therefore, higher seed water absorption is an essential characteristic for high-vigor rice cultivars.

Seed hardness can define as the maximum force applied on grain at any time during the first cycle of compression. In this study, seed hardness was significantly positively related to seed vigor (Table 3). Rice seeds are mainly composed of starch, the structure of which plays an important role in grain quality. Wang et al. [7] reported that more tightly packed and compact starch granules were beneficial for the formation of high-vigor rice seeds. Chun et al. [30] found that starch morphology and structure would directly affect the rice quality. Thus, higher seed hardness indicates the superior starch quality of rice seeds and ultimately increases seed vigor. Moreover, seed hardness provides benefits in terms of resistance against seed coat pathogens and the avoidance of seed spoilage from poor storage conditions [31]. Therefore, higher seed hardness is also an essential characteristic for high-vigor rice cultivars. However, several studies reported that seed hardness delayed seed germination. Zhang et al. [32] and Zhang et al. [31] found that seed germination had a significant negative correlation with seed hardness. This may be the reason that the plant materials, water absorption time, and water absorption characteristics are different. Therefore, an effort should be made to elucidate the mechanism between seed hardness and seed vigor of rice.

Many previous studies used the principal component analysis for indices regarding seed vigor, and found that the first principal component could mainly explained the variance of seed vigor [7,33,34]. Also, Li et al. [33] and Luo et al. [34] reported that the first principal component could predict the seed vigor to a large extent. In seed vigor indicators measured under different germination tests in this study, the first principal component explained 90.5% of the variance on average of seed physical properties and loaded on seed hardness and water absorption rate (Table 4), which indicates that seed hardness and water absorption rate can be regarded as indicators for rice seed vigor. In this study, we found that seed thickness and width were significantly related to seed vigor in 2014, while non-significant correlations were recorded in 2015 and 2016. These results indicate that the relationship between seed thickness, width, and seed vigor is influenced by the genetic factor. Moreover, several previous studies found that big size seeds had higher germination potential and germination rate [26,35]. Notably, it is achieved that seed vigor is related to seed size in the same cultivar, while there is no direct evidence determining that seed vigor is related to seed thickness and width among different rice cultivars. Hence, seed thickness and width cannot be regarded as indicators for screening rice cultivars with high vigor.

## 5. Conclusions

Significant differences were recorded in germination rate, salt-stressed germination rate and drought-stressed germination rate among different rice cultivars. Consistently, significant differences were obtained in seed thickness, width, length, projection area, hardness, and water absorption rate. Moreover, correlation analysis and principal component analysis showed seed hardness and water absorption rate were significantly positively related to seed vigor indicators. These results suggest that seed hardness and water absorption rate can be recommended as efficient indicators for screening rice seeds with high vigor.

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