Relationship of size and shape rice seed to early seedling vigor traits

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Abstract. Rice crop improvement program for direct-seeded has been directed at identifying the quality of seeds with rapid uniform germination and biomass accumulation during the initial phase of seedling establishment. Seed and embryo size are thought as two critical factors in the emergence of faster and vigorous seedlings. For the reason, this study was purposed to evaluate the relationship between the size and shape of rice seed with embryo size and its effects on germination. This study was designed using rice seeds from 55 genotypes, which were grouped in three sizes (medium, long, and extra-long) and two shapes (medium and slender). Germination was conducted with the top of paper method in a controlled germinator (24h lighting, 25 ± 2°C, 95%). We have found that the size and shape of seed significantly affected (α < 0.05) to embryo length, the time of radicles and plumules emergence, and seedling dry weight. Longer seeds tended to have longer embryos and emerged of radicles and plumules faster. Meanwhile, longer seeds with slender shape tend to have greater seedling dry weight. The results informed that the size and shape of rice seeds could be considered important characters for early seedling vigor traits in direct-seeded systems.

1. Introduction
Vigorous crop establishment since early growth is the key success in further plant growth and development. Huang et al. [1] described that early vigor is a combination between uniformity and emergence of seedlings after seeding (seed vigor) with the ability of the young plants to grow and develop after emergence (seedling vigor). Some researchers reported that plants have better early vigor traits, reduced losses of rice yield due to competition with weeds and drought [2], and increased biomass and dry grain yield of wheat in the Meditteranean environment [3]. Therefore, early vigor has become an agronomic trait and signifies the potential of seed germination, seedling growth, and tolerance to adverse environmental factors.

Early vigor (EV) was reported to relate to the ability of seedlings to grow and develop well since their emergence and directly connect to growth and plant yield [4][5][6][7]. One of the important characters in determining the success of crop cultivation is the fast of seedling establishment and...
development that uniform in various field conditions or known as early seedling vigor (ESV) [8][9]. For the reasons, researchers suggested that for direct seeding of rice using varieties that have better EV and ESV traits [10][11][12][7].

The success of seedling establishment is determined by the size of the seed and embryo, such as in wheat [13][14][15], barley [16][17], and rice [18][19][2]. It was confirmed by the results of the review carried out by Kesavan et al. [20], and Ambika et al. [21] concluded that seed size was one of the crucial indicators of seed quality that affected vegetative growth (plant height and number of tillers) and often related with crop yield. Thus, seed size is one of the essential characters to be considered in a crop improvement program.

A previous study showed that was correlated between Indica rice seed weight with embryo weight, and embryo weight had a strong correlation with embryo length [22]. Embryo length was positive associated with seed quality parameters, and seedling vigor consists of germination percentage, germination rate, shoot length, root length, and seedling dry weight [23]. The information indicated that embryo length could be a variable in the improvement of seed quality for Indica rice. However, there is research that mentioned that seed size and embryo length of rice not significantly affected seedling vigor [1]. Therefore, this study aimed to evaluate the relationship size and shape of rice seed to the traits of ESV in the germination phase.

2. Materials and methods

2.1. Seed materials

The genetic materials in this study consisted of fifty rice genotypes from the rice breeding program of IPB University and five national rice varieties (Ciherang, INPARI 33, Jatiluhur, IPB 3S, dan IPB 8G). The genotypes were produced at the experimental farm of IPB University, Darmaga, Bogor (6°33'52.7"S 106°44'06.4"E) in the planting period from April-May 2019 and the harvesting period from late August to early September 2019. A total of six hills of each genotype were harvested and dried 3-4 days in the sun. Spikelets were shed manually and then sorted between filled and empty spikelets using a seed blower machine. The sorted seeds were stored in closed and dry boxes at room temperature (average 27°C).

2.2. Laboratorium experiments

A total of 400 seeds of each genotype and variety were taken to observe the size and shape characters by image analysis using the SmartGrain software application [24]. Eight seeds from 400 seeds were hulled and carried out observation of embryo length using a fluorescence microscope (Olympus microscope BX-51 series; Olympus Corp.) at 1.25x magnification. Measurement was carried out in September-November 2019. Seed size (length of seed) and seed shape (length-to-width ratio) data were used as a basis for grouping the characters. The grouping was according to the handbook of rice standard evaluation system (SES) from International Rice Research Institute [25] which classified into four groups seed size: short (< 5.50 mm), medium (5.51 – 6.60 mm), long (6.61 – 7.50 mm), and extra-long (> 7.51 mm). Meanwhile, seed shape grouped into round (< 1.0), bold (1.0-2.0), medium (2.1 – 3.0), dan slender (> 3.0).

Germination test was carried out at Seed Quality Analysis Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The fifty seeds (replicated three times) in which healthy was taken from each genotype and variety. The germination test was conducted by the top of paper method using the paper towel [26]. Fifty seeds have been arranged the method in a transparent box measuring 145 mm x 90 mm and then germinated in a controlled germinator with 24 h illumination, T 25±2°C, and RH 95% (seedburo germinator; Seedburo Equipment Company) for 13 days (21 February – 4 March 2020). Seed vigor was measured with radicle emergence analysis according to the method used by Onwimol et al. [27]. The radicle and plumule emergence (2 mm in length and healthy) were measured at 12 hours interval.
| No | Genotype               | Seed length (mm) | Seed shape (length to width ratio) |
|----|-----------------------|------------------|-----------------------------------|
|    |                       | Mean ± SD        |                                   |
| 1  | IPB187-F-101-1       | 7.04 ± 0.07      |                                   |
| 2  | IPB187-F-102-1       | 7.55 ± 0.05      |                                   |
| 3  | IPB187-F-37-1-3      | 7.70 ± 0.02      |                                   |
| 4  | IPB187-F-41-2-1      | 7.68 ± 0.02      |                                   |
| 5  | IPB187-F-43-1-1      | 7.70 ± 0.12      |                                   |
| 6  | IPB187-F-44-2-2      | 7.63 ± 0.12      |                                   |
| 7  | IPB187-F-46-2-2      | 7.61 ± 0.12      |                                   |
| 8  | IPB187-F-49-2-2      | 7.58 ± 0.05      |                                   |
| 9  | IPB187-F-50-1-3      | 7.53 ± 0.14      |                                   |
| 10 | IPB187-F-52-2-3      | 7.43 ± 0.02      |                                   |
| 11 | IPB187-F-55-2-1      | 7.22 ± 0.09      |                                   |
| 12 | IPB187-F-56-3-2      | 7.35 ± 0.04      |                                   |
| 13 | IPB187-F-56-3-2      | 7.65 ± 0.13      |                                   |
| 14 | IPB187-F-68-3-2      | 6.95 ± 0.06      |                                   |
| 15 | IPB187-F-75-1-1      | 7.36 ± 0.04      |                                   |
| 16 | IPB187-F-76-2-3      | 7.40 ± 0.01      |                                   |
| 17 | IPB187-F-76-2-3      | 7.29 ± 0.03      |                                   |
| 18 | IPB187-F-88-1-2      | 6.77 ± 0.08      |                                   |
| 19 | IPB187-F-90-1-2      | 6.91 ± 0.16      |                                   |
| 20 | IPB189-F-33-1-2      | 6.81 ± 0.17      |                                   |
| 21 | IPB189-F-35-2-1      | 7.55 ± 0.02      |                                   |
| 22 | IPB189-F-42-1-1      | 7.53 ± 0.01      |                                   |
| 23 | IPB189-F-42-1-1      | 7.55 ± 0.01      |                                   |
| 24 | IPB189-F-43-1-1      | 7.41 ± 0.01      |                                   |
| 25 | IPB190-F-103-1       | 7.08 ± 0.16      |                                   |
| 26 | IPB190-F-112-1       | 7.10 ± 0.17      |                                   |
| 27 | IPB191-F-17-1-2      | 7.26 ± 0.06      |                                   |
| 28 | IPB191-F-24-2-1      | 6.65 ± 0.03      |                                   |
| 29 | IPB191-F-24-2-1      | 6.65 ± 0.03      |                                   |

**Note:** The numbers that followed the same letters in the same column are not significantly different at α = 0.05 level Tukey/HSD test.
The normal seedling was assessed to refer to a handbook seedling evaluation issued by ISTA (the International Seed Testing Association) [26]. The variables observed consist of vigor index (%) counted at five days after seeding/DAS (number of normal seedlings / total number of seeds), germination percentage (%) calculated from first count at 5 DAS, and final count at 13 DAS ((first count+final count)/Total number of seeds x 100%), and germination velocity (% day⁻¹). Both radicle and plumule length were determined by taking three samples from each replication using caliper on the 13th day, while seedling dry weight was determined using electronic balance after dried by oven at 60°C for 72 hours.

2.3. Statistical analysis
Data were analyzed by analysis of variance and Pearson correlation using Minitab (17th version). Means were compared followed by calculation of honest significance difference or Tukey test at a significant level of p ≤ 0.05.

3. Results
The ANOVA result on seed length and seed shape of 50 genotypes and five national varieties showed that the genotypes had a significant effect on both characters at an error rate of 5%. The average size (seed length) and shape (length-width ratio) of 50 genotypes had longer (7.30±0.09 mm) and larger (3.05±0.07) than five national varieties tested, 6.62±0.04 and 2.69±0.03, respectively (Table 1). This variation could be the basis for seed grouping according to the criteria issued by IRRI (the International Rice Research Institute). Based on the standard evaluation system in rice [25], 55 genotypes tested, was grouped into three groups of seed size (medium, long, and extra-long) and two groups of seed shape (medium and slender). The seed size consists of five genotypes grouped in medium seed, 36 genotypes were classified in long seed group, and 14 genotypes were classified in extra-long seed group. Meanwhile, seed shape consists of 29 genotypes grouped in medium shape, and 26 genotypes grouped in slender shape (Table 2). Based on the groups, there were five combinations that long and extra-long seeds had both of seed shape while all medium-sized genotypes only had medium shape. The combination informed that seeds in which long to extra-long sizes have a different shape. Meanwhile, the uniform shape of medium-sized genotypes might not be undoubtedly accurate to describe all genotypes of all medium genotypes because of the small number of genotypes tested. Therefore, exploration and evaluation of the seed shape variation from medium-sized genotypes need to be done with a larger number of genotypes.

| Table 2. Group of size and shape rice seeds from 55 genotypes |
|---------------------------------------------------------------|
| **Seed Shape (length-to-width ratio)** | **Seed Length (mm)** |
| No | Medium (B1) (LWR 2.1 – 3.0) | Long (S2) (6.61–7.50 mm) | Extra-Long (S3) (> 7.50 mm) |
| IPB3S | IPB187-F-55-2-1 | IPB187-F-6-2-3 |
| IPB191-F-24-2-1 | IPB187-F-85-1-1 | IPB187-F-52-2-2 |
| IPB187-F-49-1-2 | IPB194-F-40-2-2 | IPB187-F-74-1-3 |
| JATILUHUR | IPB189-F-18-2-1 | |
| IPB 8G | IPB187-F-88-1-2 | |
| IPB187-F-46-2-3 | IPB187-F-101-2-1 | |
| CIHERANG | |
| IPB194-F-58-3-3 | |
| IPB193-F-38-2-1 | |
| IPB194-F-92-3-1 | |
| IPB194-F-36-2-3 | |
| IPB189-F-35-1-1 | |
| IPB187-F-46-2-3 | |
| IPB189-F-31-1-2 | |
| INPARI 33 | |
| IPB187-F-68-3-2 | |
The variation in seed size had a significant effect on embryo length (Table 3). The longer seeds (long and extra-long) had longer embryos than medium seeds, while the seed shape had no significant effect (Tukey test $p < 0.05$). Embryo length is also significantly affected by the combination of seed size and shape. The Tukey test ($\alpha < 0.05$) showed that long-sized rice seeds with both shapes had longer embryo...
than medium seeds, but not significantly different from extra-long seed. These results indicate that the longer seeds tend to have longer embryo compared with other sizes of rice seeds.

Analysis of variance on the germination test for both of rice seed characters had a significant effect on the emergence time of radicle and plumule, also seedling dry weight (Figure 1 and Table 3). Different sizes of rice seed produced germination percentage, germination velocity, and vigor index was not significantly different from each other. It means that all seed lots had germination ability (seed vigor) that were as good. But, different sizes resulted in further emergence of radicle and plumule. Longer seed (long and extra-long) emerged radicle 4-5 hours faster for 50% of total seeds (TRE50) and 7-10 hours faster for a maximum of total seed number (TREMax) than medium size. As the emergence of plumule, which the medium-sized seeds were latest to emerge of plumule than another size (Figure 1). The slender shape emerged radicle faster than medium shape (Table 3) and had greater seedling dry weight in both sizes, long and extra-long (Table 4). The difference in seed shape resulted in different radicle lengths in which slender seeds had 10% longer than medium, but both shapes not different for plumule length (Table 3 and Figure 2). The results indicate that the physical character of seeds, such as size and shape, contributed to the rapid emergence of radicle and plumule also accumulate greater biomass so that they have the potential to grow and develop faster and more robust. These traits are needed for the direct-seeded system to suppress weed growth and rapidly adapt to sub-optimum conditions.

**Figure 1.** Effect of size (i) and shape (ii) rice seeds to time of 50% radicule and plumule emergence (TRE50, TPE50) and time of maximum radicle and plumule emergence (TREMax, TPE Max)

**Table 4.** Effect of combination between size and shape of rice seeds to embryo length, emergence time of radicle (TRE) and plumule (TPE), and seedling dry weight

| Treatment | Length of Embryo (mm) | TRE50 (hour) | TREmax (hour) | TPE50 (hour) | SDW (g) |
|-----------|-----------------------|--------------|---------------|--------------|---------|
| S1B1      | 1.53 b                | 69.31 a      | 101.99 a      | 86.37 a b    | 0.44 b  |
| S2B1      | 1.65 a                | 66.06 ab     | 96.51 ab      | 83.01 b      | 0.44 b  |
| S2B2      | 1.68 a                | 64.37 b      | 91.24 b       | 81.20 b      | 0.45 a b|
| S3B1      | 1.64 a b              | 68.25 a b    | 94.27 ab      | 75.34 b      | 0.47 a b|
| S3B2      | 1.63 a b              | 64.21 b      | 91.37 b       | 90.21 a      | 0.48 a  |

*Note: the numbers that followed the same letters in the same column are not significantly different at a 5% level Tukey/HSD test*

**4. Discussion**

Rice varieties that have the ability to rapid germination and produce robust seedling are suitable for the direct seeded (DSR) system. Rapid growing and strong seedling could support well plant growth and adapt to environmental changes [28]. Varieties with these traits have become a priority in crop improvement programs, especially rice variety for the DSR system [9]. The rapid seedling form ability has been reported to be closely related to seed physical characters [29][30]. This study focused on evaluating the relationship between early seedling vigor traits and seed physical characters consist of
three sizes (medium, long, and extra-long) and two shapes (medium and slender) on germination ability on a laboratory scale.

As mentioned above, longer seeds tend to have a longer embryo. It was confirmed by a positive correlation between seed size with embryo length (Table 5). Namuco et al. [2] stated that larger seeds tend to have bigger embryos. Longer seeds and longer embryos are reported to have good germination ability [22][23]. However, the results of our study showed that seed vigor characters such as vigor index, germination velocity, and germination percentage were not significantly different from both the size and shape of the seed (Table 3). Seed’s ability to germinate is related to many factors, such as the storage period of seeds, germination environment, and physiological activity in seeds [31][32]. Physiological traits of seeds might affect germination ability, including the influence of starch content and enzyme activity, where seeds that amylose content and high α-amylase enzyme activities can increase rice seeds vigor [33].

The speed and uniformity of seedling emergence are important keys for the assessment of early seedling vigor varieties [9]. Both of these traits could be measured quickly through the radicle emergence [34] and the percentage of normal seedling in the first count at five days after seeding (DAS), or called vigor index. According to Figure 1 dan Table 3, genotypes with long and slender seeds were faster to emerge radicle either single factor or in combination factor (Figure 2). Onwimol et al. [27] and Luo et al. [35] reported that the radicle emergence time of rice seeds had been a useful parameter for the classification of rice seed vigor. On the other hand, long seeds resulted in a higher germination percentage than different sizes (Table 3). It showed that long seeds have better germination uniformity.

Table 5. Correlation between size and shape of rice seeds and embryo length with seedling vigor traits

|                  | EL  | GP  | GV  | TRE50 | TREP | TPE50 | TPEP  | SDW  |
|------------------|-----|-----|-----|-------|------|-------|-------|------|
| Seed size        |     |     |     |       | **   |       |       | **   |
| Seed shape       | ns  | ns  | ns  |       | **   |       |       | ns   |
| Embryo length    | ns  | ns  | ns  |       | **   |       |       | ns   |

*significant at α 5%; **significant at α 1%; *not significant

The strong morphological and physiological characters of the seedling are also critical traits for early seedling vigor varieties [1]. The longer radicle length of slender seeds (Table 3) indicated that slender seeds had a potential better adaptation in sub-optimum conditions. Whereas a higher plumule-radicle length ratio in the medium-shaped seed group indicated more seed reserves mobilized from seeds to seedling shoot. According to Huang et al. [1], a higher ratio of plumule-radicle length indicated seed
had strong vigor. However, a combination of long-sized and medium-shaped gave a plumule-radicle length ratio (> 1.0; data not shown) higher than other combinations. Nutrient mobilization also depends on environmental factors during the germination phase. Unfortunately, this study was conducted on a laboratory scale under optimum conditions, so that our results cannot be generalized to stress conditions.

Biomass accumulation in the early stage of growth is the other important trait of early seedling vigor genotypes. This accumulation reflects strong seedling vigor. Biomass accumulation in this study is reflected by the seedling dry weight (SDW) variable (Table 3 and Figure 2). The longer and slender seeds produced the highest SDW, but not significantly different from the medium ones. Our result disagrees with the previous study mentioned that SDW was not influenced by seed size and seed shape (width) [36]. Accumulation of biomass at the germination stage can support the seedling to grow photosynthetic organs, and it will help to suppress competition with weeds in the direct-seeded system. Banik et al. [37] mentioned that rice cultivar with high seedling vigor traits and rapid leaf area development at early vegetative growth could suppress weeds growth. Correlation analysis in this study showed that SDW was positively correlated with the size and shape of seeds, but not with embryo length (Table 5). These results reinforce the information that the size and shape of seeds are closely related to early seedling vigor traits of rice plants.

5. Conclusion
Evaluation of rice genotypes with various sizes and shapes of seeds on seedling vigor characters provides valuable information. We conclude from our results that rice genotypes have various sizes and shapes of seeds potential to become varieties that have the ability to the early seedling. The genotypes which have longer size and slender seeds have shown superior initial seedling vigor characters in terms of the rapid emergence of radicle and plumule and higher biomass accumulation. These characters can be used to increase early seedling vigor after further testing. The genotypes are included in the prominent early seedling vigor group, need to be validated in field conditions before being applied in the plant improvement programs.

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