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*by Rifqi Fauzi*

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*Research article***Seed Attribute Evaluation of Genotypes for Developing Direct Seeding of Rice Cultivation**

**Ahmad Rifqi Fauzi<sup>1,2</sup>, Ahmad Junaedi<sup>3,\*</sup>, Iskandar Lubis<sup>3</sup>, Munif Ghulmahdi<sup>3</sup>, Hajrial Aswiddinoor<sup>3</sup>, Jun-Ichi Sakagami<sup>4</sup>**

<sup>1</sup>Study Program of Agronomy and Horticulture, Graduate School, IPB University (Bogor Agricultural University), Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

<sup>2</sup>Agroecotechnology Study Program, Faculty of Bioindustry, Trilogi University, Jl. TMP. Kalibata No.4, Duren Tiga, South Jakarta 12760, Jakarta, Indonesia

<sup>3</sup>Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti, IPB Dramaga Campus, Bogor 16680, West Java, Indonesia

<sup>4</sup>Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan;

\* **Correspondence:** junaediagh@gmail.com; Tel: +622518629353; Fax: +622518629353.

**Abstract:** Direct seeding of rice (DSR) may give benefit in using water and labor more efficient and reduce production costs. This study purposes to investigate early vigor (EV) and related attributes of the seed for developing suitable cultivars of DSR system. The research has been conducted in four stages: the measurement of the size of the seed, endosperm, and embryo; the germination test in the laboratory; seedling test using experimental pots; and testing the agronomic performance on transplanting and direct seeding methods in a plastic house. Seed material used eight breeding lines of IPB University and two released varieties. The results showed that each genotype had different characteristics of seed, endosperm, and embryo in both weight and area. Seed weight becomes the most dominant in the emergence of superior EV traits, whereas the more seed weight indicates faster radicle emergence and more weight of seedling. DSR method compared to transplanting showed superiority in the variables of height, leaf area, photosynthesis rate, heading time, and plant dry weight until 65 days old. The seed characters have positively correlation to dry weight of seedlings, number of leaves, leaf area, and canopy dry weight. Candidate genotypes for DSR would be further investigate in the field trial agronomically.

**Keywords:** Early vigor; germination test; seedling emergence; seedling vigor; transplanting

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## 1. Introduction

<sup>14</sup> Rice is one of the important staple foods in the world besides wheat and corn. Global rice production reached 759.6 million tons in 2017, and 90.4% was produced in Asia [1] and consumed by more than 60% of the world's population [2]. The increase in world population to 9.8 billion people by 2050 requires an increase of rice productivity by 30% in order to meet global demand [3]. However, this effort to increase productivity faces challenges related to the availability of water resources and a decrease in the number of workers that may disrupt rice production.

Transplanted cultivation systems dominate (> 50%) world rice production [4]. This cultivation system required a large amount of irrigation water and a large labor requirement [5] that would be unprofitable when faced with limited water and labor scarcity because it will reduce yield productivity and farmers' income. One alternative solution is to develop a cultivation system that is more efficient in management and production inputs. Direct seeding of rice (DSR) to be an alternative technology that is very important to maintain profitable rice production in current and future agricultural scenarios [6]. DSR provided benefits in the form of efficiency in labor and water inputs, thereby reducing production costs [7][8] and producing lower methane emissions than transplanting rice cultivation (TPR) [9]. DSR has been practiced in several countries such as the United States, Australia, Japan, India, and several Southeast Asian countries such as Thailand and Vietnam [4]. However, the development of this system still faces challenges, one of which is the limited availability of suitable cultivars [7][10][11]. The rice plant breeding program for the DSR system has recently been directed at identifying the quality of rice seeds that have the power to grow and develop more quickly and uniformly (early vigor, EV) and could accumulate high biomass during the growth phase [12].

The cultivar development with specific traits such as early vigor (EV), tolerance to immersion, and resistance to drought are very considered for DSR systems [13]. EV is very important for the establishment of robust plants and the ability to compete with weeds [14] so that it becomes a key factor in plant growth and development [15]. Meanwhile, submersion and drought tolerance traits were needed for the prone to flooding areas and long dry seasons. These traits are directly related to the morphological and physiological characters of plants since the early stages [16][17][15]. Other characters identified as contributing to the EV characteristics were seedling length, wet and dry weight of the seedling, number of tillers per plant, mesocotyl length, germination rate, amylase activity, root activity, and chlorophyll content [10][17][12].

The ability of plants to emerge earlier and grow and develop faster was informed to be related to seed quality, especially the physical characters of seeds [18]. Seed weight and size were informed to contribute to the EV properties. Shi et al. [19] stated that the variety with superior early vigor was contributed by the weight of 1000 seeds and seed width higher compared to other tested variety. It indicates that there is a relationship between seed characters and attributes inherent in whole grains such as endosperm and embryos with early vigor traits. As far as we know, information regarding the relationship of seed characters and their attributes to early vigor and performance during the growth period is still rare. Therefore, we designed this study in several series using rice-breeding lines to

investigate the character of the seeds and their germination ability both on a laboratory test and in a pot experiment. In addition, we also observed the growth and development of rice plants on the transplanting and direct-seeded systems until the heading phase.

9

## 2. Materials and Methods

### 2.1. Time and location of the experiment

The research was designed in four stages. The first stage was the characterization of the physical traits of seeds, endosperm, and embryos during September-November 2019 at the Agronomy and Horticulture Laboratory, IPB University. The second stage was the germination testing carried out during February - March 2020 at the Agronomy and Horticulture Laboratory, IPB University. Seeding test in a pot experiment as the third stage was carried out in a plastic house of the field experiment station, IPB University, Babakan Dramaga, Bogor (6°33'52.7"S 106°44'06.4"E). Observations on the performance of rice plants in two cultivation systems, i.e. transplanting and direct seeding, as the last stage have been carried out during February to May 2021 in a pot experiment in the same plastic house as the previous stage.

### 2.2. Genetic materials

The genetic material used consist of 10 (ten) rice genotypes, which are 8 (eight) breeding lines of IPB University rice breeding program and 2 (two) released varieties in Indonesia, i.e. Ciherang and IPB 3S varieties. Lines from the rice breeding program of IPB University consist of IPB187-F-41-2-1, IPB187-F-43-1-1, IPB187-F-75-1-3, IPB189-F-42-1-1, IPB189-F-6-2-3, IPB190-F-12-1-2, IPB193-F-17-2-3, IPB193-F-38-2-1, and IPB193-F-38-2-1. Seeds of the genotypes were obtained from the experimental rice field of IPB University, Babakan, Bogor. The seed material for the first and second experiments harvested on August 2019, while the last two experiments used genetic material from the November-December 2020 harvest period. Meanwhile, one other national variety used the Ciherang variety obtained from the Indonesian Rice Research Center, Agricultural Research and Development Agency, Ministry of Agriculture, Sukamandi, West Java.

### 2.3. Seeds, endosperm, and embryo physical characterization

Rice seeds were taken from six hills rice plants of each genotype and then sun dried for 3-4 days until the moisture content reached 11-13%. The grain was threshed manually and then separated between the filled grain and empty grain using a seed blower. The filled grains with the results of the sorting are used as samples for seed measurement.

The measurement was carried out from September to November 2019 at the Laboratory of Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The tools used for this experiment were a Canon G3010 series scanner to scan seeds and endosperm, an Olympus BX 51

microscope, and a laptop to process scanned data. Two-dimensional measurements for seeds and endosperm using the SmartGrain (SG) software developed by Tanabata et al. [20] from the National Institute of Agrobiological Science, Tsukuba, Japan. As for the measurement of embryos using DP2-BSW from Olympus®.

Seed measurement using SmartGrain (SG) software. The database of SG used is RGB (red, green, blue). A total of 400 seeds of each genotype were taken and scanned using a Canon G3010 series scanner (600 dpi resolution) and stored in the form of a joint photographic expert group (JPEG). The scan results were used to perform measurements on the SG application. The scale used is 0.030 mm/pixel (calibrated by manual measurement). The SG system will detect grains according to the basic color used for measurement. Measuring of shapes characters is also carried out by the system according to the desired level of accuracy. The grain that had been measured is then separated from the skin to take the endosperm. A total of 40 selected endosperms were measured using the same procedure as measuring seed characters.

The endosperm was stored in a transparent and closed container and stored in dry conditions at room temperature ( $\pm 27^{\circ}\text{C}$ ). Eight white endosperms and had embryos, and were not broken were taken to measure the character of the embryos. Measurements were carried out at the Department of Agronomy and Horticulture Laboratory, IPB University. Embryo visualization using a microscope (Olympus BX-51 series) at 1.25x magnification, while the measurement uses the DP2-BSW application from Olympus®. Measurement of the length and width of the embryo was drawing a straight line between the two points with the longest size. The circumference was drawing a rectangle at each point following the embryo shape that appears on the visualization of the microscope. All embryo characters were observed on a scale of 500  $\mu\text{m}$  (0.5 mm) with a brightness level of ISO 50, and a resolution of 2560 x 1920. Observations of the characters needed to determine the size of rice seeds were carried out visually (descriptively) and through direct measurements. Variables such as weight of 1000 seeds (g), area of seed ( $\text{mm}^2$ ), area of endosperm ( $\text{mm}^2$ ), area of embryo size ( $\text{mm}^2$ ), and endosperm/embryo ratio (EER).

#### 2.4. Germination test

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 were taken from ten genotypes and varieties. The germination test was conducted by the top of the paper method using the paper towel [21]. Fifty seeds had been placed in a transparent box (145 mm x 90 mm x 150 mm) and then germinated in a controlled germinator (Seedburo Equipment Company) with 24 h illumination,  $T 25 \pm 2^{\circ}\text{C}$ , and RH 95% for 13 days (21 February – 4 March 2020). Seed vigor was measured with radicle emergence analysis according to the method used by [22]. The radicle and plumule emergence (2 mm in length and healthy) were observed at 12 hours intervals.



The normal seedling was assessed to refer to a handbook seedling evaluation issued by ISTA (the International Seed Testing Association) [21]. The variables observed consist of vigor index (%) counted at five days after seeding/DAS ( $\text{number of normal seedlings} / \text{total number of seeds} \times 100\%$ ), germination percentage (%) calculated from the first count at 5 DAS, and final count at 13 DAS ( $(\text{first count} + \text{final count}) / \text{Total number of seeds} \times 100\%$ ). Seedling dry weight was determined using electronic balance after dried by oven at 60°C for 72 hours.

## 2.5. Seedling emergence in pot experiment

This experiment was conducted using the pot experiment in a plastic house at the experimental field of IPB University, Dramaga, Bogor (6°33'47.8"S 106°44'9.75"E). The dark pots used a top diameter of 32 cm and a height of 35 cm. The soil used was silted soil with a dominant texture of clay (61%). The planting medium was in a wet condition before sowing the seeds. A total of 75 seeds of each genotype and variety were spread on the soil surface and each genotype was replicated three times. The early vigor characters observed in this pot experiment consisted of germination percentage, number of leaves, dry weight of seedlings, and total leaf area of seedlings. The microclimate during the experiment in the plastic house was recorded to have an average daily temperature 29.9°C and 71.9% relative humidity and lighting between 11-12 hours per day.

The germination percentage variable (%) was calculated by observing the number of emerged seedlings on the surface divided by the number of seeds sown multiplied by 100%. The variables were observed 14 days after seeding. On the same day, fifteen uniform and healthy growth seedlings from each genotype were taken to measure the number of leaves and dry weight of the seedlings. The seedlings were dried in an oven at 60°C for 72 hours and weighed using a digital scale with an accuracy of .00 g. The number of leaves was observed 25 days after sowing. At the end of the observation (30 days after sowing) the total leaf area of the seedlings was measured using a leaf area meter (LI-COR 3000). The data obtained were then analyzed using statistical analysis software.

## 2.6. Characterization of agronomy traits in different planting method

The experiment was designed using a pot (32 cm diameter and 35 cm height in a plastic house with a randomized complete block design (RCBD). The study used two factors: first, the genotype of rice which consisted of eight breeding lines from the IPB University rice breeding program, and two national varieties. The different planting methods as the second factor are transplanted and direct seeding. A total of twenty treatment combinations were replicated three times. Each replication consisted of three experimental pots so that agronomy performance observations were carried out on 180 experimental units. The pots were filled with soil (pH 5.96) with a texture of sand (19.2%); dust (19.7%); clay (61.05%) and contained 1.92% C-Organic, 0.22% total N, 127.57 ppm P<sub>2</sub>O<sub>5</sub>, and 39.74 mg K<sub>2</sub>O. Twelve kilograms of mashed soil had been filled into a pot and inundated in water until the soil puddled.

Seeds of each genotype soaked for 18 hours and dried for 24 hours were seeded using two different methods. In the direct seeding method, a total of five seeds of each genotype were sown on the wet soil surface in pots, and then the two selected plants were to be maintained until the heading phase. Meanwhile, the seeds in the transplanting method were sown in the nursery, and 15-days seedlings old were transplanted to pots as many as two seedlings per hole. Maintenance was done by irrigating up to a height of 2-3 cm from the surface three weeks after sowing and kept it until it enters the heading phase. Fertilization was carried out by applying N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O fertilizers as much as 1.5 g, 0.6 g, and 0.6 g per pot for both methods. The microclimate during the experiment showed an average daily temperature of 31°C with a maximum and minimum temperature of 22.5°C and 43.5°C, respectively. While the average daily relative humidity (RH) was 71.2% with a minimum and maximum were 31% and 99%, respectively.

The traits of agronomic observed in this experiment were plant height (cm), total leaf area (cm<sup>2</sup>), photosynthesis rate (mmol m<sup>-2</sup> s<sup>-1</sup>), plant biomass (g plant<sup>-1</sup>), and heading time (days after sowing/DAS). Plant height was observed every ten days from 25 days after sowing to 65 days after sowing. Leaf area was measured at 28 DAS using a leaf area meter (Li-Cor 3000), while the photosynthesis rate was measured at 32 DAS using a photosynthetic meter (Li-Cor 6400XT) at a PAR of 400 mmol s<sup>-1</sup>. Plant biomass was observed at 45 and 65 DAS and heading. The plants were dried using an oven at 80°C until the plant weight was stable and weighed using a digital scale. The biomass measurement consisted of shoot dry weight, root dry weight, and total dry weight. Observation of heading time was carried out by counting the number of days from seeding until the first panicle appeared from each genotype.

## 2.7. Data analysis

The data obtained from all experiments were analyzed using a *t*-test at an error level of 5% (*α* level 0.05) using the application of *statistical tools for agricultural research* (STAR) from IRRI. The mean comparison for variables with a significant effect was tested using the Tukey test. Pearson correlation was analyzed using STAR software.

## 3. Results and Discussion

### 3.1. Seed, endosperm, and embryo physical traits

Based on the measurement of different seed characters (Table 1), each genotype tested had different characteristics of seed, endosperm, and embryo. The seven genotypes from rice breeding program of IPB University has a grain weight of 1000 grains higher than the two national varieties. While the IPB-190-F-12-1-2 genotype had the lightest weight (<27 g) compared to other genotypes. The genotype and the two national varieties consistently had smaller endosperm and broad attributes than the genotype with a weight > 29 g. Meanwhile, the genotype with the heaviest size, IPB193-F-38-2-1, had

the highest endosperm area among the other genotypes but had a smaller embryonic area than genotypes IPB-187-F-41-2-1 and IPB189-F- 6-2-3. Differences in the size of the endosperm other than genetic factors, according to Morita et al. [23] were also influenced by environmental temperature where the endosperm area at high temperatures at night is 10% smaller than at normal temperature conditions.

**Table 1.** Physical characters of seed, endosperm, and embryo of rice genotypes

Genotype	1000-GW g	Area of seed mm <sup>2</sup>	Area of endosperm mm <sup>2</sup>	Area of embryo mm <sup>2</sup>	Endosperm/ Embryo Ratio
IPB3S	27.02 f	12.81 cd	9.69 cd	0.6323 c	15.39 bcd
CIHERANG	27.26 f	12.52 d	10.05 cd	0.4713 c	21.34 a
IPB190-F-12-1-2	26.91 f	12.7 d	10.1 bcd	0.6007 c	16.93 abc
IPB187-F-75-1-3	27.71 e	12.16 d	9.94 cd	0.5863 c	17.16 abc
IPB189-F-6-2-3	28.1 de	15.63 a	10.99 ab	0.8607 ab	12.95 cd
IPB187-F-41-2-1	28.3 cd	13.46 bcd	9.3 d	0.8777 a	10.71 d
IPB193-F-17-2-3	28.58 bc	12.61 d	10.46 bc	0.661 bc	15.94 bc
IPB189-F-42-1-1	28.81 b	15.28 a	10.2 bcd	0.614 c	16.65 abc
IPB187-F-43-1-1	29.63 a	14.53 abc	10.37 bc	0.6113 c	17.06 abc
IPB193-F-38-2-1	29.86 a	15.16 ab	11.55 a	0.6423 c	18.15 ab
Means	28.22	13.69	10.27	0.66	16.23

Note: the numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%.

Measurements of other variables showed that the Ciherang variety was the tested genotype with the highest endosperm/embryo ratio than others. This indicates that, besides having a smaller weight, the Ciherang variety has a 15% smaller embryo size than its seed area. On the other hand, genotype IPB-187-F-41-2-1 had the smallest endosperm/embryo ratio because it had the smallest endosperm size but the largest embryo size among other genotypes. Hong et al. [24] reported that endosperm development limits embryo size whereas decreasing endosperm size increases embryo size which is the result of enlargement of the scutellum. Other reports on wheat suggest that large embryo sizes can be targets for the development of high-vigor lines [25].

The difference in these characters will be differences in each genotype to grow earlier. The number of reserves in the seed will determine the speed of seed development and the endosperm as the largest energy provider has a role in encouraging seedling growth. Therefore, the size of the seed becomes important as a trait that can contribute to the speed at which seeds emerge and encourage seedlings to grow and develop faster and stronger. Several reports stated that seeds that have good vigor are closely related to seed and embryo size [26][27][28]. Seed size and shape, apart from being a parameter used to analyze plant biodiversity [29] are also important physical indicators of seed quality that can affect



plant growth and yield [30] and have a major impact on the market value of seed products in the market [31].

### 3.2. Germination test

The germination test was carried out in this study to obtain information regarding the initial strength of the seed in seedling establishment and the accumulation of seedling biomass. The study showed that each genotype had the same initial seed vigor (vigor index and germination) but differed in the time variable for radicle emergence (Table 2). The magnitude of the vigor index and germination percentage did not show statistically significant differences indicated that all of the tested genotypes had good seed quality. Different things were shown in the speed at which the seeds germinated using the radicle emergence measurement method. On average, each genotype emerges to a radicle at 65 hours after seed germination. Genotype IPB-187-41-2-1 was the fastest to produce radicles in less than 57 hours or about 24.5% and 16.1%, respectively, faster than the Ciherang variety and the genotype with the largest 1000 grain weight. This genotype had the largest embryonic area and the smallest endosperm/embryo ratio among the tested genotypes (Table 1). The finding can be an indication that the character of the embryo as the center point of the emergence of the radicle plays a role in promoting faster germination. Pandey and Seshu [32] mentioned that a larger embryo was an important attribute for seedling vigor improvement of indica rice cultivar.

The existing seed reserves will be mobilized for seedling growth when the seed dormancy has broken and seedling has emerged. The seed reserves remobilization will accumulate in the seeds can be measured in the sprout biomass. Therefore, the character of seed weight becomes important in supporting the growth of seedlings that have emerged. Our findings showed that the seed with the lightest 1000 grain weight (IPB190-F-12-1-2) produced the lowest seedling dry weight, almost 20% lower than the genotype with the highest dry weight (IPB-193-F-17-2-3). An interesting finding from the seed characteristics of the two varieties tested was that although IPB 3S weighted 1000 grains lower than Ciherang, the dry weight produced was 10% higher. The ability of seeds to germinate is related to the physiological activities of the seeds [33][34] such as starch content and enzyme activity, were seeds that have amylose content and high-amylase enzyme activities [35]. The character of the larger embryonic area (Table 1) and the faster emergence time of the radicle (Table 2) were thought to contribute to the accumulation of biomass in seedlings because, with rapid emergence, mobilization of seed reserves to seedlings would be fast so that biomass accumulation occurred quickly. The genotype with the heaviest grain (> 29 g) produced a dry weight of 9.3 and 9.5 mg seedling<sup>-1</sup>, respectively, and was higher than the genotype with 1000 grain weight less than 28 g.

**Table 2.** Vigor index, germination percentage, seedling dry weight, and time of radicle and plumule emergence of rice genotypes

Genotype	Vigor index	Germination percentage	Time of RE50	Time of PE50	Seedling dry weight
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	(%)	(%)	hours	hours	(mg seedling <sup>-1</sup> )
IPB3S	95 a	98 a	66.11 ab	83.06 a	9.19 abc
CIHERANG	94 a	94.5 a	71.37 a	87.82 a	8.59 bc
IPB190-F-12-1-2	92 a	94.5 a	66.83 ab	89.28 a	8.04 c
IPB187-F-75-1-3	90 a	91.5 a	64.68 ab	75.13 a	8.45 bc
IPB189-F-6-2-3	96 a	98 a	70.56 a	76.56 a	9.71 ab
IPB187-F-41-2-1	98 a	99.5 a	56.13 b	87.68 a	8.91 abc
IPB193-F-17-2-3	88 a	92 a	57.35 b	83.47 a	10.19 a
IPB189-F-42-1-1	72 a	85 a	66.27 ab	95.22 a	9.75 ab
IPB187-F-43-1-1	83 a	89 a	65.94 ab	74.4 a	9.36 ab
IPB193-F-38-2-1	86 a	91 a	65.18 ab	75.63 a	9.53 ab
Means	89.40	93.30	65.04	82.83	9.17

Note: RE50 = radicle emergence of 50% germinated seeds. PE50 = plumule emergence of 50% germinated seeds. The numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%.

### 3.3. Seedling emergence in the pot experiment

The early vigor character is a combination of the seed vigor and seedling vigor characters [15]. The Evaluation of seedling vigor character to rice genotypes was conducted in a pot experiment and the results are showed in Table 3. In general, each genotype has different early vigor characters from another one. The germination percentage of the IPB-187-F-41-2-1 genotype was the highest (78.9%) of all tested genotypes. This percentage was not significantly different with the Ciherang variety (70.2%), as well as the number of leaves produced at the age of 14 days after seedling (4 leaves/seedling). However, the two genotypes differed in other seedling vigor characters like seedling dry weight, the number of leaves at 25 days after sowing, and total leaf area. the IPB193-F-17-2-3 showed consistent response on the seedling dry weight variable in laboratory testing. This genotype produced the highest seedling dry weight among other genotypes in the two tests (Table 2 and Table 3). The result also informed that the heavier genotypes had a higher seedling dry weight. The finding agrees with Teng et al. [36] that longer, wider, and heavier seeds produced higher seedling dry weight. This high accumulation of biomass resulted in the highest number of leaves and the largest total leaf area. The strength of the seedling vigor will provide an advantage in faster canopy formation and greater photosynthetic capacity so that it is expected to be able to compete with weeds, especially in the direct seeding cultivation method. Rice cultivars with high seedling vigor traits and rapid leaf area development at early vegetative growth could suppress weeds growth [37].

**Table 3.** Early vigor traits (germination percentage, number of leaves, seedling dry weight, and leaves area) of rice genotypes at pot experiment

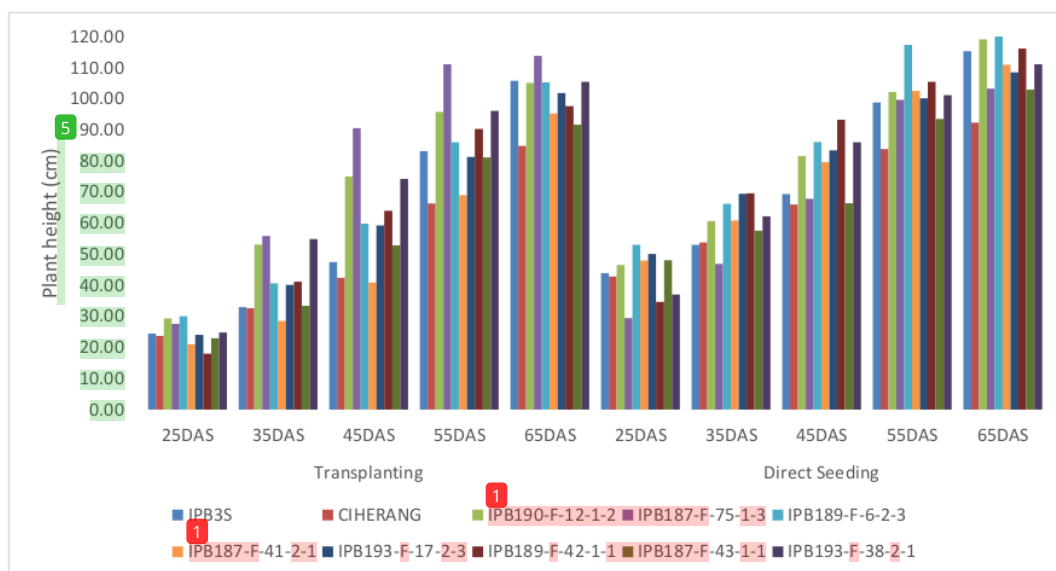
Genotype	Germination		14 <sup>th</sup> DAS		25 <sup>th</sup> DAS		Total leaf area (cm <sup>2</sup> )			
			number of leaves (leaves)	Seedling dry weight (mg seedling <sup>-1</sup> )	number of Leaves (leaves)					
	%									
IPB3S	32.41	bc	3.89	abc	42.45	c	10.02	bc	73.28	cde
CIHERANG	70.19	a	3.89	abc	45.34	bc	14.28	ab	85.22	bcd
IPB190-F-12-1-2	8.06	c	3.5	bc	44.67	bc	12.17	bc	101.35	bc
IPB187-F-75-1-3	67.04	a	3.33	c	42.45	c	8.79	c	48.98	e
IPB189-F-6-2-3	28.52	bc	3.78	abc	48	bc	12.85	bc	87.46	bcd
IPB187-F-41-2-1	78.89	a	4	abc	62.67	abc	11.81	bc	112.77	b
IPB193-F-17-2-3	55	ab	4.89	a	85.58	a	18.2	a	167.64	a
IPB189-F-42-1-1	34.26	bc	3.22	c	40.19	c	8.98	c	54.71	de
IPB187-F-43-1-1	55.19	ab	4.55	ab	66.44	ab	11.04	bc	128.41	b
IPB193-F-38-2-1	77.59	a	4	abc	60.78	bc	8.73	c	79.67	bcd
Means	50.72		3.91		53.86		11.69		93.95	

Note: DAS = days after seeding. The numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%.

### 3.4. Agronomy performance of genotypes in two cultivation methods

Preliminary evaluation of seed characters and their effects on some early vigor characters is one of the critical points in cultivar development for direct seeding cultivation methods. For this reason, the results of the evaluation of germination tests in the laboratory and pot experiments need to be assessed on their performance when planted with transplanting and direct seeding cultivation methods. Studies on the agronomy performance of the previously tested genotypes are presented in tables and figures. The growth performance on the plant height was measured since the plants were 25 days after seeding (Figure 1). The results of our study showed that the height of the rice plant in the direct seeding (DS) cultivation method was higher than the rice plant grown with the transplanting system (TP) at all ages of observation. At the end of the observation (65 DAS), the height of the rice plant in the DS method was 111.2 cm compared to 100.7 cm in the TP method. While the character of the plant height of each genotype is different from one another. The IPB187-F-75-1-3 genotype was the highest (113.8 cm) in the TP method and IPB189-F-6-2-3 was the highest (132 cm) in the DS method at the end of the observation. Both genotypes were also still higher than the two national varieties in both cultivation methods. The height of rice plants with direct seeding in the flooding condition tends to be taller than in the saturated and anaerobic conditions [38]. This difference, apart from being influenced by the cultivation system, may also be influenced by the phenology of each genotype because from our observations in the field, the two genotypes entered the heading phase the fastest in each cultivation

method (Table 4).



**Figure 1.** Plant height of rice genotypes cultivated by transplanting and direct seeding method (DAS: days after seeding)

Measurement of leaf area and photosynthesis rate was carried out at the end of the seedling phase to see the character of seedling vigor towards the next growth phase. The results of the two variables indicate that the cultivation method affects the total leaf area and photosynthesis rate of rice plants (Table 4). In general, the leaf area of rice plants under the DS method was 2.4x higher than that of rice grown using the TP method. This is presumably because the plants were only 13 days old after transplanting, but also because of the transplanting shock, where the plants experienced growth retardation due to transfer to a new growing environment. On the other hand, plants grown using the DS method have adapted since the early stages of germination, so that seedling growth can continue without any mechanical resistance as in TP. The report from Chen et al. [39] stated that rice planted in the DS system has the advantage of faster leaf area formation so that the formation of more tillers compared to the TP system.

The rate of photosynthesis of rice plants in the 10% DS method was greater than that of the TP method (Table 4). However, different things were shown by the IPB190-F-12-1-2 where the photosynthetic rate of plants grown in the DS method was about 15% lower than plants grown with the TP method. We suspected that the large growth from the beginning of the emergence of seedlings by increasing the number of leaves and leaf area caused a decrease in photosynthetic capacity at the end of the seedling phase (30 days after seeding). This heading time was calculated from the beginning of the seedling until the first panicle of each genotype appeared. Almost all genotypes planted with the DS method entered the heading phase 6-19 days earlier than those planted with the TP method. These

results explain that the absence of mechanical barriers such as transplanting can encourage plants to develop faster as shown in the DS method. However, we found that the IPB187-F-75-1-3 genotype gave a different response compared to other genotypes on the total leaf area and time of heading variables. We have not been able to explain whether the cause is genetic or environmental influences, further testing is needed for these genotypes.

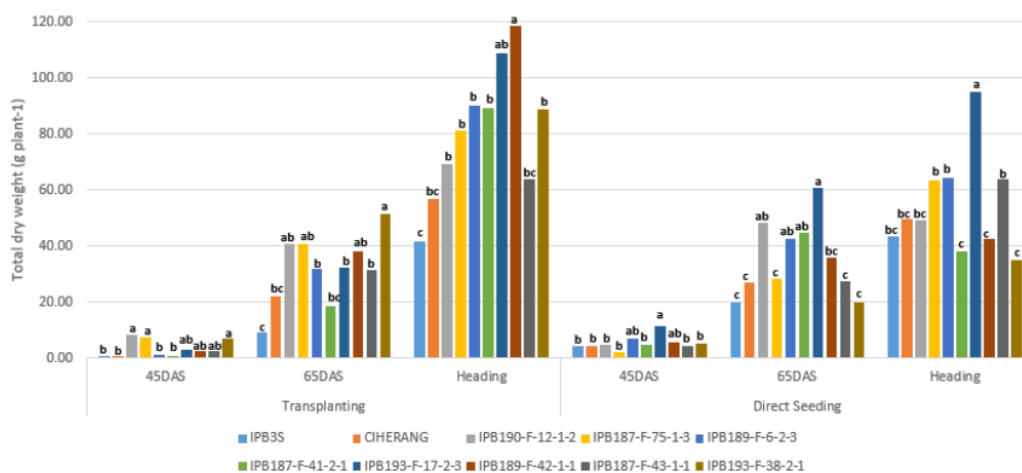
**Table 4.** Leaf area, photosynthetic rate, and time to heading of rice genotype on transplanting (TP) and direct-seeding (DS) method

Genotype	Total Leaf Area (cm <sup>2</sup> )				Photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )				Time of Heading (DAS)			
	TP		DS		TP		DS		TP		DS	
IPB3S	10.53	b	74.65	cd	20.42	ab	22.95	a	83.3	cd	74.7	c
CIHERANG	17.79	b	89.57	bcd	21.60	ab	23.38	a	100.7	a	73.7	c
IPB190-F-12-1-2	35.74	b	102.76	bc	22.62	a	19.51	c	78.7	de	72.3	c
IPB187-F-75-1-3	101.76	a	41.00	e	20.23	b	20.25	bc	74.0	e	84.7	a
IPB189-F-6-2-3	23.42	b	88.75	bcd	21.30	ab	23.22	a	90.2	bc	71.7	c
IPB187-F-41-2-1	12.32	b	116.51	b	20.63	ab	23.18	a	95.3	ab	76.0	bc
IPB193-F-17-2-3	21.47	b	175.94	a	21.13	ab	23.06	a	93.3	ab	83.3	ab
IPB189-F-42-1-1	36.49	b	59.32	de	20.19	b	21.48	abc	87.5	bc	72.0	c
IPB187-F-43-1-1	30.08	b	121.01	b	20.54	ab	23.24	a	90.0	bc	72.0	c
IPB193-F-38-2-1	99.95	a	86.02	bcd	19.68	b	22.27	ab	82.0	cde	73.7	c
Means	38.95	B	95.552	A	20.83	B	22.26	A	87.5	A	75.4	B

Note: the numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%. Means are compared TP to DS.

The accumulation of biomass during plant growth is reflected in the dry weight of the plant. This biomass is an energy source for the vegetative and generative growth of plants. We observed the dry weight of this plant to get an overview of the dry weight yields produced by the genotypes of rice grown in the transplanting and direct seeding planting methods. Our finding showed that the highest dry weight production of rice plants grown in the direct seeding method was at 45 and 65 days after sowing (Figure 2). However, when entering the heading phase, the dry weight production of the plant in the transplanting method was 48% greater than that of the direct seeding method. One of the reasons for this difference was caused by the longer duration of vegetative growth in rice plants using the transplanting method so that the biomass accumulated in the dry weight gain of shoots and plant roots (Tables 5 and 6).





**Figure 2.** The total dry weight of rice genotypes cultivated by transplanting and direct seeding method (DAS: days after seeding)

**Table 5.** Shoot dry weight (g) of rice genotype on transplanting (TP) and direct-seeding (DS) method

Genotype	45 DAS		65 DAS		Heading	
	TP	DS	TP	DS	TP	DS
IPB3S	0.63 b	2.68 ab	7.07 c	14.70 b	18.56 b	34.09 ab
CIHERANG	0.68 b	3.03 ab	14.32 bc	19.26 b	56.86 a	36.50 ab
IPB190-F-12-1-2	4.73 ab	2.57 ab	26.8 ab	28.82 ab	58.18 a	40.58 ab
IPB187-F-75-1-3	5.55 a	1.40 b	33.05 a	22.28 ab	67.58 a	57.60 a
IPB189-F-6-2-3	0.88 ab	4.64 ab	19.33 bc	29.60 ab	47.53 a	48.05 ab
IPB187-F-41-2-1	0.54 b	3.31 ab	12.73 c	30.60 ab	70.53 a	27.98 b
IPB193-F-17-2-3	2.01 ab	6.67 a	21.14 ab	36.49 a	85.77 a	68.10 a
IPB189-F-42-1-1	1.43 ab	3.96 ab	27.77 ab	27.80 ab	51.94 a	35.82 ab
IPB187-F-43-1-1	1.47 ab	2.54 ab	20.57 bc	18.35 b	51.31 a	43.37 ab
IPB193-F-38-2-1	4.87 ab	3.68 ab	38.07 a	15.87 b	69.31 a	29.16 b
Means	2.28 B	3.45 A	22.09 B	24.38 A	57.76 A	42.13 <sup>3</sup> B

Note: the numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%. Means are compared TP to DS.

In terms of genotype, IPB193-F-17-23 consistently produced the highest dry weight since the plant was 45 days after sowing. Even at the time of heading, the dry weight production of the genotype was 1.5 – 2.5 times greater than the other genotypes in the direct seeding method. Meanwhile, in the transplanting method, the dry weight of the genotype was only 10% lower than IPB189-F-42-1-1

which resulted in a dry weight of 118.5 g plant<sup>-1</sup>. The two genotypes identified have similar characteristics of 1000 grain weight, endosperm, and embryo (Table 1). These two genotypes can be considered as the main characters that contribute to the superior early vigor trait. Even if sorted from germination testing in the laboratory and pot experiment, the IPB193-F-17-2-3 genotype consistently produced high dry weight and strong early seedling vigor characters. On the other hand, the genotypes with the 1000 grain weight heavier in this observation showed the superiority of plant dry weight in the transplanting system until the plant age was 65 days. Whereas in the direct seeding method, the genotype did not show superior characters on the dry weight variable of the plant. The heavier seed weight character in this series of tests contributed to the early phase of plant growth but was not consistent enough when the plant entered the generative stage. The finding can be considered in the development of DSR cultivars in the future.

**Table 6.** Root dry weight (g) of rice genotype on transplanting (TP) and direct-seeding (DS) method

Genotype	45 DAS		65 DAS		Heading	
	TP	DS	TP	DS	TP	DS
IPB3S	0.16 b	1.62 ab	2.23 b	5.15 c	7.23 b	9.29 b
CIHERANG	0.31 b	1.42 ab	7.82 b	7.95 bc	10.72 b	12.94 ab
IPB190-F-12-1-2	3.51 a	2.09 ab	13.76 a	19.39 ab	10.87 ab	8.56 b
IPB187-F-75-1-3	1.98 ab	0.54 b	7.74 b	6.03 c	13.78 ab	15.66 ab
IPB189-F-6-2-3	0.29 b	2.54 ab	12.67 a	13.14 abc	22.93 a	16.48 ab
IPB187-F-41-2-1	0.28 b	1.46 ab	5.88 b	14.21 ab	18.48 ab	9.94 b
IPB193-F-17-2-3	1.15 ab	4.77 a	11.04 ab	24.30 a	23.13 a	26.67 a
IPB189-F-42-1-1	1.03 ab	1.51 ab	10.57 ab	8.07 bc	25.06 a	6.65 b
IPB187-F-43-1-1	0.89 b	1.62 ab	11.04 ab	9.19 bc	12.55 b	20.67 ab
IPB193-F-38-2-1	2.35 ab	1.77 ab	13.55 a	4.09 c	19.27 ab	5.83 b
Means	1.20 B	1.93 A	9.63 B	11.15 A	16.40 A	13.27 B

Note: the numbers followed the same letter in the same column show no significant difference refer to Tukey test at the level  $\alpha$  level 5%. Means are compared TP to DS.

The study found that the dry weight of shoots and roots of rice plants had a similar response to the total dry weight of plants. The dry weight of shoots and roots in the direct seeding method was higher than the plants in the TP method at 45 and 65 days after seeding (Tables 5 and 6). On the other hand, the dry weight of shoots and roots at the heading phase was 37% and 23.6% greater, respectively, in the TP method than in the DS method. The highest increase in shoot dry weight in both methods occurred at the age of 45 days to 65 days as much as 7 times for the DS method and 9 times for the TP method. While on the root dry weight variable, the highest increase in weight occurred at the age of the plant from 45 days to 65 days old in both cultivation methods. This is in line with the findings

of Nangklang et al. [40] who reported that the dry weight of the crown and paddy planted with direct seeding in wetlands resulted in higher shoot and root dry weight during panicle initiation towards the flowering phase compared to cultivars grown in the upland and transplanting method.

The dry weight of the shoot of rice plants differed between genotypes. The dry weight of the shoot of the two varieties of Ciherang and IPB 3S showed almost the same weight in the DS method but different in the TP method, where Ciherang had a dry weight almost three times greater than IPB 3S at the time of heading. The highest dry weight at heading was produced by genotype IPB193-F-17-2-3 in both cultivation methods (Table 5). Meanwhile, on the root dry weight variable, this genotype also had the highest weight in the DS method (Table 6).

### 3.5. *Correlation between seed attributes with early vigor characters and agronomy traits*

The observation results from the four stages of our research, some of the variables we did correlation analysis to see the relationship between the variables. The results of the correlation analysis of the observed variables are presented in Tables 7a and 7b. The correlation results showed that the character of the weight of 1000 grains had a strong positive correlation with seed area, endosperm area, and dry weight of sprouts in laboratory testing ( $r > 0.50$ ;  $p\text{-value} < 0.001$ ) while the moderate correlation was with the germination variable ( $r = 0.41$ ), seedling dry weight in the pot experiment ( $r = 0.48$ ), and total leaf area ( $r = 0.46$ ). The result is in line with the report of Botwright et al. [41] reported that large grain (large grain) accelerated the development of leaf area which has an effect on increasing biomass production by 25% at anthesis and 15% at maturation and can significantly increase crop yields by 15%.

The character of seed area and endosperm area were positively correlated with seedling dry weight on germination testing in the laboratory with correlation coefficients of 0.41 and 0.46, respectively. Endosperm area had a positive correlation with plant height, total leaf area, and canopy dry weight. The character of the area of the embryo is positively correlated with plant height. With the results of this correlation, the seed attributes have a contribution in determining the characteristics of the initial formation of plants. These results are in line with our previous findings [42] that the size and shape of the seed play a role in determining the early vigor trait.

**Table 7a.** Pearson correlation between seed, endosperm, and embryo physical traits with early vigor traits and agronomy attributes

	1000GW	AS	Aen	Aem	EER	VI	GP_1	SDW_1	TRE	TPE	GP_2	NoL_1	SDW_2
1000GW	1.00												
AS	0.62**	1.00											
Aen	0.52**	0.49*	1.00										
Aem	0.15	0.36	0.04	1.00									
EER	-0.02	-0.20	0.24	-0.93**	1.00								
VI	-0.36	-0.24	-0.08	0.25	-0.22	1.00							
GP_1	-0.35	-0.13	-0.12	0.35	-0.33	0.97**	1.00						
SDW_1	0.56**	0.41*	0.45*	0.26	-0.20	-0.13	-0.06	1.00					
TRE	-0.22	0.22	0.24	-0.21	0.35	-0.02	-0.04	-0.29	1.00				
TPE	-0.25	-0.07	-0.37*	-0.17	0.05	-0.43*	-0.34	-0.11	-0.06	1.00			
GP_2	0.41*	-0.15	0.00	-0.04	0.12	-0.07	-0.16	0.01	-0.31	-0.16	1.00		
NoL_1	0.32	-0.08	0.16	0.12	-0.07	0.03	0.00	0.37*	-0.34	-0.22	0.31	1.00	
SDW_2	0.48*	-0.01	0.17	0.19	-0.15	-0.02	-0.03	0.39*	-0.38	-0.01	0.29	0.70	1.00
NoL_2	-0.15	-0.24	-0.08	-0.08	0.09	0.08	0.06	0.19	-0.15	0.19	-0.02	0.40*	0.46*
Heigh_1	-0.21	0.01	0.15	0.37*	-0.28	0.37*	0.39*	0.07	0.07	-0.14	-0.34	0.42*	0.33
Heigh_2	0.18	0.28	0.46*	-0.03	0.11	-0.23	-0.23	0.04	0.13	-0.03	-0.23	-0.35	-0.02
TLA	0.46*	-0.12	0.40*	0.01	0.04	0.05	-0.04	0.30	-0.35	-0.25	0.28	0.44*	0.64**
FS	-0.05	0.02	-0.03	0.16	-0.10	0.20	0.21	0.23	0.03	0.02	0.07	0.44*	0.35
TDW_45	0.17	0.01	0.42*	0.00	0.08	0.02	-0.03	0.24	-0.09	-0.20	-0.16	-0.01	0.16
SDW_45	0.23	0.05	0.48*	-0.01	0.10	0.00	-0.06	0.23	-0.09	-0.18	-0.09	-0.07	0.17
RDW_45	0.10	-0.04	0.33	0.01	0.04	0.04	0.00	0.23	-0.07	-0.22	-0.23	0.05	0.14
ToHead	0.05	-0.20	-0.18	0.17	-0.14	0.25	0.21	0.29	-0.30	-0.07	0.41*	0.48*	0.36

Note: 1000GW=1000 grain weight, AS=area of seed, Aen=area of endosperm, Aem=area of embryo, EER=endosperm/embryo ratio, VI=vigor index, GP\_1=germination percentage in laboratory experiment, SDW\_1=seedling dry weight of laboratory test, TRE=time of radicle emergence, TPE=time of plumule emergence, GP\_2=germination percentage at pot experiment, NoL\_1=number of leaves in germination test at 14 DAS, NoL\_2= number of leaves at 25 DAS, Height\_1= plant height at 25 DAS, Height\_2= plant height at 45 DAS, TLA=total leaf area, FS=photosynthetic rate, TDW\_45=total dry weight at 45 DAS, SDW\_45=seedling dry weight at 45 DAS, RDW\_45=root dry weight at 45 DAS, ToHead=time of heading

**Table 7b.** Pearson correlation between seed, endosperm, and embryo physical traits with early vigor traits and agronomy attributes

	NoL_2	Height_1	Height_2	TLA	FS	TDW_45	SDW_45	RDW_45	ToHead
1000GW	-0.15	-0.21	0.18	0.46*	-0.05	0.17	0.23	0.10	0.05
AS	-0.24	0.01	0.28	-0.12	0.02	0.01	0.05	-0.04	-0.20
Aen	-0.08	0.15	0.46*	0.40*	-0.03	0.42*	0.48*	0.33	-0.18
Aem	-0.08	0.37*	-0.03	0.01	0.16	0.00	-0.01	0.01	0.17
EER	0.09	-0.28	0.11	0.04	-0.10	0.08	0.10	0.04	-0.14
VI	0.08	0.37*	-0.23	0.05	0.20	0.02	0.00	0.04	0.25
GP_1	0.06	0.39*	-0.23	-0.04	0.21	-0.03	-0.06	0.00	0.21
SDW_1	0.19	0.07	0.04	0.30	0.23	0.24	0.23	0.23	0.29
TRE	-0.15	0.07	0.13	-0.35	0.03	-0.09	-0.09	-0.07	-0.30
TPE	0.19	-0.14	-0.03	-0.25	0.02	-0.20	-0.18	-0.22	-0.07
GP_2	-0.02	-0.34	-0.23	0.28	0.07	-0.16	-0.09	-0.23	0.41*
NoL_1	0.40*	0.42	-0.35	0.44*	0.44*	-0.01	-0.07	0.05	0.48*
SDW_2	0.46*	0.33	-0.02	0.64**	0.35	0.16	0.17	0.14	0.36
NoL_2	1.00	0.52	-0.14	0.24	0.46*	0.29	0.22	0.35	0.46*
Height_1		1.00	-0.11	0.13	0.55**	0.13	0.04	0.22	0.14
Height_2			1.00	0.21	-0.42*	0.53**	0.63**	0.39*	-0.46*
TLA				1.00	-0.06	0.56**	0.59**	0.50**	0.10
FS					1.00	-0.11	-0.20	-0.01	0.53**
TDW_45						1.00	0.97**	0.97**	-0.06
SDW_45							1.00	0.88**	-0.08
RDW_45								1.00	-0.02
ToHead									1.00

Note: 1000GW = 1000 grain weight, AS=area of seed, Aen=area of endosperm, Aem=area of embryo, EER=endosperm/embryo ratio, VI=vigor index, GP\_1=germination percentage in laboratory experiment,

SDW\_1= seedling dry weight of laboratory test, TRE=time of radicle emergence, TPE=time of plumule emergence, GP\_2=germination percentage at pot experiment, NoL\_1=number of leaves in germination test at 14

DAS, NoL\_2= number of leaves at 25 DAS, Height\_1= plant height at 25 DAS, Height\_2= plant height at 45 DAS, TLA=total leaf area, FS=photosynthetic rate, TDW\_45=total dry weight at 45 DAS,

SDW\_45=seedling dry weight at 45 DAS, RDW\_45=root dry weight at 45 DAS, ToHead=time of heading



#### 4. Conclusions

Evaluation of seed characters is the first step in screening seed physical traits related to early vigor. Early vigor characters have been identified as suitable for cultivar development for direct seeding of rice systems. Based on the results, the characteristics of seed weight, seed area, endosperm area, and embryonic area have a direct relationship with early vigor characteristics. Seeds that have more weight, larger seed area, and larger endosperm area have superior early vigor characters on the variables of germination test, seedling dry weight, and total leaf area. Tests on direct seeding showed that several genotypes from IPB University rice breeding have the potential to be developed into DSR cultivars. Candidate genotypes for DSR would be further investigated in the field trial agronomically.

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8

#### Conflict of interest

All authors declare that have no conflict of interest in this paper.

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