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Agronomic Performance and Selection of Doubled-Haploid Rice Lines for Rainfed Lowland Paddy Field

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Abstract

Rainfed lowland rice cultivation is an alternative to increase national rice production. Breeding of high yielding rice varieties suitable for rainfed lowland condition can be accelerated by using doubled-haploid (DH) as genetic materials. This study aimed at obtaining information on the agronomic performance including yields in several DH rice lines and selecting DH lines suitable for rainfed lowland paddy field. The experimental design used was a Randomized Complete Block Design with 3 replications. The treatment was thirty DH lines and 4 check varieties namely RJ31 Ciherang, RJ32 Inpari 18, RJ33 Inpari 40, and RJ43 Inpari 41. The results showed that there were variability in all agronomic performances, i.e., plant height, number of tillers, days to heading and to harvest, panicle length, number of filled and empty grains, 1000-grain weight and grain yield. The DH lines, namely RJ19 DR8-43-3-1 and RJ25 DR10-14-1-1, gave the same productivity as 4 check varieties. Index selection showed that twelve DH lines with medium number of productive tillers, early maturing, and productivity of more than 4.40 tons.ha^{-1} were selected for further evaluation.

Keywords: Doubled-haploid, rainfed rice, selection index, yield component.

Introduction

Rice (*Oryza sativa* L.) is the main staple food for most Indonesian and Asian people. Rice has been cultivated for a long time in Indonesia. Preserved ancient botanical evidence in the form of rice phytoliths has confirmed that people farmed domesticated rice in the interior of Sulawesi Island, Indonesia, by at least 3,500 years ago (Deng et al., 2020). Recently, national rice consumption reaches 98.40 kg.capita^{-1}.year^{-1} with a population of approximately 252,165,000 people (BPS, 2019). The increasing population of Indonesia can be used as an indication that the need for rice will also increase. Between 2010-2014, the increase of Indonesia's population was 1.40% per year, thus Indonesia's rice needs will reach for more than 70 million tons in the year of 2025 (BPS, 2015).

An effort to increase rice production in Indonesia is generally prioritized for land with irrigation facilities, rice paddies where water is always available at every season. The level of national production has not yet been fulfilled and there is even a shortage due to drought, weeds, pests and diseases, and natural disasters such as floods (Rahayu and Harjoso, 2010). Rice can be cultivated in rainfed fields. The development of rainfed lowland rice lines is one of the efforts, programs, or strategies to increase national rice production, in addition to irrigated and dry land.

The problems of productivity, drought tolerance, and short duration rice, among others, can be overcome by plant breeding activities to get the desired lines. Obtaining these lines can be done with conventional breeding or anther culture. According to Dewi and Purwoko (2001; 2011) breeding carried out conventionally takes a very long time from 8 to10 generations. Shortening of pure line development time can be done by utilizing the haploid system through anther culture technique. It is expected that the process of obtaining pure lines as fully homozygous lines (doubled-haploid /DH) is accelerated by only one to two generations. Thus, it can increase efficiency of the selection process and accelerate the acquisition of varieties for release as well as saving costs, time, and labor (Dewi and Purwoko, 2012; Mishra and Rao, 2016; Purwoko, 2017).

New improved varieties can be determined by the agronomic performance (phenotypic appearance) of a line. The quantitative character of genotypes can vary from one environment to another. A variety that is consistent with high yields in all environments is generally the desired genotype in breeding programs (Pabendon and Takdir, 2000). Plant breeding techniques are not only applied to the development of high yielding varieties but also the ability of varieties to adapt in various environments (Mulusew et al., 2009; Akter, 2014). Estimation of plant adaptation in various locations and the magnitude of the influence of interactions in the typology of land: biophysical (climate specifically), edaphic and biotic diversity (Sitaresmi et al., 2012) in a good agroecological environment is one technique for obtaining superior varieties from several rice lines tested. Munarso et al. (2010) stated that to identify stable and high yielding genotypes, evaluation in several target production environments should be conducted.

In previous research, Dewi et al. (2017) obtained 275 DH rice lines by using anther culture of F1 derived from crossing of parents that had high yield and drought tolerance characters. Those DH lines were then evaluated based on their agronomic characters. The results showed high genetic variability as well as broad sense heritability (more than 90%) for all variables tested. Anther culture could generate high genetic variability for further selection (Syafii et al., 2018). In this study 30 DH lines were evaluated for their agronomic performance. Then, potential DH lines suitable for rainfed lowland paddy fields were selected.

Material and Methods

This research was carried out at Sukamandi Rice Field Experiment Station, West Java, The Indonesian Center for Rice Research, from May to September 2019.

Plant Materials

Genetic material used in this study were 34 genotypes, i.e., 30 doubled-haploid (DH) rice lines, designated as RJ1 to RJ30, and 4 checks varieties, designated as RJ31 to RJ34 (Table 1). The doubled-haploid plants obtained from anther culture of 6 F1s derived from Inpari 18/B12825E-TB-1-25//Gajah Mungkur (DR7), Inpari18/IR87795-14-11- B- SKI- 12//Gajah Mungkur (DR8), Inpari 18/IR83140-B-11-B/Gajah Mungkur (DR9), Inpari 22/IR87705-14-11-B-SKI-12// Gajah Mungkur (DR10), Inpari 22/IR83140- B-11-B// Gajah Mungkur (DR11), and Inpago 8/B12825E-TB-1-25//Gajah Mungkur (DR12). Check varieties used were Ciherang, Inpari 18, Inpari 40, and Inpari 41. Ciherang is a mega-variety, while the other three are newly released lowland rice varieties adaptive to rainfed paddy field.

Planting and Harvesting

For each line, seeds were sowed in two 0.5 m long grooves with a distance between lines 10 cm. Land preparation involved clearing, ploughing, and harrowing. Transplanting was done by planting 18 day-old rice seedlings, 2 to 3 seedlings for each hill. Plant spacing used was 25 cm x 25 cm, so that in each plot there were 8 rows with 20 hills for each row.

Fertilization was done by giving NPK in the form of Urea (250 kg.ha^{-1}), KCl (150 kg.ha^{-1}), and SP36 (150 kg.ha^{-1}). Fertilizers were given in three stages. The first fertilization was carried out at transplanting by giving Urea 83.3 g.plot^{-1}, KCl 150 g.plot^{-1}, and SP36 150 g.plot^{-1}. The second fertilization was carried out at 4 weeks after planting (WAP) by giving urea 83.3 g.plot^{-1}. The last fertilization was carried out at 8 WAP or during flowering stage by giving Urea 83.3 g.plot^{-1}. Maintenance includes weeding and controlling plant pests and diseases. Weeding was done intensively when the plant was in the vegetative and reproductive phases. Pest and disease control was carried out by mechanical and chemical methods. In mechanical method, pest was killed directly or the environment was made unsuitable for it. For example, traps for pest animals and insects; or barriers such as screens or fences to keep animals and insects out, while in chemical control, chemical pesticides are applied to protect plants from pests and diseases. Harvesting was carried out when 90% of rice panicles in one plot turned yellow. Seeds were harvested to estimate grain yield per plot. The grains were sun-dried for approximately 3 to 4 days to reach ± 14% moisture content as measured by grain moisture tester DMC550 and later converted to dry grain yield per hectare (ton.ha^{-1}).

Experimental Design and Data Collection

The experimental design used was a completely randomized complete block design (RCBD) in the form of genotypes as a treatment (Table 1) which was repeated three times. The experimental unit was a 2 m x 5 m rice plot.

Observations and measurements of the yield components were carried out on 3 hills for all lines tested. The agronomic characters observed according to Standard Evaluation System for Rice from IRRI (2013) were as follows:

1. Vegetative plant height (cm) was measured from ground level to the tip of the highest leaf. This character was observed at 45 days after planting.

Table 1. Lines and check varieties used in the experiment

DH lines	DH lines	DH lines	Check varieties
RJ1 DR7-3-4-1	RJ11 DR7-69-1-3	RJ21 DR9-11-1-1	RJ31 Ciherang
RJ2 DR7-7-6-1	RJ12 DR7-69-1-4	RJ22 DR9-11-1-2	RJ32 Inpari 18
RJ3 DR7-26-3-1	RJ13 DR7-95-1-1	RJ23 DR9-58-1-1	RJ33 Inpari 40
RJ4 DR7-31-2-1	RJ14 DR8-18-1-1	RJ24 DR9-69-2-2	RJ34 Inpari 41
RJ5 DR7-35-1-1	RJ15 DR8-20-1-1	RJ25 DR10-14-1-1	
RJ6 DR7-37-1-1	RJ16 DR8-23-2-3	RJ26 DR10-26-1-1	
RJ7 DR7-43-1-5	RJ17 DR8-31-1-1	RJ27 DR10-27-3-1	
RJ8 DR7-44-1-2	RJ18 DR8-32-2-1	RJ28 DR10-42-1-1	
RJ9 DR7-67-2-1	RJ19 DR8-43-3-1	RJ29 DR11-36-1-1	
RJ10 DR7-69-1-1	RJ20 DR9-4-1-1	RJ30 DR12-52-2-1	

Note: Inpari 18/B12825E-TB-1-25//Gajah Mungkur (DR7), Inpari18/IR87795-14-11- B- SKI- 12//Gajah Mungkur (DR8), Inpari 18/IR83140-B-11-B/Gajah Mungkur (DR9), Inpari 22/IR87705-14-11-B-SKI-12//Gajah mungkur (DR10), Inpari 22/IR83140- B-11- B//Gajah Mungkur (DR11), and Inpago 8/B12825E-TB-1-25//Gajah Mungkur (DR12)

2. Generative plant height (cm) was measured from ground level to the longest panicle tip. This character was observed before harvest.
3. Number of productive tillers (tillers/hill) was measured before harvest by counting tillers that produce panicles.
4. The days to heading was calculated from the days of sowing until 50% of rice panicle in a plot was fully visible.
5. The days to harvest was calculated from the days of sowing to the days when 90% of rice panicles in one plot turned yellow.
6. Panicle length (cm) was measured from the panicle neck to the panicle tip of each panicle.
7. The number of filled grains per panicle (grains) was observed by counting the number of filled
8. grains in each panicle.
9. The number of empty grains per panicle (grains) was observed by counting the number of empty grains in each panicle.
10. Total number of grains per panicle (grains) was observed by counting the number of grains in
11. each panicle.
12. Percentage of unfilled grains per panicle (%), was obtained by comparing the number of unfilled grains per panicle to the number of grains per panicle then multiplying by one hundred.
13. Percentage of filled grains per panicle (%), was obtained by comparing the number of filled grains per panicle to the number of grains per panicle then multiplying by one hundred.
14. Weight of 1,000 grains (g), was done by weighing 1,000 filled grains with ± 14% moisture content.
15. Productivity (tons.ha^{-1}) was calculated using the formula: (160,000 hills.ha^{-1} / number of hills harvested per plot) x (grain yield per plot(kg)/ 1,000)

Data Analyses

Data obtained was analyzed by the normality test, if the tested trait distributed normally then continued with the analysis of variance. Furthermore, if the F test was significant, then Duncan's multiple range test was performed (Gomez and Gomez, 1984). Selection index was used to select genotypes suitable for further yield trials in rainfed lowland environment. Several agronomic important traits representing yield and yield components were chosen simultaneously and economic weightage was given to the phenotypic values of each trait in such a way that expected gain in aggregate genotypic value would be maximized (Ramos et al., 2014; Gazal et al., 2017). Determination of the selection index were conducted based on Falconer and Mackay (1996) as follows:

$$I = b_1X_1 + b_2X_2 + b_3X_3 + ... + b_nX_n$$

where: I is the selection index
b_n is the weight of the variable-n
X_n is a standardized phenotype value (z) for variable-n
$z = \frac{x - \bar{x}}{s}$
x is the means of each genotype
$\bar{x}$ is the means of the variable
s is the standard deviation of the variable

Then, the I values were ranked and used to select the best lines. The analysis of variance and the construction of the weighted selection index were carried out using SAS 9.0 and STAR programs.

Results and Discussion

Agronomic Characters of Doubled-Haploid Lines

The agronomic performance of DH rice lines showed different responses. This result can be seen in the recapitulation of variance observed in 12 characters (Table 2). Genotypes have a significant effect on all agronomic traits observed, except for the number of grains per panicle and the percentage of filled grains per panicle. The analysis of variance in Table 2 shows the character such as days to harvest has the lowest coefficient of variation (CV) value, while the number of empty grains has the highest CV value. The CV is the ratio of the standard deviation to the mean. When we are presented with estimated values, the CV relates the standard deviation of the estimate to the value of this estimate. However, the magnitude of the CV value depends on the experimental, plant, and observed factors. The lower the value of the CV, the more precise the estimate, so that it will reduce the error of the experiment (Gomez and Gomez, 1984).

Plant height character was measured at vegetative and reproductive stages. The average vegetative plant height of 34 rice genotypes ranged from 65.4-115.7 cm, while for reproductive plant height ranged from 75.6-141.9 cm (Table 3). Vegetative plant height will increase until the plant reached the reproductive stage. According to Rachmawati and Retnaningrum (2013), plant height increase is influenced by the availability of water and nutrients. Plant height at reproductive stage is one of the important criteria in selection process because it is related to the ease of maintenance and harvesting process. Tall rice plants (> 125 cm) are very easy to lodge and lodging can cause a decrease in grain yield, while the short one (< 80 cm) is relatively difficult for farmers to harvest. Therefore, plant height at reproductive stage is also an important character to farmers' acceptance of new varieties (Dewi et al., 2015). In general, according to Akbar et al. (2018) intermediate plant height (90-124 cm) was categorized as a good agronomic character for rainfed rice.

The average number of productive tillers ranged from 11.6 to 35.2 tillers per hill (Table 3). From this experiment, there is one DH line, RJ23DR9-58-1-1, that has a desirable number of productive tillers (35 tillers per hill), 6 DH lines have an acceptable number of productive tillers (20-25 tiller per hill), and the rest have medium number of productive tillers (10-19 tillers per hill). All DH lines, except for RJ23DR9-58-1-1 line, have non-significantly different number of productive tillers with the check varieties for rainfed lowland, i.e. Inpari 18, Inpari 40 and Inpari 41. The number of tillers has been reported to have a positive association with plant biomass and economic yields in rice (Wang et al., 2017). Besides the genetic and environmental conditions, the number of rice tillers is strongly influenced by age of seedling at transplanting time and plant spacing which will also determine its population density (Donggulo et al., 2017). Especially for rice, the number of productive tillers often became the main character in the selection process because it directly influences rice yields through the number of panicles, the number of grains, and filled grains per panicle (Fukushima, 2019). However, the large number of productive tillers can result in the non-synchronized panicle maturity, thus causing the quality of rice to decline (Abdullah et al., 2008).

Table 2. Summary of statistical analyses on the influence of genotypes on agronomic characters

Variables	Mean Square	F-value	CV (%)
Vegetative plant height (VPH)	443.80	9.56**	8.20
Generative plant height (GPH)	877.81	34.74**	5.10
Number of productive tillers (NPT)	65.32	1.55*	13.30
Days to 50% flowering (DTF)	69.10	20.79**	2.20
Days to harvest (DTH)	35.29	11.05**	1.60
Panicle Length (PL)	6.10	4.90**	4.80
Number of filled grains per panicle (NFG)	154.21	2.57**	16.10
Number of unfilled grains per panicle (NUG)	959.14	4.36**	20.20
Number of grains per panicle (NTG)	181.86	1.89ns	13.70
% Filled grain (%FG)	522.40	2.28ns	7.60
1000-grain weight (GWE)	12.68	21.08**	2.90
Productivity (GY)	4.61	13.47**	14.20

Note:** significant at P < 0.01;* significant at P < 0.05; ns= non-significant

Table 3. Plant height and number of reproductive tillers of doubled haploid lines and check varieties

Genotype	Plant height (cm)				Number of productive tillers (tiller.hill^{-1})	
	Vegetative		Generative			
RJ1 DR7-3-4-1	98.7	bcd	127.2	b	15.3	c
RJ2 DR7-7-6-1	96.1	bcde	109.1	fg	23.8	bc
RJ3 DR7-26-3-1	76.3	ghij	83.2	mnopq	14.0	c
RJ4 DR7-31-2-1	102.2	bc	114.1	def	13.4	c
RJ5 DR7-35-1-1	68.0	j	91.6	ijklm	21.4	bc
RJ6 DR7-37-1-1	84.2	efghi	109.9	efg	13.7	c
RJ7 DR7-43-1-5	83.9	efghi	88.9	klmno	19.1	bc
RJ8 DR7-44-1-2	90.0	cdef	94.4	ijkl	18.8	bc
RJ9 DR7-67-2-1	65.4	j	75.6	q	16.3	c
RJ10 DR7-69-1-1	67.4	j	78.2	pq	19.3	bc
RJ11 DR7-69-1-3	72.7	ij	80.4	nopq	17.1	c
RJ12 DR7-69-1-4	86.2	defgh	93.7	ijkl	15.8	c
RJ13 DR7-95-1-1	76.3	ghij	125.8	bc	11.6	c
RJ14 DR8-18-1-1	70.4	j	79.4	opq	12.4	c
RJ15 DR8-20-1-1	86.8	defgh	99.0	hi	16.2	c
RJ16 DR8-23-2-3	71.4	ij	83.56	mnopq	18.0	bc
RJ17 DR8-31-1-1	83.9	efghi	98.4	hij	17.9	bc
RJ18 DR8-32-2-1	73.9	hij	83.0	mnopq	20.8	bc
RJ19 DR8-43-3-1	74.8	ghij	94.4	ijkl	19.0	bc
RJ20 DR9-4-1-1	115.7	a	141.9	a	15.0	c
RJ21 DR9-11-1-1	97.6	bcd	108.3	fg	17.2	c
RJ22 DR9-11-1-2	92.2	bcde	119.8	bcd	16.6	c
RJ23DR9-58-1-1	77.3	fghij	85.1	lmnopq	35.2	a
RJ24 DR9-69-2-2	75.7	ghij	89.1	jklmn	17.9	bc
RJ25 DR10-14-1-1	74.3	ghij	83.6	mnopq	18.4	bc
RJ26 DR10-26-1-1	75.6	ghij	86.9	klmnop	22.3	bc
RJ27 DR10-27-3-1	103.2	b	125.1	bc	20.4	bc
RJ28 DR10-42-1-1	69.6	j	78.8	pq	14.7	c
RJ29 DR11-36-1-1	94.6	bcde	118.1	cde	14.7	c
RJ30 DR12-52-2-1	69.1	j	75.6	q	18.2	bc
RJ31 Ciherang	89.9	cdef	99.3	hi	30.6	ab
RJ32 Inpari 18	87.3	defg	96.1	hijk	18.0	bc
RJ33 Inpari 40	87.7	defg	104.0	gh	19.1	bc
RJ34 Inpari 41	78.3	fghij	99.3	hi	19.7	bc

Note: The values followed by the same letters within the same column are not significantly different according to DMRT at α 5%.

Analysis of variance and means tested (Table 4) show that the days to 50% flowering (DTF) varied between 77.3 to 87.3 days after sowing (DAS), while the days to harvest (DTH) varied between 110 to125 DAS. According to BB Padi (2015), the days to harvest or harvest time of rice plants can be grouped as follows: long (more than 151 HSS), moderate (125-150 HSS), early (105-124 HSS), very early (90-104 HSS), and ultra-early (less than 90 HSS). Presented in Table 4, all check varieties and the tested DH lines were categorized as early maturing except for RJ13-DR7-95-1-1 line. Early maturing rice is highly

Table 4. Days to 50% flowering, days to harvest, and panicle length of doubled haploid lines and check varieties

Genotype	Days to				Panicle length (cm)	
	50% flowering (DAS)		Harvest (DAS)			
RJ1 DR7-3-4-1	83.3	defg	111.0	hi	23.7	bcdefg
RJ2 DR7-7-6-1	82.7	efgh	112.0	fghi	24.9	abc
RJ3 DR7-26-3-1	83.7	cdefg	112.0	fghi	23.0	cdefgh
RJ4 DR7-31-2-1	76.7	klmnop	110.3	i	24.9	abc
RJ5 DR7-35-1-1	85.7	abcde	113.3	efghi	23.3	cdefgh
RJ6 DR7-37-1-1	83.7	cdefg	112.0	fghi	26.2	a
RJ7 DR7-43-1-5	75.7	lmnopq	115.0	cdef	23.4	bcdefgh
RJ8 DR7-44-1-2	75.0	mnopq	112.3	fghi	24.2	abcd
RJ9 DR7-67-2-1	87.3	ab	116.7	cde	22.9	cdefgh
RJ10 DR7-69-1-1	86.7	abcd	115.3	cdef	24.1	abcd
RJ11 DR7-69-1-3	87.3	ab	118.3	bc	22.8	cdefgh
RJ12 DR7-69-1-4	82.7	efgh	117.0	bcd	23.3	cdefgh
RJ13 DR7-95-1-1	87.0	abc	125.0	a	22.8	cdefgh
RJ14 DR8-18-1-1	69.3	r	111.3	ghi	21.7	fgh
RJ15 DR8-20-1-1	79.3	hijk	113.3	efghi	21.3	hi
RJ16 DR8-23-2-3	81.0	fghi	112.0	fghi	23.8	bcdef
RJ17 DR8-31-1-1	86.7	abcd	110.0	i	22.0	defgh
RJ18 DR8-32-2-1	76.7	klmno	110.7	i	19.7	i
RJ19 DR8-43-3-1	83.0	efg	116.0	cde	24.1	abcde
RJ20 DR9-4-1-1	85.3	abcde	114.3	defgh	26.0	a
RJ21 DR9-11-1-1	84.7	bcde	120.0	b	25.6	ab
RJ22 DR9-11-1-2	80.3	ghij	110.0	i	23.0	cdefgh
RJ23 DR9-58-1-1	82.67	efgh	112.0	fghi	22.3	defgh
RJ24 DR9-69-2-2	80.3	ghij	117.7	bcd	22.5	defgh
RJ25 DR10-14-1-1	73.3	oq	110.3	i	21.5	ghi
RJ26 DR10-26-1-1	73.3	opq	110.3	i	21.6	fgh
RJ27 DR10-27-3-1	80.3	ghij	114.3	defgh	23.8	bcdef
RJ28 DR10-42-1-1	78.3	ijklm	110.0	i	21.9	efgh
RJ29 DR11-36-1-1	84.0	bcdef	114.7	defg	24.9	abc
RJ30 DR12-52-2-1	78.7	ijkl	110.0	i	22.1	defgh
RJ31 Ciherang	88.7	a	114.7	defg	23.6	bcdefg
RJ32 Inpari 18	74.7	nopq	111.3	ghi	22.4	defgh
RJ33 Inpari 40	77.3	jklmn	110.3	i	22.3	defgh
RJ34 Inpari 41	80.7	fghij	110.3	i	22.1	defgh

Note: The values followed by the same letters within the same column are not significantly different according to DMRT at α 5%. DAS= days after sowing.

recommended to be planted in rainfed rice fields. This is in accordance with the conditions of rainfed rice fields where the source of water for irrigation is limited from rainfall which is difficult to predict. Early maturity rice is also preferred by farmers at irrigated lowland rice fields because it can increase cropping index from two to three times a year (Fatimah et al., 2014). The panicle length of all tested genotypes varied between 19.7 to 26.2 cm (Table 4). RJ6 DR7-37-1-1 has the longest panicle length (26.16 cm), followed by RJ20 DR9-4-1-1 (26.0 cm), RJ21 DR9-11-1-1 (25.6 cm), RJ2 DR7-7 -6-1 (24.9 cm) and RJ29 DR11-36-1-1 (24.9 cm). Factors that can affect the length of rice panicles are sudden environmental changes during planting season as well as the amount of nutrients available in the soil (Mulsanti et al., 2014; Zahrah, 2011). Based on the panicle length classification by Rahmah and Aswidinnoor (2013), panicle length can be grouped into short (<20 cm), medium (20-30 cm) and long (> 30 cm) panicle. There is one line, i.e. RJ18 DR8-32-2-1, classified as short panicle, while the other 29 lines and all check varieties classified as medium panicle. RJ6 DR7-37-1-1 and RJ20 DR9-4-1-1 have 9% to 15% longer panicles than all check

Table 5. Number of grains per panicle in doubled haploid lines and check varieties

Genotype	No. filled grains.		No. unfilled grains		Total no. grains	
RJ1 DR7-3-4-1	126.6	bcd	82.7	abcdefg	208.8	bcdef
RJ2 DR7-7-6-1	142.9	abcd	97.3	abc	240.7	abcd
RJ3 DR7-26-3-1	163.8	abc	59.3	fghi	223.4	abcdef
RJ4 DR7-31-2-1	130.5	bcd	87.7	abcdefg	207.8	bcdef
RJ5 DR7-35-1-1	156.5	abc	60.0	efghi	216.4	abcdef
RJ6 DR7-37-1-1	151.2	abcd	66.8	defghi	217.4	abcdef
RJ7 DR7-43-1-5	115.2	cd	96.7	abc	212.0	bcdef
RJ8 DR7-44-1-2	189.2	a	64.1	defghi	254.0	abcd
RJ9 DR7-67-2-1	140.4	abcd	84.6	abcdefg	225.3	abcde
RJ10 DR7-69-1-1	141.5	abcd	87.0	abcdef	228.6	abcde
RJ11 DR7-69-1-3	141.6	abcd	89.5	abcde	231.0	abcde
RJ12 DR7-69-1-4	168.1	ab	106.3	a	274.7	a
RJ13 DR7-95-1-1	161.7	abc	57.6	fghi	219.6	abcdef
RJ14 DR8-18-1-1	116.2	cd	46.4	hij	162.7	f
RJ15 DR8-20-1-1	155.1	abc	64.8	defghi	219.7	abcdef
RJ16 DR8-23-2-3	146	abcd	68.6	cdefghi	214.7	abcdef
RJ17 DR8-31-1-1	102.7	d	75.3	bcdefgh	177.8	ef
RJ18 DR8-32-2-1	146.8	abcd	62.6	efghi	209.2	bcdef
RJ19 DR8-43-3-1	142.1	abcd	56.4	ghi	199.0	cdef
RJ20 DR9-4-1-1	125.9	bcd	89.1	abcde	215.4	abcdef
RJ21 DR9-11-1-1	184.6	a	62.7	efghi	247.5	abcd
RJ22 DR9-11-1-2	150.3	abcd	81.8	abcdefg	231.4	abcde
RJ23 DR9-58-1-1	116.2	cd	89.5	abcde	205.6	bcdef
RJ24 DR9-69-2-2	157.5	abc	98.1	ab	256.2	abcd
RJ25 DR10-14-1-1	182.5	a	83.1	abcdefg	266.3	ab
RJ26 DR10-26-1-1	163.3	abc	93.3	abcd	257.0	abcd
RJ27 DR10-27-3-1	180.2	a	81.1	abcdefg	261.5	abc
RJ28 DR10-42-1-1	145.6	abcd	72.1	bcdefghi	217.6	abcdef
RJ29 DR11-36-1-1	155.6	abc	87.2	abcdef	242.5	abcd
RJ30 DR12-52-2-1	167.7	ab	43.8	ij	211.5	bcdef
RJ31 Ciherang	181	a	55.8	ghi	236.9	abcde
RJ32 Inpari 18	174.6	ab	63.1	efghi	237.6	abcde
RJ33 Inpari 40	190	a	60.4	efghi	250.3	abcd
RJ34 Inpari 41	169	ab	27.8	j	196.2	def

Note: Values followed by the same letters within the same column are not significantly different according to DMRT at α 5%.

varieties. Despite of the grain size, the total number of grains per panicle depends on the panicle length. In general, the total number of grains tends to increase in a longer panicle (Nazirah and Damanik, 2015).

The number of filled grains per panicle is one of the selection criteria to obtain lines with high yields. The results of the analysis showed the average number of filled grains per panicle of DH lines ranged from 102.7-189.2 grains, while the number of unfilled grain ranged from 27.8 - 106.3 grains (Table 5). In this experiment, twenty-three DH lines has non-significantly different number of filled grains compared to the four check varieties. The number of filled and unfilled grains is influenced by genetic and environmental factors. Spikelet fertility and seed yield per panicle were severely reduced by extreme temperature in the 14 days period before anthesis (Eixarch and Ellis, 2015). Too low temperature affected spikelet fertility (Zeng et al., 2017), while too high temperature will inhibit the development of pollen grains in the anther resulted in sterile spikelets and unfilled grains, thus decrease the number of filled grains (Khamid, 2016). Other factors that influence the amount of filled and unfilled grains include the level of pests and diseases attacks during planting season.

The number of grains per panicle ranged between 162.7 - 274.7 grains (Table 5). Among the check variety, RJ34 Inpari 41 has the least number of grains per panicle, 196.2 grains. There are 27 lines that have grains number per panicle not significantly different from check variety RJ33 Inpari 40. Abdullah et al. (2008) stated that the high number of grains per panicle will extend maturation phase, thus it will increase the number of empty grains due to imbalance of source and sink, especially in new plant type (NPT) of rice.

The results of the analysis of the percentage of filled grain are presented in Table 6. Check variety RJ34 Inpari 41 has the highest average percentage of filled grain of 86.9%. There are 12 DH lines that have percentage of filled grain not significantly different from RJ34 Inpari 41. The grain yield is strongly influenced by several factors, namely genetic and environmental factors. Determination of the variety used, the choice of growing environment, the amount of light intensity, and the presence of pest and disease determine how much yield to be harvested (Wahyuni et al., 2013).

Seed index or 1,000-grain weight ranges from 22.5-31.0 g (Table 6). The lowest 1,000-grain weight was found in RJ25 DR10-14-1-1 line which was not significantly different from the RJ26 line DR10-26-1-1, and check variety RJ33 Inpari 40. The RJ11 DR7-69-1-3 had the highest 1,000-grain weight, not different from RJ10 DR7-69-1-1, RJ9 DR7-67-2-1, RJ5 DR7-35-1-1 and check variety RJ32 Inpari 18. Juhriah et al. (2013) classified 1,000-grain weights as light (<25 g), moderate (25-30 g), and heavy (> 30 g). Four DH lines were classified as light, 24 DH lines as moderate, and two DH lines as heavy in 1000-grain weight. The 1,000-grain weights of check varieties RJ33 Inpari 40 classified as light, while RJ31 Ciherang, RJ32 Inpari18, and RJ34 Inpari 41 were classified as moderate. Factors influencing the weight of 1,000-grain include the availability of water in the pollination and grain filling phases. Sujinah and Jamil (2016) stated that the lack of water will reduce the weight of 1,000 grains.

Productivity of DH lines ranges from 2.10 to 7.12 tons.ha^{-1} (Table 6). RJ7 DR7-43-1-5 has the lowest productivity while the check variety RJ31 Ciherang has the highest productivity. There are two DH lines, i.e., RJ19 DR8-43-3-1 (7.12 tons.ha^{-1}) and RJ25 DR10-14-1-1 (6.45 tons.ha^{-1}) which has the same productivity as the four check varieties. Mahmud and Purnomo (2014) stated that productivity is closely related to the yield components such as the number of productive tillers per hill, the number of grains per panicle, the percentage of filled grains, and the 1,000-grain weight.

Correlation Analysis for Determining Good Agronomic Performance

The relationship between agronomic traits and yield is known from the correlation analysis values presented in Table 7. The generative plant height correlates positively and very significantly with the vegetative plant height (r=0.85, P<0.01). The high plant height at reproductive stage is a result from high plant height at vegetative stage, which is not desirable (Dewi et al., 2015). The heading character correlates positively and very significantly with days to harvest (r=0.52, P<0.01). Faozi et al. (2010) stated that days to harvest was determined by the speed of heading, because in general rice plants have different duration of vegetative period, while the grain filling period is relatively similar.

The number of grain was positively and very significantly correlated to the character of the number of filled grains per panicle (r=0.71, P<0.01). The results of this analysis are in line with the results of Kartina et al. (2016) which stated that an increase in the total number of grains per panicle is accompanied by an increase in the overall number of filled grain. Increasing the number of filled grains per panicle will be followed by the addition of grain yield per hill. Moreover, grain yield per hectar or productivity positively correlates with the number of vegetative

Table 6. Percentage of filled grain, 1000-grain weight and productivity of doubled haploid lines and check varieties

Genotype	Percentage of filled grain (%)		1000-grain weight (g)		Productivity (tons.ha^{-1})	
RJ1 DR7-3-4-1	60.7	bc	26.3	hijklm	3.26	ijk
RJ2 DR7-7-6-1	58.9	c	28.9	bcd	4.10	efghij
RJ3 DR7-26-3-1	72.9	ab	25.0	mnop	5.25	bcde
RJ4 DR7-31-2-1	62.0	bc	28.7	bcde	4.60	cdefgh
RJ5 DR7-35-1-1	73.0	ab	29.6	abc	5.12	cdefg
RJ6 DR7-37-1-1	69.4	ab	27.1	fghijk	3.77	hij
RJ7 DR7-43-1-5	54.0	c	26.5	hijklm	2.10	k
RJ8 DR7-44-1-2	74.3	ab	26.2	ijklmn	4.50	cdefghi
RJ9 DR7-67-2-1	62.2	bc	29.6	abc	5.17	cdef
RJ10 DR7-69-1-1	62.2	bc	30.5	a	5.25	bcde
RJ11 DR7-69-1-3	60.8	bc	31.0	a	3.99	efghij
RJ12 DR7-69-1-4	61.3	bc	24.8	nop	3.13	jk
RJ13 DR7-95-1-1	73.7	ab	26.5	hijklm	4.70	cdefgh
RJ14 DR8-18-1-1	71.3	ab	27.2	efghijk	2.40	k
RJ15 DR8-20-1-1	70.4	ab	27.6	defghi	5.05	cdefgh
RJ16 DR8-23-2-3	68.0	bc	27.1	fghijk	4.18	defghij
RJ17 DR8-31-1-1	57.9	c	26.3	hijklm	4.08	efghij
RJ18 DR8-32-2-1	70.0	abc	28.1	defg	4.05	efghij
RJ19 DR8-43-3-1	70.9	ab	28.9	bcd	7.12	a
RJ20 DR9-4-1-1	58.0	bc	26.1	jklmno	3.83	ghij
RJ21 DR9-11-1-1	74.4	ab	27.8	defgh	4.62	cdefgh
RJ22 DR9-11-1-2	64.7	bc	25.1	mnop	3.74	hij
RJ23 DR9-58-1-1	56.6	bc	27.3	efghij	3.18	ijk
RJ24 DR9-69-2-2	61.0	bc	25.4	lmno	5.29	bcde
RJ25 DR10-14-1-1	68.3	bc	22.5	q	6.45	ab
RJ26 DR10-26-1-1	63.0	bc	22.8	q	5,62	bc
RJ27 DR10-27-3-1	69.0	ab	28.4	cdef	5.55	bcd
RJ28 DR10-42-1-1	67.3	bc	24.7	op	4.75	cdefgh
RJ29 DR11-36-1-1	63.1	bc	28.0	defg	3.89	fghij
RJ30 DR12-52-2-1	79.2	ab	25.8	klmno	4.40	cdefghij
RJ31 Ciherang	75.5	ab	26.8	ghijkl	7.00	a
RJ32 Inpari 18	73.8	ab	30.0	ab	6.88	a
RJ33 Inpari 40	76.2	ab	23.8	pq	6,92	a
RJ34 Inpari 41	86.9	a	27.3	efghijk	7.24	a

Note: The values followed by the same letters within the same column are not significantly different according to DMRT at α 5%.

tillers, the number of filled grains per panicle, and the percentage of filled grain, but negatively correlated with the number of unfilled grains and the percentage of unfilled grains.

The large correlation coefficient shows a close relationship between the characters observed (Prabowo et al., 2014). In this experiment a close relationship appeared between vegetative plant height and generative plant height, days to 50% flowering and days to harvest, number of unfilled

Table 7. Pearson correlation analysis between traits in doubled haploid rice lines

	VPH	GPH	NVT	NPT	DTF	DTH	PL	NFG	NUG	NTG	%NFG	%NUG	GWE	GY
VPH	1													
GPH	0.853**	1												
NVT	-0.395*	-0.342*	1											
NPT	-0.063	-0.215	0.44**	1										
DTF	0.062	0.246	0.114	0.1	1									
DTH	-0.029	0.162	-0.043	-0.16	0.522**	1								
PL	0.562**	0.551**	-0.417*	-0.142	0.43*	0.288	1							
NFG	0.092	0.043	0.236	0.033	-0.133	0.095	0.013	1						
NUG	0.255	0.118	0.017	0.077	0.102	0.164	0.235	-0.285	1					
NTG	0.273	0.127	0.228	0.087	-0.047	0.208	0.186	0.706**	0.477**	1				
%NFG	-0.188	-0.09	0.093	-0.065	-0.153	-0.084	-0.192	0.666**	-0.896**	-0.051	1			
%NUG	0.188	0.09	-0.093	0.065	0.153	0.084	0.192	-0.666**	0.896**	0.051	-1**	1		
GWE	-0.05	-0.026	0.041	0.068	0.397*	0.301	0.256	-0.23	-0.06	-0.255	-0.047	0.047	1	
GY	-0.082	-0.072	0.485**	0.242	0.058	-0.028	-0.119	0.623**	-0.386*	0.286	0.574**	-0.574**	-0.003	1

Note: ** Significant at $P < 0.01$, * significant at $P < 0.05$. VPH= vegetative plant height; GPH= generative plant height; NVT= number of vegetative tiller; NPT= number of productive tiller; DTF= days to 50% flowering; DTH= days to harvest; PL= panicle length; NFG:= number of filled grains; NUG= number of unfilled grains; NTG= number of grains per panicle; %NFG= percentage of filled grain; %NUG= percentage of unfilled grain; GWE= 1000-grain weight; GY= productivity.

grain to percentage of filled grain, number of unfilled grain to percentage of unfilled grain, % number of filled grain to grain yield, percentage of unfilled grain to grain yield per hectar.

Selection Index to Obtain Superior Lines

Table 8 shows that the check variety RJ34 Ciherang has the highest index value of 15.49 while the RJ7 DR7-43-1-5 line has the lowest index value of -14.74. Selection of doubled-haploid rice lines with superior agronomic character and high yield can be done through indirect selection using characters correlated to productivity (Aryana, 2009). The selection method used is weighted selection index which involves productivity (GY) and selected variables that have a significant effect to grain yield from analysis correlation, i.e., number of filled grains (NFG) and percentage of filled grain (%FG) and characters suitable for rainfed lowland paddy field, i.e. number of productive tiller (NPT) and days to harvest (DH). Before establishing the selection index model, the selected traits were given a weight of 1 to 5 to maximize the model as suggested by Sabouri et al. (2008). Therefore, the model of selection index (I) was formulated as follow: I = (5*GY) + (2*NPT) + (2* NFG) + (1*%FG) – (1*DH). The high weighting of the character of productivity and agronomic characters related to productivity in that formulation was based on the economic value (Ramos et al., 2014; Gazal et al., 2017). In addition, positive and negative signs indicated the direction of selection, i.e. increased number of filled grain and decreased days to harvest, respectively.

Based on the positive index value and good agronomic performance including phenotypic acceptability, i.e., medium number of productive tiller, early maturing and productivity more than 4.40 t/ha, 12 DH lines can be selected as materials for further yield trials in the rainfed lowland rice fields (Table 8). They are RJ3 DR7-26-3-1, RJ5 DR7-35-1-1, RJ8 DR7-44-1-2, RJ13 DR7-95-1-1, RJ15 DR8-20-1-1, RJ19 DR8-43-3-1, RJ21 DR9-11-1-1, RJ25 DR10-14-1-1, RJ26 DR10-26-1-1, RJ27 DR10-27-3-1, RJ28 DR10-42-1-1, RJ30 DR12-52-2-1. All selected DH lines had good and acceptable agronomic characters similar to the released varieties used as checks (Table 3, 4, 5, and 6). In addition, in Indonesia rice varieties under cultivation in rainfed lowlands were low yielding ranged from 2.00 to 3.50 $tons.ha^{-1}$ as reported by Kasno et al. (2016). Thus, those twelve selected DH lines are potential to be tested in further yield trials program.

Conclusion

The DH lines tested in this study have variability in agronomic traits. Number of tillers at vegetative stage, number of grains and filled grain per panicle, and percentage of filled grain positively and significantly correlated to grain yield or productivity, while plant height at vegetative and generative stages, days to harvest, panicle length, and number of unfilled grains

Table 8. Ranks, selected agronomic performance and selection index (I) of DH lines and check varieties

Rank	Genotype	Agronomic characters*					I**
		NPT	NFG	% NFG	DTH	GY	
1	RJ31 Ciherang	30.6	181.0	75.5	114.7	7.00	15.49
2	RJ34 Inpari 41	19.7	169.0	86.9	110.3	7.24	12.99
3	RJ33 Inpari 40	19.1	190.0	76.2	110.3	6.92	11.58
4	RJ32 Inpari 18	18.0	174.6	73.8	111.3	6.88	9.96
5	RJ25 DR10-14-1-1	18.4	182.5	68.3	110.3	6,45	8.27
6	RJ19 DR8-43-3-1	19.0	142.1	70.9	116.0	7.12	7.26
7	RJ3 DR7-26-3-1	14.0	163.8	72.9	112.0	5.25	3.93
8	RJ27 DR10-27-3-1	20.4	180.2	69.0	114.3	5.55	3.49
9	RJ26 DR10-26-1-1	22.3	163.3	63.0	110.3	5,62	3.38
10	RJ8 DR7-44-1-2	18.8	189.2	74.3	112.3	4.50	2.03
11	RJ5 DR7-35-1-1	21.4	156.5	73.0	113.3	5.12	1.95
12	RJ30 DR12-52-2-1	18.2	167.7	79.2	110.0	4.40	1.70
13	RJ15 DR8-20-1-1	16.2	155.1	70.4	113.3	5.05	1.26
14	RJ28 DR10-42-1-1	14.7	145.6	67.3	110.0	4.75	0.69
15	RJ13 DR7-95-1-1	11.6	161.7	73.7	125.0	4.70	0.37
16	RJ21 DR9-11-1-1	17.2	184.6	74.4	120.0	4.62	0.00
17	RJ24 DR9-69-2-2	17.9	157.5	61.0	117.7	5.29	-0.49
18	RJ10 DR7-68-1-1	19.3	141.5	62.2	115.3	5.25	-0.74
19	RJ9 DR7-67-2-1	16.3	140.4	62.2	116.7	5.17	-1.22
20	RJ4 DR7-31-2-1	13.4	130.5	62.0	110.3	4.60	-1.63
21	RJ2 DR7-7-6-1	23.8	142.9	58.9	112.0	4.10	-2.20
22	RJ18 DR8-32-2-1	20.8	146.8	70.0	110.7	4.05	-2.34
23	RJ16 DR8-23-2-3	18.0	146.0	68.0	112.0	4.18	-2.56
24	RJ6 DR7-37-1-1	13.7	151.2	69.4	112.0	3.77	-2.75
25	RJ22 DR9-11-1-2	16.6	150.3	64.7	110.0	3.74	-3.69
26	RJ29 DR11-36-1-1	14.7	155.6	63.1	114.7	3.89	-3.94
27	RJ23 DR9-58-1	35.2	116.2	56.6	112.0	3.18	-4.66
28	RJ17 DR8-31-1-1	17.9	102.7	57.9	110.0	4.08	-6.24
29	RJ11 DR7-69-1-3	17.1	141.6	60.8	118.3	3.99	-6.47
30	RJ20 DR9-4-1-1	15.0	125.9	58.0	114.3	3.83	-6.96
31	RJ12 DR7-69-1-4	15.8	168.1	61.3	117.0	3.13	-7.10
32	RJ1 DR7-3-4-1	15.3	126.6	60.7	111.0	3.26	-7.58
33	RJ14 DR8-18-1-1	12.4	116.2	71.3	111.3	2.40	-9.27
34	RJ7 DR7-43-1-5	19.1	115.2	54.0	115.0	2.10	-14.74

Note: *NPT=number of productive tiller (tiller per hill); NFG= Number of filled grains (grain.panicle-1); % NFG= percentage of filled grains (%); DTH= days to harvest (DAS);
*I: index value based on standardized selection index.

negatively correlated to grain yield. There were twelve DH lines that have potential to be tested further in yield trials program for rainfed lowland paddy field. The selected DH lines have good agronomic characters, especially in number of productive tiller, early maturing, and productivity of more than 4.40 tons.ha^{-1}.

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