



*Research article*

## **Agronomic performance, yield stability and selection of doubled haploid rice lines in advanced yield trials**

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**Abstract:** Anther culture is a widely utilized technique in rice breeding because of its simplicity and effectiveness in rapidly obtaining pure lines in the form of doubled haploid plants. The selection of doubled haploid (DH) rice lines derived from anther culture in advanced yield trials is an important step for obtaining superior DH lines. We aimed to determine agronomic performance, including yield and yield stability in order to select lowland DH rice lines that are high yield and have good agronomic performance based on the selection index method. The research was conducted in Indonesia at three locations, i.e., Bogor (West Java), Indramayu (West Java) and Malang (East Java) from July to December 2022. The genotypes tested were 29 DH lines and three check varieties (Inpari-42 Agritan GSR, Inpari-18 Agritan and Bioni63 Cihayang Agritan) using a randomized complete block design (RCBD) with genotypes as a single factor and three replications. High heritability values are found in all agronomic characters, except the percentage of filled grain/panicle, the percentage of empty grain/panicle and productivity. The yield stability based on the Kang method showed that 15 lines

were stable and had high productivity. Phenotypic correlation analysis showed that the number of productive tillers, days to flowering, days to harvesting, number of filled grains/panicle and percentage of filled grains all had positive values and significantly correlated with productivity. Phenotypic path analysis showed that the character of days to harvesting, number of filled grains/panicle, number of productive tillers and percentage of filled grains/panicle directly affected the productivity. Based on the weighted selection index, 12 DH lines were selected due to having a positive and higher index (8.54 to 0.28) than the Bioni63 Agritan and Inpari 18 check varieties. Among those lines, 9 DH lines were also stable based on the Kang Method.

**Keywords:** anther culture; correlation; heritability; high yielding; selection index; yield stability

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## 1. Introduction

Rice is a staple food consumed by most of the world's population, especially in Asia [1]. In 2021, rice production in Asia reached 708.08 million tons [2]. According to the United States Department of Agriculture (USDA) [3], global rice production reached 510.31 million metric tons as of February 2021/2022. This amount increased by 0.09% compared to the previous month and by 0.56% compared to 2020. The USDA predicts a decrease of 5.09 million tons in global rice production for 2022/2023 compared to 2021/2022. The decline in rice production is attributed to global climate change in several rice-producing countries and land-use changes [4–6].

Climate change and global warming induce environmental stresses, including salinity, drought, warmer temperatures, alterations in precipitation patterns, fluctuations of weather events and increasing insect and disease infestations that significantly affect rice production [7,8]. Changes in climate can affect insect pests in several ways, such as increased number of generations, altered synchrony between plants and pests, altered interspecific interaction, increased risk of invasion by migratory pests, increased incidence of insect-transmitted plant diseases and reduced effectiveness of biological control, especially natural enemies [9]. Therefore, new high-yielding rice varieties with multiple stress resistance to abiotic stresses and tolerance to abiotic stresses need to be developed [10].

The efforts to develop such varieties can be achieved through both conventional and non-conventional plant breeding methods. Conventional plant breeding usually requires 6-8 generations to produce pure lines [11]. However, in non-conventional plant breeding and by applying the anther culture technique, the process of obtaining pure lines can be shortened into two generations [12]. Pure lines in the form of doubled haploid (DH) lines derived from anther culture have been proven to accelerate the development of drought-tolerant rice lines in a short period of time [11]. In the previous preliminary yield trial, 29 rice lines were selected from 60 DH lines obtained from anther culture of several F<sub>1</sub>s derived from crossing between two high-yielding genotypes that either have resistance to biotic stresses or tolerance to abiotic stresses [13]. These selected lines should undergo advanced yield trials in multiple locations to obtain more accurate information on agronomic performance, yield potential and yield stability across environments [14].

The selection of high-yielding rice lines can be based on various parameters to determine the selection criteria, such as heritability, genetic variance, phenotypic variance and coefficient of genetic variation. Productivity is a crucial trait in the development of high-yielding rice varieties. However, direct selection based solely on productivity is feasible but not effective because of the involvement

of multiple genes that control this trait [15]. Environmental factors significantly influence rice productivity; therefore, the selection of rice lines based on productivity would be more effective if other traits, such as plant height, number of productive tillers, number of total grains, percentage of filled grains and weight of 1000 grains, are also considered [14]. Index selection can be employed to select high-yielding doubled haploid rice lines by incorporating multiple desired traits [16]. The selection index combines several key traits, with weights assigned to prioritize each trait [15]. Weight values were used in the selection index for multivariate analysis [17]. We aimed to determine the agronomic performance and yield stability of selected rice DH lines, and select high-yielding DH lines across multiple locations based on the selection index.

## 2. Materials and methods

### 2.1. Plant materials

The plant materials used in the study were 29 DH lines produced from the anther culture research [13]. They were 13 DH lines (WH03, WH04, WH06, WH07, WH08, WH09, WH16, WH17, WH19, WH20, WH21, WH27, WH28) derived from the F<sub>1</sub>: KP4 × BioNL 6-1; 15 DH lines (WH36, WH37, WH38, WH39, WH40, WH42, WH43, WH44, WH45, WH47, WH48, WH49, WH50, WH52, WH54) derived from the F<sub>1</sub>: Inpari 45 Dirgahayu × KP4; and WH59, a DH line derived from the F<sub>1</sub>: Ciherang Malaka × Inpari 45 Agritan. Three check varieties were used, namely Inpari 42 Agritan GSR (WH65; Inpari 42; a green super rice variety), Inpari 18 Agritan (WH66, Inpari 18, short duration rice variety) and Bioni63 Ciherang Agritan (WH67; Bioni63; commercial rice variety) as a productivity check.

### 2.2. Procedures

The research was conducted in three locations (Bogor and Indramayu in West Java and Malang in East Java, Indonesia), from July to December 2022. The field experiment was arranged in a randomized complete block design (RCBD) with rice genotypes as the factor. The treatments used were 29 DH lines and three check varieties were tested with three replications; hence, the total experimental unit was 96. Each experimental unit was a plot of 3 m x 3 m. The seedlings were planted 21 days after sowing, with a plant spacing of 25 cm x 25 cm. Plant maintenance was performed according to rice cultivation standards [18]. Installation of nets was performed when the plants started to flower to control bird pests. The fertilizer dosage was as follows: Urea 200 kg ha<sup>-1</sup>, SP36 100 kg ha<sup>-1</sup> and KCl 100 kg ha<sup>-1</sup>. Urea fertilizer was applied three times. All SP36 and KCl along with one-third of Urea were given as a basal fertilizer one day prior to planting. The remaining Urea was given at 4 weeks and 7 weeks after planting [18].

The observations of agronomic characters were as follows: Plant height was measured from ground level to the longest panicle tip before harvest; number of productive tillers was measured before harvest by counting tillers that produce panicles; days to flowering was observed from sowing to a stage when 50% of plants in each plot have begun to flower; days to harvesting was observed from sowing to a stage when 90% of rice panicles of the rice plant in one plot turned yellow; panicle length was measured at the maturity stage from the panicle neck to the apex; number of filled grains per panicle and number of unfilled grains per panicle was observed by counting the number of filled and

unfilled grains in each panicle; number of total grains per panicle was observed by adding number of filled and unfilled grains in each panicle; weight of 1000 grains was calculated by weighing 1,000 filled grains with  $\pm 14\%$  moisture content; and productivity was obtained from conversion from weight per plot to dry grain yield per hectare ( $\text{ton ha}^{-1}$ ).

### 2.3. Climate data in three locations

Average climate from July to December 2022 at three research locations. Bogor, West Java: Rainfall (355.68 mm), temperature ( $26^\circ\text{C}$ ), relative humidity (84.17%) and altitude (192 m), latitude and longitude coordinates are ( $6^\circ33'47.9''\text{ S}$ ,  $106^\circ44'10.8''\text{ E}$ ). Indramayu, West Java: rainfall (311.2 mm), temperature ( $27.67^\circ\text{C}$ ), relative humidity (82%) and altitude (34 m), latitude and longitude coordinates are ( $66^\circ2'34.3''\text{ S}$ ,  $107^\circ92'20.4''\text{ E}$ ). Malang, East Java: rainfall (234.17 mm), temperature ( $24.73^\circ\text{C}$ ), relative humidity (78.25%) and altitude (395 m), latitude and longitude coordinates are ( $8^\circ4'\text{ S}$   $112^\circ35'\text{ E}$ ).

### 2.4. Data analysis

Data were analyzed using combined analysis of variance (ANOVA) to study the effects of the environment, genotype, and genotype  $\times$  environment interaction. The analysis was performed using SAS On Demand for Academics software (welcome.oda.sas.com). Information on heritability helps determine the characters of the selection process. Estimations of broad-sense heritability ( $h^2_{\text{bs}}$ ) were calculated through the formula as follows:

$$h^2_{\text{bs}} = \frac{\sigma_g^2}{\sigma_p^2} \quad (1)$$

$h^2_{\text{bs}}$  is the broad sense of heritability;  $\sigma_g^2$  and  $\sigma_p^2$  are the genotypic variance and phenotypic variance. Categories of heritability values are: Low ( $h^2_{\text{bs}} \leq 0,20$ ), moderate ( $0,20 \leq h^2_{\text{bs}} \leq 0,50$ ) and high ( $0,50 \leq h^2_{\text{bs}} \leq 1,00$ ) [19]. The experimental coefficient of variation (CV) and the genotypic coefficient of variation (GCV) were calculated as follows:

$$\text{CV} = \frac{\sqrt{\text{MSE}}}{\bar{x}} \times 100\% \quad (2)$$

$$\text{GCV} = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100\% \quad (3)$$

where MSE is the mean square error;  $\sigma_g^2$  is the genotypic variance and  $\bar{x}$  is the grand mean. Couto et al. [20] grouped the coefficient of variation (CV) into four groups namely low ( $<10\%$ ), moderate (10–20%), high (20–30%) and very high ( $>30\%$ ). The genotypic coefficient of variation (GCV) is categorized into three categories: low (0–10%), moderate (10–20%) and high ( $>20\%$ ) [21].

Stability analysis was performed using the methods of Kang [22]. The method entailed using a multi-location data calculation approach to determine the selected genotypes based on the variance-stability value ( $\sigma_i^2$ ) and average yield. The genotype with the highest mean yield was given the highest rank, whereas that having the lowest mean yield was given the lowest rank. The sum of the two rankings produces the final index. Genotypes with high index values are considered high-yielding and stable.

Correlation analysis was used to identify the characters that had a significant effect on

productivity. Correlation and path analysis were analyzed using R software with the ‘*agricolae*’ package. The characters that have a positive association with productivity are selected simultaneously and given a certain weight and then the genotypes are ranked based on their index value [16]. The selection index is symbolically written by the formula according to Kang [23] as follows:

$$I = \sum b_i x_i = b_1 x_1 + b_2 x_2 + \dots + b_n x_n \quad (4)$$

Where  $I$  is the selection index;  $b_n$  is the weight given to the selected character; and  $x_n$  is the standardized phenotype value calculated using the standardization formula [24] as follows:

$$p_{ij} = \frac{(x_{ij} - \bar{x}_i)}{s_i} \quad (5)$$

Where  $p_{ij}$  = standardized genotype mean value;  $x_{ij}$  = genotype mean value;  $x_i$  = average value of a character; and  $s_i$ : standard deviation of a character.

### 3. Results and discussion

#### 3.1. Combined analysis of variance of DH lines

Combined variance analysis of DH rice lines showed that genotype (G) had a significant effect on all observed agronomic characters. Environment (E) had a significant effect on all agronomic characters, except on panicle length. The interaction between genotype and environment ( $G \times E$ ) significantly affected all agronomic characters (Table 1). The significant interaction between genotype and environment ( $G \times E$ ) shows that the tested genotypes respond differently to different environmental conditions. This is in line with Konate et al. [25], where characters having significant  $G \times E$  interactions showed that the genotype responded differently to different environmental conditions. The  $G \times E$  interaction showed significant variability in all characters where genotypes gave different responses to different environmental conditions [26].

The coefficient of variation (CV) of the plant height characters, days to flowering, days to harvesting, panicle length, number of total grains per panicle, percentage of filled grains, weight of 1000 grains and productivity is in a low category (1.08–9.97%), number of productive tillers (13.09%) and number of filled grains per panicle (11.57%) is in the moderate category, while the number of unfilled grains per panicle (28.32%) and the percentage of unfilled grains (25.43%) is in the high category (Table 1). This showed that the lower the coefficient of variation for a certain trait the higher the degree of accuracy for the trait measured, indicating high reliability of experimental data for selection purposes. In addition, the higher coefficient of variation for a certain character indicates that the expression of the character is more influenced by the environment [27].

#### 3.2. Variance component and heritability

Agronomic character expression is determined by genetic factors, environmental factors and genetic and environmental interaction factors. The genotypic coefficient of variance (GCV) on agronomic characters was found from the lowest (1.37%) in days to harvesting to the highest (13.51%) in the number of unfilled grains per panicle (Table 2). Genotypic coefficient of variation (GCV) is categorized into three categories, namely low (0–10%), moderate (10–20%) and high (>20%) [27], of

all the observed characters have low and moderate categories. Characters with low GCV values may not be considered in the selection process because of low diversity [28]. A similar result was also reported by Tuhina-Khatun et al. [29] who obtained a low GCV value in days to flowering and days to harvesting.

**Table 1.** Combined analysis of variance of DH rice lines.

Agronomic characters	F values of			Coefficient of variation (%)
	Genotype (G)	Environment (E)	G × E Interaction	
Plant height (cm)	283.36**	814.80**	19.61**	3.26
Number of productive tillers	23.39**	1,912.38**	7.81*	13.09
Days to flowering (DAS)	54.40**	8,323.90**	8.07**	2.24
Days to harvesting (DAS)	33.88**	7,107.98**	9.84**	1.08
Panicle length (cm)	10.53**	4.80tn	1.20**	3.41
Number of filled grains/panicle	1,722.87**	53,994.10**	744.78**	11.57
Number of unfilled grains/ panicle	391.20**	7,932.28**	180.49**	28.32
Number of total grains/panicle	2,367.25**	48,037.83**	805.90**	9.97
Percentage of filled grains (%)	97.67**	3,263.22**	55.02**	6.98
Percentage of unfilled grains (%)	97.67**	3,263.22**	55.02**	25.43
Weight of 1000 grains	30.62**	118.88**	3.63**	5.05
Productivity (ton ha <sup>-1</sup> )	0.86**	28.17*	0.74**	9.38

Note : DAS: days after sowing; \*\* = significantly different at  $\alpha$  0.01; \* = significantly different at  $\alpha$  0.05; ns = not significantly different at  $\alpha$  0.05.

**Table 2.** The components of variance and heritability of the agronomic characters of DH rice lines.

Agronomic characters	$\sigma_p^2$	$\sigma_g^2$	$\sigma_{ge}^2$	GCV	Heritability	
					$h_{bs}^2$	Criteria
Plant height (cm)	31.48	29.31	2.56	5.11	0.93	high
Number of productive tillers	2.60	1.73	0.74	7.29	0.67	high
Days to flowering (DAS)	6.04	5.15	1.32	2.51	0.85	high
Days to harvesting (DAS)	3.77	2.67	2.72	1.37	0.71	high
Panicle length (cm)	1.17	1.04	0.16	4.12	0.89	high
Number of filled grains/panicle	191.43	108.68	170.54	7.90	0.57	high
Number of unfilled grains/ panicle	43.47	23.41	25.89	13.51	0.54	high
Number of total grains/panicle	263.02	173.48	175.31	7.85	0.66	high
Percentage of filled grains (%)	10.86	4.74	8.35	2.77	0.44	moderate
Percentage of unfilled grains (%)	10.86	4.74	8.35	10.11	0.44	moderate
Weight of 1000 grains	3.40	3.00	0.50	5.97	0.88	high
Productivity (ton ha <sup>-1</sup> )	0.10	0.01	0.10	1.64	0.14	low

Note:  $\sigma_p^2$  = Phenotypic variance,  $\sigma_g^2$  = Genotypic variance (G),  $\sigma_{ge}^2$  = Variance of genotype and location interaction,  $h_{bs}^2$  = Broad sense heritability, GCV = Genotypic coefficient of variation.

The heritability value of the agronomic characters of the DH rice lines was between 0.14–0.93 (Table 2). Most of the agronomic traits had high heritability. However, the characteristics of the

percentage of filled grains and the percentage of empty grains had moderate heritability, and the productivity had low heritability. The low heritability of the productivity was due to the high proportion of the genotype by environment variance (33.2%) and experimental error variance (52.4%) to the phenotypic variance, relative to the genetic variance (14.4%). According to [16], characters that are strongly influenced by the environment, such as productivity, usually have low heritability values, while characters that have high heritability values indicate that these characters can be inherited because the genetic factors contributed more to the characters than environmental factors. Similar results were also reported by Akbar et al. [28] and Alsabab et al. [19] in which almost all the agronomic characters in doubled haploid lines had high heritability values.

### 3.3. Agronomic characters and yield stability

The average plant height of the DH lines is presented in Table 3. The DH line with the lowest plant height was WH04 (96.49 cm), whereas the DH line with the highest plant height was WH59 (114.36 cm). The plant height of lowland rice is classified into three categories, namely short (<110 cm), medium (110–130 cm) and tall (>130 cm) [18]. The check varieties had an average plant height of less than 110 cm as shown by Inpari 42 Agritan GSR (99.62 cm), Inpari 18 Agritan (91.29 cm) and Bioni63 Ciherang Agritan (104.58 cm). Thus, generally, the plant height of the tested DH lines in the three locations falls within the short and medium categories, while the check varieties are categorized as short. There were 22 DH lines categorized as short (<110 cm) and seven DH lines categorized as medium (110–130 cm). Short and medium-height plants are less prone to lodging, especially during grain filling, resulting in consistently high yields [30]. Lodging can reduce cereal yield by reducing the grain size and number or by reducing the amount of crop that can be recovered at harvest [31].

The average number of productive tillers for the tested DH lines and the check varieties at the three locations was 18.0 tillers (Table 3). The DH lines with the highest number of productive tillers were WH21 (21.1 tillers), WH04 (20.5 tillers) and WH28 (20.0 tillers). The check varieties, namely Inpari 42 Agritan GSR, Inpari 18 Agritan and Bioni63 Ciherang Agritan had 18.2, 14.1 and 20.6 productive tillers, respectively. The number of productive tillers per plant is one of the important agronomic characteristics associated with the grain yield of rice [16]. In this research, the tested DH lines predominantly exhibited a similar number of productive tillers as the check varieties, falling within the moderate category (10–19 tillers) and the high category (20–25 tillers).

The average days to flowering for the tested DH lines and check varieties at the three locations were more than 80 DAS (Table 3). The WH40 (87.4 DAS) exhibited the earliest days of flowering, whereas the WH17 (93.7 DAS) had the latest days of flowering. The check varieties, i.e. Inpari 42 Agritan GSR, Inpari 18 Agritan and Bioni63 Ciherang Agritan have an average days to flowering of 91.1 DAS, 80.6 DAS and 90.0 DAS, respectively. The average days to harvesting for the tested DH lines ranged from 117.4–121.3 DAS (Table 3). The earliest days of harvesting were observed in WH39 (117.4 DAS), while WH50 (121.3 DAS) had the latest days to harvesting. The check varieties had average days to harvesting ranging from 110.3 DAS to 120.2 DAS. According to IRRI [18], the grouping of days to harvesting (P) is divided into four groups: very early ( $P \leq 110$  DAS), early ( $110 < P \leq 115$  DAS), medium ( $115 < P \leq 125$  DAS) and long ( $125 < P \leq 150$  DAS). Thus, all the DH lines had moderate days to harvesting similar to those of the two check varieties Inpari 42 Agritan GSR and Bioni63 Ciherang Agritan, while Inpari 18 Agritan had an early harvest period.

**Table 3.** Mean agronomic characters and yield stability of DH rice lines and check varieties at three locations.

No.	Genotype	PH	NPT	DF	DH	PL	NFG	NUG	NTG	PFG	PUG	WOG	PRD	YSi
1	WH03	97.47	19.4	92.9	120.4	22.89	132.7	33.3	166.0	79.5	20.5	28.86	7.82	34 +
2	WH04	96.49	20.5	90.4	119.3	22.92	125.0	30.4	155.5	80.4	19.6	29.32	7.55	29 +
3	WH06	108.27	18.0	91.9	119.7	25.49	129.2	33.0	162.2	79.5	20.6	32.27	7.16	15
4	WH07	109.62	18.4	92.0	120.2	25.94	145.8	33.4	179.2	81.0	19.0	31.65	7.66	31 +
5	WH08	107.31	18.2	91.7	120.8	24.99	145.4	43.1	188.5	77.1	22.9	25.73	7.68	30 +
6	WH09	105.67	19.3	92.8	120.7	24.97	149.8	38.9	188.7	79.4	20.6	26.23	7.50	28 +
7	WH16	100.29	19.5	93.1	120.6	23.44	123.3	27.7	151.0	81.5	18.5	27.38	7.06	8
8	WH17	99.93	19.8	93.7	121.0	23.82	128.7	22.7	151.5	84.9	15.1	26.85	7.03	7
9	WH19	103.78	17.5	92.9	120.6	24.09	115.4	44.9	160.3	72.3	27.7	30.39	6.91	4
10	WH20	97.76	19.8	91.4	120.7	22.93	124.3	29.2	153.6	80.5	19.5	29.02	7.39	27 +
11	WH21	97.44	21.1	91.2	119.0	22.74	118.1	29.1	147.2	80.3	19.7	29.06	7.38	23 +
12	WH27	107.71	17.4	89.3	120.3	25.32	127.8	37.7	165.5	76.9	23.1	27.89	7.15	14
13	WH28	109.13	20.0	92.3	120.4	24.56	126.0	38.4	164.4	77.0	23.1	28.03	6.93	5
14	WH36	111.76	17.6	92.1	119.4	24.67	133.2	33.5	166.7	79.5	20.5	27.99	7.29	23 +
15	WH37	110.38	17.7	90.6	119.4	26.22	166.8	34.1	200.9	82.9	17.2	26.79	7.32	24 +
16	WH38	109.76	17.2	90.1	118.6	25.59	134.2	40.0	174.2	76.9	23.1	28.17	7.15	13
17	WH39	108.69	16.3	87.7	117.4	25.87	148.8	39.6	188.4	79.3	20.8	31.53	7.09	9
18	WH40	107.47	15.5	87.4	119.2	24.94	131.3	35.8	167.0	78.2	21.8	31.64	6.99	6
19	WH42	107.76	16.9	88.1	119.8	25.30	142.3	52.5	194.8	72.5	27.5	30.68	7.12	11
20	WH43	110.91	16.1	88.2	118.2	25.04	136.4	38.7	175.1	77.0	23.0	31.22	7.17	16 +
21	WH44	113.31	15.3	87.9	118.3	26.23	136.8	42.2	179.0	75.7	24.3	31.01	6.89	2
22	WH45	107.49	18.5	88.8	119.0	24.14	118.0	21.2	139.2	84.5	15.5	28.72	7.13	12
23	WH47	108.53	17.5	90.8	118.9	25.61	130.6	48.6	179.3	72.7	27.3	29.20	6.85	1
24	WH48	109.04	18.7	90.9	120.2	24.18	135.8	39.3	175.1	77.1	22.9	25.87	7.10	10
25	WH49	110.67	18.0	90.4	120.3	25.33	124.7	35.3	160.0	77.4	22.6	28.42	7.38	26 +
26	WH50	112.09	16.4	90.9	121.3	25.89	133.6	36.0	169.6	79.5	20.5	28.28	7.65	30 +
27	WH52	106.47	18.4	90.1	120.9	25.41	112.0	38.4	150.4	74.2	25.8	31.03	7.21	20 +
28	WH54	104.51	18.1	90.4	119.7	25.28	117.5	36.2	153.8	75.9	24.1	29.12	7.23	21 +
29	WH59	114.36	17.5	89.0	118.1	25.29	128.4	31.8	160.2	79.4	20.6	28.43	7.19	17 +
30	Inpari 42	99.62	18.2	91.1	120.2	24.41	161.3	32.4	193.7	82.8	17.3	28.11	7.23	22 +
31	Inpari 18	91.29	14.1	80.6	110.3	22.33	99.5	37.6	137.0	73.5	26.5	31.31	6.21	-9
32	Bioni63	104.58	20.6	90.0	117.8	24.67	140.0	30.9	171.0	81.6	18.4	26.80	6.91	1
Average		105.92	18.0	90.3	119.4	24.70	132.0	35.8	167.8	78.5	21.5	28.97	7.20	
LSD 5%		6.55	4.48	3.8	2.5	1.6	29.0	19.3	31.8	10.4	10.4	2.78	1.28	

Note: PH = plant height (cm), NPT = number of productive tillers, DF = days to flowering (d), DH = days to harvesting (d), PL = panicle length (cm), NFG = number of filled grains/panicle, NUG = number of unfilled grains/ panicle, NTG = number of total grains/panicle, PFG = percentage of filled grains, PUG = percentage of unfilled grains, WOG = weight of 1000 grains (g), PRD = productivity (ton/ha), YSi = Kang yield-stability index.

The panicle length of DH lines ranged from 22.74 cm to 26.23 cm (Table 3). Lines WH44 (26.23 cm) had a significantly longer panicle length than the check varieties Inpari 42 Agritan GSR (24.41 cm) and Inpari 18 Agritan (22.33 cm). In general, the length of the panicle directly contributes to the



number and shape of grains per panicle, thus having a direct impact on the yield of rice plants [32]. In this research, the average number of total grains per panicle for the DH lines was more than 120.0 grains (Table 3). Among the tested DH lines, line WH37 (200.9 grains) has the highest number of total grains per panicle and significantly higher than check varieties Inpari 18 Agritan (137.0 grains), but similar to Inpari 42 Agritan GSR and Bioni63 Ciherang Agritan.

The number of grains per panicle is greatly influenced by genetic and environmental factors (Table 1). The high number of grains per panicle (150.0 to 200.9 grains) indicates that DH lines possess favorable genetic traits that will contribute to their superior grain yield potential. Similar results were reported by Sadimantara et al. [33] who found that the number of grains per panicle significantly affects rice productivity. The genetic makeup of a rice variety plays a crucial role in determining the potential number of grains per panicle, while environmental factors, such as soil fertility, water availability, temperature and sunlight, also play a role in determining the actual grain yield.

The actual grain yield was determined by the number of filled and unfilled grains, and the number of filled grains per panicle is directly proportional to the yield of a crop [34]. The average number of filled grains per panicle of the DH lines in three locations was 132.0 grains in a range of 112.0–166.8 grains, while the average number of unfilled grains per panicle was 35.8 grains in a range of 21.2–52.5 grains (Table 3). Lines WH37 (166.8 grains) has a significantly higher number of filled grains per panicle than the check varieties Inpari 18 and Bioni63 Ciherang Agritan, but it is not significantly different from the variety Inpari 42.

The percentage of filled grains and unfilled grains is presented in Table 3. If panicles contain a large number of filled grains compared to unfilled grains, it inherently indicates the high productivity or yield of a crop [34]. Therefore, the percentage of filled and unfilled grains is a crucial parameter for judging the best variety among different varieties of rice. Lines WH17 (84.9%) has the highest percentage of filled grains per panicle, while lines WH19 (72.3%) has the lowest. Generally, the tested DH lines had a high percentage of filled grains (>75%), which is similar to those of the check varieties Inpari 42 and Bioni63 Ciherang Agritan. The average percentage of unfilled grains was 21.5%, with WH17 having the lowest percentage of unfilled grains per panicle (15.1%), and lines WH19 having the highest percentage of unfilled grains (27.7%). An antagonistic interaction between ABA and GAs may be involved in mediating grain filling. It is possible that the lower ABA and higher GA content lead to the poor grain filling of inferior spikelets [35].

Grain weight is one of the important indicators of grain filling. The average weight of 1000 grains of DH lines ranged from 25.73 g to 32.27 g (Table 3). The DH lines WH06 (32.27 g), WH07 (31.65 g), WH19 (30.39 g), WH39 (31.53 g), WH40 (31.64 g), WH42 (30.68 g), WH43 (31.22 g), WH44 (31.01 g) and WH52 (31.03 g) had higher weights of 1000 grains compared to the variety Inpari 42 (28.11 g) and Bioni63 Ciherang Agritan (26.80 g), but similar to the weight of 1000 grains of the variety Inpari 18 (31.31 g). The weight of 1000 grains is a distinguishing characteristic of a variety, as it is related to the grain size and genetic traits inherited from the parent crosses [36]. The weight of 1000 grains in rice is determined not only by genetic traits, but also by factors such as husk size, sink activity and source capacity [37].

The average productivity of DH rice lines ranges from 6.85 to 7.82 ton ha<sup>-1</sup> (Table 3). The DH lines WH03 (7.82 ton ha<sup>-1</sup>) gave the highest yield than the varieties Inpari 42 Agritan GSR (7.23 ton ha<sup>-1</sup>), Inpari 18 Agritan (6.21 ton ha<sup>-1</sup>) and Bioni63 Ciherang Agritan (6.91 ton ha<sup>-1</sup>). The analysis using the method of Kang [22] indicates that DH lines WH03 (7.82 ton ha<sup>-1</sup>), WH04 (7.55 ton ha<sup>-1</sup>), WH07 (7.66 ton ha<sup>-1</sup>), WH08 (7.68 ton ha<sup>-1</sup>), WH09 (7.50 ton ha<sup>-1</sup>), WH20 (7.39 ton ha<sup>-1</sup>), WH21

(7.38 ton ha<sup>-1</sup>), WH36 (7.29 ton ha<sup>-1</sup>), WH37 (7.32 ton ha<sup>-1</sup>), WH43 (7.17 ton ha<sup>-1</sup>), WH49 (7.38 ton ha<sup>-1</sup>), WH50 (7.65 ton ha<sup>-1</sup>), WH52 (7.21 ton ha<sup>-1</sup>), WH54 (7.23 ton ha<sup>-1</sup>) and WH59 (7.19 ton ha<sup>-1</sup>) are stable lines with high productivity (Table 3).

**Table 4.** Productivity (ton/ha) of DH rice lines and check varieties at three locations (Malang, Indramayu and Bogor).

No.	Genotype	Locations		
		Malang	Indramayu	Bogor
1	WH03	8.53	7.46	7.47
2	WH04	8.53	7.22	6.91
3	WH06	7.95	7.20	6.34
4	WH07	8.32	7.25	7.42
5	WH08	8.98	7.05	7.02
6	WH09	8.47	7.25	6.79
7	WH16	7.76	6.40	7.02
8	WH17	7.58	6.95	6.57
9	WH19	7.81	6.71	6.23
10	WH20	7.60	7.84	6.74
11	WH21	7.38	7.24	7.53
12	WH27	8.09	6.56	6.79
13	WH28	7.50	6.54	6.74
14	WH36	8.45	6.69	6.74
15	WH37	7.88	7.11	6.96
16	WH38	7.25	7.45	6.74
17	WH39	7.40	7.36	6.51
18	WH40	7.82	6.42	6.74
19	WH42	7.78	6.99	6.57
20	WH43	7.59	7.13	6.79
21	WH44	7.32	6.28	7.08
22	WH45	7.33	7.56	6.51
23	WH47	8.05	6.61	5.89
24	WH48	8.22	6.92	6.17
25	WH49	7.81	7.55	6.79
26	WH50	8.68	7.66	6.62
27	WH52	7.28	7.54	6.79
28	WH54	7.54	7.65	6.51
29	WH59	7.83	6.65	7.08
30	Inpari 42	7.50	7.47	6.74
31	Inpari 18	6.44	4.76	7.42
32	Bioni63	7.39	6.16	7.19
Average		7.81	6.99	6.79
LSD 5%		2.22	2.22	2.22

Table 4 shows that each tested line varied in yield at each location because each location has different environmental conditions (see 2.3. Climate data at three locations). Environmental factors have a significant effect on productivity [38] as indicated by significant  $G \times E$  interaction (Table 1). The average productivity of the DH lines tested at the Malang location ranges from 7.25 ton ha<sup>-1</sup> to 8.98 ton ha<sup>-1</sup>, while the varieties Inpari 42 Agritan GSR (7.5 ton ha<sup>-1</sup>), Inpari 18 Agritan (6.44 ton ha<sup>-1</sup>) and Bioni63 Ciherang Agritan (7.39 ton ha<sup>-1</sup>). In the Indramayu location, the average productivity of the DH lines ranges from 6.28 ton ha<sup>-1</sup> to 7.84 ton ha<sup>-1</sup>, while for the Inpari 42 Agritan GSR (7.47 ton ha<sup>-1</sup>), Inpari 18 (4.76 ton ha<sup>-1</sup>) and Bioni63 Ciherang Agritan (6.16 ton ha<sup>-1</sup>) varieties. In the Bogor location, the average productivity of the DH lines ranges from 5.89 ton ha<sup>-1</sup> to 7.53 ton ha<sup>-1</sup>, while for the Inpari 42 Agritan GSR (6.74 ton ha<sup>-1</sup>), Inpari 18 Agritan (7.42 ton ha<sup>-1</sup>) and Bioni63 Ciherang Agritan (7.19 ton ha<sup>-1</sup>) varieties (Table 4). The Malang location had the highest average productivity compared to Indramayu and Bogor.

### 3.4. Phenotypic and genotypic correlations

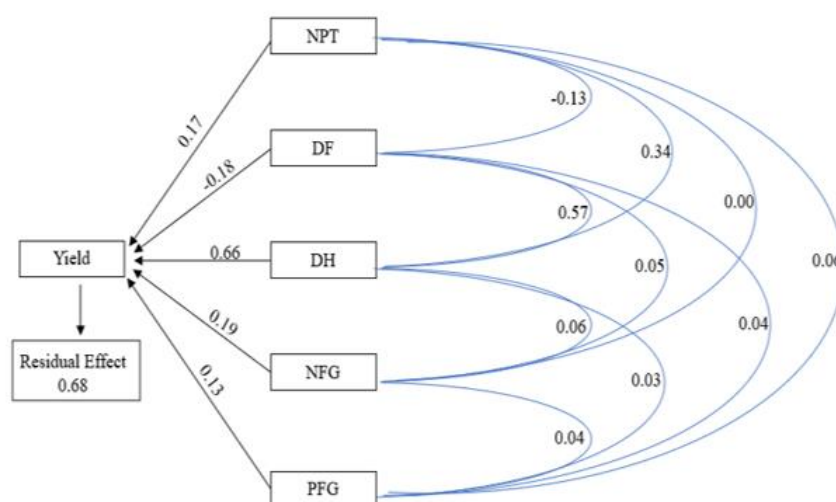
The phenotypic correlation analysis and genotypic correlation indicate the relationships between the observed agronomic characters (Table 5). The results of the phenotypic correlation analysis showed that the number of productive tillers, days to flowering, days to harvesting, number of filled grains per panicle and percentage of filled grains were positive and had significant correlations with productivity. On the other hand, the percentage of unfilled grains had a negative and significant correlation with productivity. Similar findings were reported by Thippani et al. [39], who stated that the character of days to flowering and the number of productive tillers were positively and significantly correlated with grain yield per plant. Plant height, panicle length, number of unfilled grains per panicle, number of total grains per panicle and weight of 1000 grains do not show any correlation with productivity. This shows that the phenotypic correlation is strongly influenced by genetic and environmental conditions [28].

**Table 5.** Phenotypic correlation (below diagonal) and genotypic correlation (above diagonal) between agronomic traits on DH rice lines.

	PH	NPT	DF	DH	PL	NFG	NUG	NTG	PFG	PUG	WOG	PRD
PH		-0.44*	0.08	0.35*	0.91**	0.45**	0.43*	0.51**	-0.22	0.22	-0.03	0.44*
NPT	-0.33		0.91**	0.75**	-0.47**	0.08	-1.00**	-0.33	1.00**	-1.00**	-0.58**	0.91**
DF	0.08	0.71**		1.00**	0.04	0.40	-0.39	0.17	0.69**	-0.69**	-0.57**	1.00**
DH	0.27	0.51**	0.86**		0.34	0.66**	-0.03	0.51**	0.48**	-0.48**	-0.49**	1.00**
PL	0.87**	-0.41*	0.01	0.22		0.55**	0.56**	0.64**	-0.31	0.31	0.11	0.32
NFG	0.37*	0.01	0.25	0.3	0.5**		0.41*	0.94**	0.13	-0.13	-0.34	0.99**
NUG	0.33	-0.48**	-0.22	-0.07	0.43*	0.15		0.69**	-0.86**	0.86**	0.30	-0.73**
NTG	0.45**	-0.19	0.13	0.22	0.6**	0.92**	0.54**		-0.21	0.21	-0.16	0.52**
PFG	-0.13	0.48**	0.35*	0.24	-0.17	0.35	-0.86**	-0.05		-1.00**	-0.55**	1.00**
PUG	0.13	-0.48**	-0.35*	-0.24	0.17	-0.35	0.86**	0.05	-1.00**		0.55**	-1.00**
WOG	0.00	-0.5**	-0.46	-0.35	0.12	-0.29	0.21	-0.16	-0.37*	0.37*		-0.57**
PRD	0.12	0.44*	0.6**	0.68**	0.1	0.39*	-0.17	0.27	0.37*	-0.37*	-0.26	

Note: PH = plant height, NPT = number of productive tillers, DF = days to flowering, DH = days to harvesting, PL = panicle length, NFG = number of filled grains/panicle, NUG = number of unfilled grains/panicle, NTG = number of total grains/panicle, PFG = percentage of filled grains, PUG = percentage of unfilled grains, WOG = weight of 1000 grains, PRD = productivity, \*\*: significant at  $\alpha = 0.01$ ; \*: significant at  $\alpha = 0.05$ .

The results of the genotypic correlation analysis indicated that the number of productive tillers, days to flowering, days to harvesting, number of filled grains per panicle and percentage of filled grains showed a positive and significant correlation with productivity. The percentage of unfilled grains was negatively correlated with productivity. These genotypic correlations provide insights into the genetic factors that influence the observed agronomic characters and their relationship with overall productivity. Genotypic correlations indicate that the character has a genetic relationship, even though it may not be apparent in their phenotypic correlations [28]. High genotypic correlations in the yield component characters indicate that these characters have a direct genetic influence on productivity. Karim et al. [40] explained that genotypic correlations are generally higher than phenotypic correlation coefficients, indicating that the correlations are primarily due to genetic effects. This suggests that genetic factors play a significant role in determining the relationships between the studied traits and their impact on productivity.



**Figure 1.** Diagram of path coefficients of several characters towards yield of DH rice lines.

Note:  $\leftarrow$  = direct effect;  $\curvearrowright$  = indirect effect; NPT = number of productive tillers, DF = days to flowering, DH = days to harvesting, NFG = number of filled grains/panicle, PFG = percentage of filled grains.

### 3.5. Path analysis

The results of path analysis showed that the characters with the largest direct effect on productivity were days to harvesting, number of filled grains per panicle, number of productive tillers and percentage of filled grains, whereas days to flowering had a negative direct effect on productivity (Figure 1). Characters such as the number of productive tillers and the percentage of filled grains per panicle, which have indirect effects on the coefficient of yield correlation, can be used to improve yield-related traits. By targeting these traits and selecting individuals with desirable values, breeders can indirectly enhance the overall yield potential of plant breeding programs. Akter et al. [41] explained that the number of tillers is an effective selection criterion for improving yield, while in Tirtana et al. [42], the number of filled grains per panicle has the highest direct impact on productivity. The residual value (0.68)

in the path analysis model indicated a significant environmental influence, suggesting that the explained variation was not substantial. Phenotypic path analysis results provide insight into agronomic traits that directly affect productivity. These findings can serve as a reference for determining the selection criteria to be employed.

### 3.6. Selection index on DH rice lines

The selection index of agronomic traits in DH rice lines was determined based on several analyses, namely heritability values, genetic coefficient of variation, phenotypic and genotypic correlation analyses and path analysis. In addition to these analyses, the selection criteria for agronomic traits also considered breeding objectives and the importance of a particular character. The selection index was determined by assigning weights to the selected agronomic characters according to the economic value of each trait [17]. In this research, the productivity trait is given a weight that is three times higher than the other traits, whereas the plant height trait is given a weight of  $-1$ , i.e., productivity (3), plant height ( $-1$ ), number of productive tillers (1), number of total grains per panicle (1), percentage of filled grains (1) and weight of 1000 grains (1). This weighting scheme is aimed at optimizing the selection index model to achieve the rice breeding program's target in producing high-yielding DH lines ( $>7.00$  t/ha), short category plant height ( $<110$  cm), high number of productive tillers ( $>20$  tillers), large number of total grains per panicle ( $>140$  grains), high percentage of filled grains ( $>75\%$ ) and the ideal weight of 1000 grains ( $>25.00$  g). Therefore, selection using the selection index method will be more efficient and relevant because it is determined by several important agronomic characters compared to selection that only uses only yield characters.

The highest weighted index value was observed in the lines WH03 (8.54), while WH66 (Inpari 18 Agritan) had the lowest index value of  $-11.59$  (Table 6). Based on the weighted index value, 12 DH lines were selected. The lines had positive index values and ideal agronomic performance in three locations as compared to the check varieties Bioni63 Agritan and Inpari 18 Agritan. In this research, the selection of DH lines was not too strict after considering the low heritability value for the productivity character. The productivity character was given three times greater weight than other characters so that potential lines would not be eliminated. Using the selection index method 9 out of 12 DH lines with a positive selection index were also stable, based on the yield-stability index of the Kang method [22]. Thus, when choosing a DH line, not only yield character is determined, but several other agronomic characters are also considered. Similar results using DH rice lines were reported by Hidayatullah et al. [16] where the selected lines were based on index selection using good agronomic performance and high yield. Htwe et al. [43] reported that index selection based on the combination of number of tillers, number of filled grains per panicle, percentage of filled grains and seed yield per plant resulted in the highest genetic gain and relative efficiency. Agronomic components related to yield have been used for selection efficiency in the development of high-value economic varieties [44].

**Table 6.** A weighted selection index of 29 DH rice and 3 check varieties.

Genotype	Standardized and weighted genotype mean value for						Index
	PRD	PH	NPT	NTG	PFG	WOG	
WH03	2.02	-1.51	0.83	-0.11	0.32	-0.06	8.54
WH07	1.50	0.66	0.21	0.71	0.77	1.45	6.99
WH04	1.14	-1.68	1.54	-0.76	0.59	0.19	6.67
WH21	0.59	-1.51	1.88	-1.27	0.56	0.05	4.50
WH20	0.62	-1.45	1.09	-0.88	0.63	0.03	4.19
WH65, Inpari 42	0.10	-1.12	0.11	1.60	1.30	-0.47	3.98
WH09	0.98	-0.05	0.77	1.29	0.28	-1.48	3.85
WH08	1.57	0.25	0.12	1.28	-0.40	-1.76	3.69
WH37	0.40	0.79	-0.22	2.04	1.33	-1.18	2.37
WH50	1.47	1.10	-0.99	0.11	0.32	-0.37	2.37
WH06	-0.12	0.42	0.00	-0.35	0.30	1.79	0.96
WH17	-0.54	-1.07	1.06	-1.01	1.96	-1.15	0.30
WH39	-0.35	0.49	-1.08	1.27	0.24	1.39	0.28
WH49	0.59	0.85	0.00	-0.48	-0.31	-0.30	-0.16
WH16	-0.45	-1.00	0.88	-1.03	0.93	-0.86	-0.42
WH36	0.30	1.04	-0.25	-0.07	0.32	-0.53	-0.67
WH45	-0.22	0.28	0.28	-1.76	1.83	-0.13	-0.73
WH54	0.10	-0.25	0.04	-0.86	-0.79	0.08	-0.96
WH42	-0.25	0.33	-0.70	1.67	-1.80	0.93	-0.99
WH52	0.04	0.10	0.20	-1.07	-1.28	1.12	-1.02
WH67, Bioni63	-0.93	-0.24	1.56	0.20	0.94	-1.18	-1.04
WH43	-0.09	0.89	-1.22	0.45	-0.45	1.22	-1.17
WH38	-0.16	0.68	-0.54	0.40	-0.48	-0.43	-2.20
WH59	-0.03	1.50	-0.36	-0.47	0.29	-0.29	-2.40
WH27	-0.16	0.32	-0.42	-0.14	-0.48	-0.58	-2.41
WH40	-0.67	0.28	-1.57	-0.05	-0.07	1.45	-2.54
WH48	-0.32	0.56	0.39	0.45	-0.42	-1.68	-2.77
WH28	-0.87	0.57	1.24	-0.21	-0.46	-0.51	-3.12
WH19	-0.93	-0.38	-0.34	-0.46	-1.86	0.77	-4.32
WH44	-1.00	1.32	-1.71	0.69	-0.83	1.11	-5.06
WH47	-1.13	0.46	-0.34	0.71	-1.76	0.13	-5.12
WH66, Inpari 18	-3.21	-2.61	-2.44	-1.90	-1.51	1.27	-11.59

Note: PRD = productivity, PH = plant height, NPT = number of productive tillers, NTG = number of total grains per panicle, PFG = percentage of filled grains and WOG = weight of 1000 grains.

#### 4. Conclusions

The DH lines tested at three locations had 96.49–114.36 cm plant height, 15.3–21.1 productive tillers, 87.4–93.7 DAS days to flowering, 117.4–121.3 DAS days to harvesting, 22.74–26.23 cm panicle length, 112.0–166.8 filled grains per panicle, 21.2–52.5 unfilled grains per panicle, 139.2–200.9 grains per panicle, 72.3–84.9% filled grain, 15.1–27.7% empty grain, 25.73–32.27 g of 1000

grains weight and productivity in the range of 6.85–7.82 ton ha<sup>-1</sup>. The index selection resulted in selected 12 DH lines, namely WH03, WH04, WH06, WH07, WH08, WH09, WH17, WH20, WH21, WH37, WH39 and WH50, which had a better index (8.54 to 0.28) than Bioni63 Ciherang Agritan and Inpari 18 Agritan. Among those selected DH lines, 9 lines had Kang's yield-stability index higher than average.

### Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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