

Selection index and agronomic characters of doubled haploid rice lines from anther culture

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Abstract. *Hadianto W, Purwoko BS, Dewi IS, Suwarno WB, Hidayat P. 2023. Selection index and agronomic characters of doubled haploid rice lines from anther culture. Biodiversitas 24: 1511-1517.* Development of superior rice varieties requires selection of the best lines having good agronomic characters and high yields. This study aimed to select high-yielding doubled haploid rice lines obtained from anther culture through index selection and agronomic characters. The study was conducted using a single-factor randomized complete block design (RCBD) with genotype as treatment. Sixty doubled haploid lines and 5 check varieties were evaluated along with three replications, hence, the total experimental unit was 195. Analysis of variance showed that the genotypes had a significant effect on all observed characters, except for the number of productive tillers. The agronomic characters observed had a high broad sense of heritability, except for the number of productive tillers which was in the medium category. Furthermore, index selection was carried out based on productivity, the number of productive tillers, the total number of grains, as well as the percentage of filled grains. Based on the index selection, 29 lines were selected among the 60 doubled haploid rice lines tested. These lines had the range in productivity from 2.95 to 6.77 ton ha⁻¹, the number of productive tillers from 12.3 to 20.8 tillers, the total number of grains from 135 to 275.4 seeds, the filled grain percentage from 53.23 to 87.61%, and the selection index from -1.48 to 7.88. The selected lines will be further tested at the next experimental stage in several locations to produce high-yielding, brown planthopper-resistant, and drought-tolerant rice varieties.

Keywords: Anther culture, heritability, productivity, rice, selection index

INTRODUCTION

Rice is a staple food consumed by most of the population in Asian countries, including Indonesia. The national rice production in 2021 was approximately 31.69 million tons, with an increase of 0.3517 million tons or 1.12% compared to 2020 (Central Bureau of Statistics 2021). In Indonesia, the increase in rice production faces several challenges, including the conversion of paddy fields to infrastructure use as well as global climate change. Consequently, efforts are needed to produce new varieties that are adaptive to changing environmental conditions. Climate change causes fluctuations in air temperature and humidity which increasingly stimulate the growth and development of plant pests as well as diseases. The consideration of climate change impacts on agriculture is also important (Ortiz et al. 2021). Drought stress is a major constraint to rice production, particularly in water-limited environments (Mishra et al. 2014). This extreme climate ranks first as the cause of a decline in grain yield and crop failure (Sabouri et al. 2022).

It is necessary to cross parents with superior traits such as high yields, pest resistance, and drought tolerance to obtain high productivity lines. Exploiting host plant

resistance to planthoppers and incorporating resistant genes into commercial cultivars are alternatives for rice resistance mechanism adaptation (Ferrater et al. 2013). These efforts can be carried out through breeding in rice both conventionally and non-conventionally, where the conventional method takes 6 to 8 generations to produce pure lines (Dewi et al. 2017). Meanwhile, anther culture is considered a promising non-conventional method for the development of improved rice varieties because it accelerates the process of obtaining pure lines in the first generation (Mishra et al. 2015). Anther culture is an effective and efficient technique because it can reduce fixation time to obtain homozygous lines in 1-2 generations in self-pollinated plants (Purwoko 2018). This technique can produce doubled haploid lines in a shorter time than the conventional methods. Haploid plants are produced by androgenesis through anther culture, which is simpler and more efficient ways than other techniques. The process of chromosomal doubling occurs spontaneously or by chromosomal doubling agents in rice (Purwoko 2018).

The application of the technique in rice breeding begins with the selection and crossing of parents, planting of donor plants as explant sources, and anther culture of donor plants in vitro, acclimatization of the regenerants, characterization

of doubled haploid plants, seed production, and selection of desired characters (Dewi and Purwoko 2012). This technique has been used in Korea, China, India, and Japan (Tripathy et al. 2019), while in Indonesia, Bioemas Agritan (SK Mentan No.171/HK.540/C/09/2021), Bioprima Agritan (SK Mentan No. 172/HK.540/C/09/2021) and IPB 10G Tanimar varieties (SK Mentan No. 1830/HK.540/C/07/2022) have been released.

Pure lines that have been produced need to be evaluated and selected for their high productivity. Selection as part of the plant breeding process needs appropriate criteria. Selection for doubled haploid rice lines was carried out directly in the field for the main character of rice plants, namely high productivity (Abhilash et al. 2018). Various techniques to obtain crops with increased yield and better quality have been explored (Hickey et al. 2019). The selection of doubled haploid lines with high productivity might involve other agronomic characters, such as plant height, productive tillers, harvest age, panicle length, percentage of filled and empty grains, the weight of 1000 grains, and productivity (Akbar et al. 2021). The doubled haploid lines can be selected by index selection based on several agronomic characters weighted according to their economic value (Ramos et al. 2014). This study aimed to select doubled haploid rice lines obtained from anther culture based on index selection and agronomic performance.

MATERIALS AND METHODS

Plant materials

The plant materials used in the study were 60 doubled haploid lines, namely WH1 up to WH60. The WH1-WH33 lines were derivate of the FS3 (KP4 x BioNL 6-1), the WH34-WH58 lines were derivate of the FS4 (Inpari 45 x KP4), while WH59-WH60 lines were obtained from the FS7 (Ciherang Malaka x Inpari 45). All doubled haploid lines were obtained from anther culture, and five check varieties/genotypes were used, namely WH61 KP 4, WH62 BioNL 6-1, WH63 Inpari 45, WH64 Ciherang Malaka, and WH65 Inpari 42 as a productivity check. Plant materials were obtained from the cooperation between IPB University and Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD).

Procedures

The experiment was carried out from May to August 2022 and the nursery was prepared in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) greenhouse, while transplanting was carried out at the Sawah Baru Experimental Station, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, with coordinates 6°33'47.9"S, 106°44'10.8"E. The single-factor randomized complete block design (RCBD) was used with genotype as treatment. Sixty doubled haploid lines and 5

check varieties/genotypes were tested with three replications, hence, the total experimental unit was 195. Each experimental unit was a plot size of 1 m x 3.75 m. The seedlings were planted 21 days after sowing with a spacing of 25 cm x 25 cm. Furthermore, the fertilizer dosage was as follows: Urea 100 kg ha⁻¹, SP36 166 kg ha⁻¹, and KCl 125 kg ha⁻¹. Urea fertilizer was applied three times, namely 7 days after planting (DAP), 35 DAP, and 45 DAP for one-third of the dose each. KCl and SP-36 fertilizers were applied once at 7 DAP. Installation of nets was performed when the plants started to flower to control bird pests.

Maintenance of plants was performed according to rice cultivation standards (IRRI 2013). Moreover, observations were made on 3 samples (hills) per experimental unit which included plant height at the generative phase (cm), the number of productive tillers observed at the time of harvest, age calculated from the period of planting up to when 90% of plant panicles turned yellow, panicle length (cm), number of filled grains, number of empty grains, number of total grains, percentage of filled grain and percentage of empty grain (%), the weight of 1000 grains weighed with a moisture content of ±14%, as well as the productivity of each line and check varieties measured on a plot-basis and then converted to ton ha⁻¹.

Data analysis

Analysis of the variance component estimation was performed using SPSS software and Microsoft Excel 2019. A further test used the least significant difference (LSD) at the 5% level. Quantitative observational data were analyzed by estimating the genetic variability and broad-sense heritability, following Syukur et al. (2015) (Table 1).

The formula for calculating the estimated value of the broad sense heritability (h^2_{bs}) followed Syukur et al. (2015) as follows:

$$h^2_{bs} = \frac{\sigma_g^2}{\sigma_p^2}$$

Where:

$$\sigma_g^2 = (M2-M1)/r$$

$$\sigma_p^2 = \sigma_g^2 + (\sigma_e^2/r)$$

Class of heritability values in broad terms are: high ($0.50 < h^2 < 1.00$), moderate ($0.20 < h^2 < 0.50$), and low ($h^2 < 0.20$) (Elrod and Stansfield 2010).

Table 1. Expected mean square from the analysis of variance

Source of variation	Degrees of freedom	Mean square	Expected mean square
Replication	(r-1)		
Genotype	(g-1)	M2	$\sigma_{e+r}^2 (\sigma_g^2)$
Error	(r-1) (g-1)	M1	σ_e^2

Note: r: test; g: genotype; σ_e^2 : experimental error variance; σ_g^2 : genotypic variance; M1: error mean square and M2: genotype mean square

Important agronomic characters representing yield and components considered to have high economic value were selected simultaneously. The selection index is symbolically written by the formula according to Kang (2015) as follows: $I = \sum b_i x_i = b_1 x_1 + b_2 x_2 + \dots + b_n x_n$. where, I is the selection index; b_n is the weight given to the selected character, i.e., productivity: 3, number of productive tillers: 1, number of total grains: 1, and percentage of filled grain: 1. x_n is the standardized phenotype value, calculated using the standardization formula of Jolliffe and Cadima (2016) as follows: $P_{ij} = \frac{(x_{ij} - \bar{x}_i)}{s_i}$ where, p_{ij} : standardized genotype value; x_{ij} : genotype value; \bar{x}_i : average value of a character; and s_i : standard deviation of a character.

RESULTS AND DISCUSSION

Analysis of variance of doubled-haploid rice lines

Analysis of variance showed that genotype had a highly significant effect ($p < 0.01$) on characters' plant height, harvest age, panicle length, number of empty grains, the total number of grains, percentage of filled grains (%), percentage of unfilled grain (%), the weight of 1000 grains (g) and productivity (ton ha^{-1}), significant effect ($p < 0.05$) on the number of filled grains, and not significant for the number of productive tillers (Table 2). The significant genotype effect implies that these lines varied for all of the characters observed, hence, selection can be made. This indicates the existence of genetic variability, an essential condition to achieve success with selection (Smiderle et al. 2019). Genotypes in populations with high genetic variability can be selected to obtain ones with desired traits (Akbar et al. 2021).

Several of the agronomic characters tested had coefficient of variation (CV) values ranging from 1.13 to 31.95% (Table 2). The CV value is relatively small in character harvest age (1.13%), panicle length (4.06%), plant height (4.22%), and weight of 1000 grains (4.71%), moderate in character of the total number of grains (11.38%), percentage of filled grain (12.05%), productivity (16.3%), number of filled grains (17.18%), and number of

productive tillers (18.85%), and high in the number and percentage of empty grains (30.51%). Delgado et al. (2019) stated that the CV value of a character observed indicates the magnitude of environmental errors. The low value obtained from the coefficient of variation indicates high reliability because the lower the coefficient of variation, the higher the degree of accuracy.

Genetic and phenotypic variances were used to calculate broad-sense heritability for all observed agronomic characters (Table 3). All the characters showed high broad sense heritability ($>50\%$) except for the number of productive tillers which was in the moderate category. This heritability value was then used as one of the bases of selecting characters for index selection. Similar results were reported by Hidayatullah et al. (2018), where the heritability values for all observed characters were high, except for the number of tillers and productivity which were in the medium category. According to Venmuhil et al. (2020), there are wide genetic variability and greater possible genetic gain from these traits. Belay (2018) mentioned that the value of expected genetic gain is divided into 3 groups, namely small $<10\%$, medium 10-20%, and high $>20\%$.

Table 3. Genetic and phenotypic variances as well as broad sense heritability estimates for agronomic characters of doubled haploid rice lines obtained from anther culture

Character	σ_g^2	σ_p^2	h_{bs}^2 (%)
Plant height (cm)	49.98	56.42	88.58
Number of productive tillers	0.94	4.20	22.48
Harvest age (DAS)	0.74	1.34	54.98
Panicle length	1.64	2.04	80.69
Number of filled grains	310.80	511.44	60.77
Number of empty grain	162.66	271.23	59.97
Number of total grains	532.35	703.87	75.63
Percentage of filled grain (%)	27.10	51.98	52.14
Percentage of empty grain (%)	27.10	51.98	52.14
Weight of 1000 grains (g)	4.91	5.45	90.09
Productivity (ton ha^{-1})	0.30	0.51	57.79

Note: σ_g^2 : genetic variance; σ_p^2 : phenotypic variance; h_{bs}^2 : broad sense heritability

Table 2. Analysis of variance of various agronomic characters of doubled haploid rice lines from anther culture

Traits	Genotype mean square	Error mean square	Significance	Coefficient of variation (%)
Plant height (cm)	169.25	19.32	**	4.22
Number of productive tillers	12.59	9.76	Ns	18.85
Harvest age (DAS)	4.02	1.81	**	1.13
Panicle length (cm)	6.11	1.18	**	4.06
Number of filled grain	1534.31	601.91	*	17.18
Number of empty grain	813.7	325.72	**	31.95
Number of total grains	2111.62	514.57	**	11.38
Percentage of filled grain (%)	155.94	74.64	**	12.05
Percentage of empty grain (%)	155.94	74.64	**	30.51
Weight of 1000 grains (g)	16.34	1.62	**	4.71
Productivity (ton ha^{-1})	1.54	0.65	**	16.3

Note: *: significant at 5%, **: significant at 1%, Ns: not significant; DAS: days after sowing

The high heritability of most characters (Table 3) might be due to the large value of genetic variance, and this indicates the influence of the lines obtained from anther culture of F_1 individuals which had a variety of genetic backgrounds. The lower heritability value for the number of productive tillers illustrates that the character is more influenced by environmental factors. This is in line with Alsabah et al. (2019), who reported that almost all of the characters evaluated in doubled haploid lines had high heritability estimates. Moreover, Rafii et al. (2014) stated that the estimated heritability values obtained varied from low to high for the observed agronomic characters. A desired character can be used for selection when it has a high heritability (Begum et al. 2015).

Agronomic characteristics of doubled haploid rice lines from anther culture

Analysis of variance showed that doubled haploid rice lines had a highly significant effect on plant height, days to harvest, panicle length, percentage of filled grain, percentage of empty grain, 1000 grain weight, and production, but had no significant effect on the number of productive tillers (Table 4). The average plant height was 104.29 cm with a range between 87.33 and 121.11 cm. The lowest value was found in the WH22 line, while the highest was found in the check variety, Ciherang Malaka (Table 4). The plant height of the tested doubled haploid lines can be grouped into low and medium categories, with a value of 45 and 15 genotypes in each category respectively. According to the International Rice Research Institute (2013), the plant height of lowland rice is classified into three categories, namely short (<110 cm), medium (110-130 cm), and tall (>130 cm). Short doubled haploid lines tend not to lodge easily. The number of productive tillers on average was 16.6 with a range between 12.3 and 20.8 tillers, and the analysis of variance did not show any difference among the genotypes. According to IRRI (2013), the number of productive tillers of rice can be classified into five, namely very low (<5 tillers), low (5-9 tillers), medium (10-19 tillers), good (20-25 tillers) and very high (> 25 tillers). From the results of this grouping, all lines tested were in the medium category, except for the WH24 line (20.8 tillers) and the Inpari-42 variety (20.3 tillers) which had a good number of tillers.

The average days to harvest or age was 119.2 days after sowing (DAS) with a range between 117 and 121.7 DAS (Table 4). The results showed that the days to harvest of 60 doubled haploid rice lines tested were in the medium category. The days to harvesting (P) of rice varieties were classified into four categories (IRRI 2013), namely very early ($P < 110$ DAS), early ($110 < P < 115$ DAS), medium ($115 < P < 125$ DAS), and long ($125 < P < 150$ DAS). Furthermore, the average panicle length was 26.73 cm with a range between 24 and 29.22 cm, while the lines with the longest panicle length were WH40 (29.22 cm), WH37 (29.11 cm), WH50 (29.11 cm), and the shortest was found in WH04 (24 cm) (Table 4).

The average number of total grains was 199.3 with a range between 135.0 and 275.4, while the average percentage of filled grain was 71.68% with a range of 53.23 to 87.61%. Moreover, the average percentage of empty grain was 28.32% with a range between 12.39 and 46.77% as shown in Table 4. The average weight of a thousand grains was 26.99 g with a range of 21.27 to 31.75 g. The lines WH07 (31.75 g), WH06 (31.27 g), WH46 (30.88 g), WH39 (30.84 g), WH05 (30.51 g), and WH41 (30.36 g) had a thousand-grain weight heavier than the check varieties. Also, they have larger seed sizes than the other lines. The weight of 1000 grains is largely determined by the size of the rice grain. Rice grain weight depends on the husk size, sink activity, and source (Kato and Katsura 2014) as well as the genetic character of the lines. The percentage of filled grains, the total number of grains per panicle, and the weight of 1000 grains are the main characteristics related to yield, so selection conditions can be used to improve the genetic potential of yield characters (Sadimantara et al. 2021). The average productivity was 4.95 ton ha⁻¹ with a range between 2.95 and 6.77 ton ha⁻¹, where the lines WH39 (6.77 ton ha⁻¹), WH16 (6.25 ton ha⁻¹) and WH38 (6.20 ton ha⁻¹) have higher productivity than the check varieties as demonstrated in Table 4. The doubled haploid lines that have high productivity can be used further for brown planthopper resistance evaluation and drought tolerance evaluation.

Selection index on doubled haploid rice lines

The selection index was determined by weighting productivity and other characters, namely the number of productive tillers, the number of total grains, and the percentage of filled grains. The selected agronomic characters were assigned weights, namely: productivity: 3, number of productive tillers: 1, number of total grains: 1, and percentage of filled grain: 1. Productivity was given a weight 3 times greater than the other three characters to meet the objective of the breeding program. Productivity has a higher economic value, while other characters used in the selection are supporting factors (Akbar et al. 2021). The index selection model is comprised of a combination of characters and is weighed based on economic value (Anshori et al. 2021).

The highest selection index was found in the WH39 (7.88) (Table 5), while the lowest selection index was found in the WH10 (-10.80) (data not shown). Several genotypes of the selected doubled haploid rice lines have better agronomic characteristics than the check varieties. This shows that the characters of the number of productive tillers, the number of total grains, and the percentage of filled grain are appropriate to be used in index selection along with grain yield (Oladosu et al. 2018). Twenty-nine selected doubled haploid rice lines had positive weight selection index values except for WH59, indicating higher agronomic performance and grain yield compared to the other 31 doubled haploid lines (Table 5). The selected lines will be further tested at the next experimental stage in several locations to produce high-yielding, brown planthopper-resistant, and drought-tolerant rice varieties.

Table 4. Mean agronomic characters of doubled haploid rice lines and check varieties

Lines	PH	NPT	DTH	PL	NTG	PFG	PEG	WOG	PRD
WH01	103.44	14.9	120.7	26.78	199.1	72.38	27.62	26.36	4.92
WH02	92.89	17.4	119.0	25.44	193.0	63.03	36.97	26.85	4.17
WH03	97.33	19.3	120.0	24.89	197.2	77.42	22.58	28.27	5.36
WH04	92.56	16.7	118.3	24.00	179.7	74.57	25.43	27.74	5.08
WH05	105.56	15.1	119.7	26.94	185.1	74.18	25.82	30.51	4.30
WH06	111.89	14.7	119.0	28.94	220.2	69.86	30.14	31.27	5.27
WH07	110.33	17.2	119.0	28.22	215.2	63.69	36.31	31.75	5.49
WH08	106.00	17.1	119.3	28.56	235.6	84.21	15.79	25.49	5.20
WH09	109.44	17.3	120.0	26.89	216.1	76.47	23.53	24.10	5.42
WH10	99.78	17.3	118.0	26.33	182.0	56.35	43.65	28.17	2.95
WH11	95.56	17.9	118.0	25.22	185.8	79.76	20.24	23.64	3.97
WH12	94.33	16.7	118.7	25.00	174.4	67.94	32.06	28.55	3.55
WH13	96.67	18.6	117.7	25.22	169.7	68.04	31.96	28.58	4.11
WH14	97.22	16.6	118.3	25.22	172.9	59.98	40.02	28.60	4.58
WH15	100.45	16.6	117.7	26.33	187.2	69.54	30.46	28.82	4.84
WH16	103.67	18.0	120.7	25.44	184.0	85.49	14.51	26.22	6.25
WH17	102.22	16.3	118.7	26.00	192.7	80.90	19.10	25.23	5.10
WH18	102.67	13.8	120.3	27.22	215.0	75.82	24.18	29.02	4.92
WH19	107.34	15.9	117.7	27.44	214.3	71.74	28.26	28.71	5.51
WH20	96.33	16.4	121.0	25.44	184.6	75.51	24.49	27.57	5.02
WH21	94.22	19.8	121.0	24.78	172.3	81.59	18.41	28.41	4.93
WH22	87.33	16.1	119.0	24.56	185.6	63.54	36.46	25.16	4.78
WH23	95.56	19.7	119.0	24.89	156.6	74.14	25.86	23.11	4.12
WH24	92.33	20.8	118.3	25.56	170.3	78.83	21.17	23.66	4.14
WH25	102.78	18.6	119.3	25.33	165.2	87.61	12.39	25.57	4.51
WH26	103.89	16.9	120.3	25.22	176.4	70.14	29.86	25.84	4.76
WH27	111.67	16.6	118.3	27.28	212.9	80.79	19.21	25.18	5.60
WH28	105.89	19.1	121.7	25.94	154.9	74.70	25.30	25.01	5.02
WH29	97.89	19.3	119.7	25.39	157.0	70.12	29.88	24.78	4.73
WH30	104.11	19.2	118.0	25.33	167.3	75.02	24.98	25.74	4.64
WH31	99.00	17.7	119.7	24.78	135.0	81.56	18.44	25.29	3.96
WH32	96.11	18.8	118.0	25.56	167.2	72.06	27.94	25.44	3.73
WH33	105.55	14.4	120.0	27.56	212.9	71.25	28.75	26.39	4.48
WH34	93.00	17.6	121.3	24.33	213.8	73.58	26.42	22.43	4.18
WH35	94.11	17.9	119.0	24.56	218.0	66.83	33.17	21.27	4.48
WH36	105.45	15.4	120.0	28.00	204.4	79.82	20.18	26.34	5.64
WH37	110.44	15.0	117.0	29.11	241.0	76.21	23.79	24.90	5.78
WH38	107.78	16.3	117.3	27.22	198.0	67.43	32.57	27.79	6.20
WH39	112.89	12.7	117.3	27.89	230.2	79.03	20.97	30.84	6.77
WH40	115.89	14.4	119.0	29.22	258.9	70.55	29.45	28.28	4.99
WH41	111.67	12.7	120.3	27.33	200.9	65.37	34.63	30.36	4.08
WH42	115.11	13.6	121.0	28.33	262.1	64.04	35.96	27.25	5.80
WH43	115.11	13.8	118.0	28.11	228.1	68.91	31.09	29.47	5.79
WH44	112.33	13.3	118.0	28.33	216.8	76.95	23.05	29.22	5.80
WH45	107.22	17.6	119.7	28.00	195.0	77.69	22.31	26.78	5.63
WH46	112.78	12.3	119.3	26.89	232.3	63.34	36.66	30.88	4.47
WH47	105.55	17.0	117.7	25.89	195.1	67.43	32.57	26.93	5.20
WH48	103.67	16.3	121.0	26.78	199.9	70.28	29.72	24.68	5.11
WH49	115.11	18.2	119.3	28.44	214.0	78.62	21.38	26.87	5.92
WH50	116.11	14.1	121.0	29.11	220.9	78.26	21.74	27.41	5.94
WH51	104.22	16.6	120.0	27.67	208.1	63.48	36.52	28.24	4.86
WH52	109.89	19.4	120.7	28.33	208.0	67.99	32.01	29.34	5.25
WH53	102.78	18.7	120.0	26.89	189.8	69.09	30.91	29.23	4.23
WH54	106.00	18.8	118.7	27.78	203.2	53.23	46.77	28.21	5.28
WH55	99.22	15.1	118.0	27.89	205.8	63.71	36.29	29.76	4.79
WH56	104.33	14.8	120.0	27.00	179.9	61.60	38.40	27.92	4.46
WH57	102.78	18.6	119.3	26.78	173.8	65.02	34.98	28.71	4.61
WH58	104.00	14.8	119.0	27.22	187.4	62.31	37.69	28.96	4.65
WH59	112.45	13.7	117.3	28.22	207.6	62.02	37.98	25.67	5.12
WH60	110.56	14.2	118.7	28.78	233.6	72.37	27.63	24.07	4.69
WH61 KP.4	96.89	16.2	119.3	25.56	200.4	67.69	32.31	27.96	4.76
WH62 BioNiL 6-1	117.33	17.3	118.7	26.33	188.7	82.67	17.33	25.31	4.90
WH63 Inpari -45	112.67	15.0	120.3	28.67	211.7	72.64	27.36	26.28	6.17
WH64 Ciherang Malaka	121.11	15.1	119.7	28.33	215.2	72.71	27.29	26.34	5.98
WH65 Inpari -42	98.56	20.3	120.7	26.56	275.4	70.07	29.93	21.47	4.85
Average	104.29	16.6	119.2	26.73	199.3	71.68	28.32	26.99	4.95
LSD 5%	7.1	5.1	2.18	1.75	36.7	13.96	13.96	2.05	1.30

Note: PH: plant height, NPT: number of productive tillers, DTH: days to harvest, PL: panicle length, NTG: number of total grains, PFG: percentage of filled grain, PEG: percentage of unfilled grain, WOG: weight of 1000 grains, PRD: productivity, and LSD: least significant difference at the 5% level

Table 5. Results of the index selection consisting of 29 doubled haploid rice lines

Line	PRD	NPT	NTG	PFG	SI
WH03	5.36	19.3	197.2	77.42	3.77
WH04	5.08	16.7	179.7	74.57	0.24
WH06	5.27	14.7	220.2	69.86	0.93
WH07	5.49	17.2	215.2	63.69	2.05
WH08	5.20	17.1	235.6	84.21	4.41
WH09	5.42	17.3	216.1	76.47	3.63
WH16	6.25	18.0	184.0	85.49	7.47
WH17	5.10	16.3	192.7	80.90	1.52
WH19	5.51	15.9	214.3	71.74	2.57
WH20	5.02	16.4	184.6	75.51	0.20
WH21	4.93	19.8	172.3	81.60	1.84
WH27	5.60	16.6	212.9	80.79	4.49
WH28	5.02	19.1	154.9	74.70	0.28
WH36	5.64	15.4	204.4	79.82	3.66
WH37	5.78	15.0	241.0	76.21	4.90
WH38	6.20	16.3	198.0	67.43	4.46
WH39	6.77	12.7	230.2	79.03	7.88
WH40	4.99	14.4	258.9	70.55	1.21
WH42	5.80	13.6	262.1	64.04	3.40
WH43	5.79	13.8	228.1	68.91	2.85
WH44	5.80	13.3	216.8	76.95	3.38
WH45	5.63	17.6	195.0	77.70	4.00
WH47	5.20	17.0	195.1	67.43	0.50
WH48	5.11	16.3	199.9	70.28	0.39
WH49	5.92	18.2	214.0	78.62	6.36
WH50	5.94	14.1	220.9	78.26	4.67
WH52	5.25	19.4	208.0	67.99	2.47
WH54	5.28	18.8	203.2	53.23	0.04
WH59	5.12	13.7	207.6	62.02	-1.75
WH61 KP.4	4.76	16.2	200.4	67.69	-1.48
WH62 BioNiL 6-1	4.90	17.3	188.7	82.67	1.26
WH63 Inpari -45	6.17	15.0	211.7	72.65	4.95
WH64 Ciharang Malaka	5.98	15.1	215.2	72.71	4.32
WH65 Inpari -42	4.86	20.3	275.4	70.07	4.08

Note: PRD: productivity, NPT: number of productive tillers, NTG: number of total grains, PFG: percentage of filled grain, and SI: selection index

The selected lines had a productivity range of 4.76 to 6.77 ton ha⁻¹, a number of productive tillers of 12.7 to 20.3 tillers, a number of total grains of 154.9 to 275.4 seeds, a filled grain percentage of 53.23 to 85.49%, and a selection index of -1.48 to 7.88 (Table 5). Furthermore, information on the relationship between productivity and other yield components is important for conducting efficient selection for the development of new varieties by combining important traits that can increase yields economically (Kumar et al. 2014).

In conclusion, 29 lines with good agronomic characteristics, namely productivity (4.93-6.77 ton ha⁻¹), number of productive tillers (12.7-19.4 tillers), number of total grains (154.9-262.1 seeds), and percentage of filled grain (53.23-85.49%) were selected in this study. The selected lines from index selection will be further tested in advanced yield trials in three locations (Bogor, Indramayu and Malang), brown planthopper resistance, a molecular marker for brown planthopper resistance, and drought tolerance evaluation, to identify promising lines with high yields, brown planthopper resistance, and drought tolerance.

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REFERENCES

- Abhilash R, Thirumurugan T, Sassikumar D, Chitra S. 2018. Genetic studies in F2 for biometrical traits in rice (*Oryza sativa* L). *Electron J Plant Breed* 9 (3): 1067-1076. DOI: 10.5958/0975928X.2018.00133.3.
- Akbar MR, Purwoko BS, Dewi IS, Suwarno WB, Sugiyanta, Anshori MF. 2021. Agronomic and yield selection of doubled haploid lines of rainfed lowland rice in advanced yield trials. *Biodiversitas* 22 (7): 3006-3012. DOI: 10.13057/biodiv/d220754.
- Alsabah R, Purwoko BS, Dewi IS, Wahyu Y. 2019. Selection index for selecting promising doubled haploid lines of black rice. *SABRAO J Breed Genet* 5: 430-441.
- Anshori MF, Purwoko BS, Dewi IS, Ardie SW, Suwarno WB. 2021. A new approach to select doubled haploid rice lines under salinity stress using

- indirect selection index. *Rice Sci* 28: 368-378. DOI: 10.1016/j.rsci.2021.05.007.
- Begum H, Spindel JE, Lalusin A, Borromeo T, Gregorio G, Hernandez J, Virk P, Collard B, McCouch SR. 2015. Genome-wide association mapping for yield and other agronomic traits in an elite breeding population of tropical rice (*Oryza sativa*). *PLoS ONE* 10: 1-19. DOI: 10.1371/journal.pone.0119873.
- Belay N. 2018. Genetic variability, heritability, correlation and path coefficient analysis for grain yield and yield component in maize (*Zea mays* L.) hybrids. *Adv Crop Sci Technol* 6 (5): 399. DOI: 10.4172/2329-8863.1000399
- Central Bureau of Statistics. 2021. Rice Production in 2021 Increases 1.14 percent (Provisional Figures). bps.go.id. [Indonesian]
- Delgado ID, Gonçalves FMA, da Costa Parrella RA, de Castro FMR, Nunes JAR. 2019. Genotype by environment interaction and adaptability of photoperiod-sensitive biomass sorghum hybrids. *Bragantia* 78: 509-521. DOI: 10.1590/1678-4499.20190028.
- Dewi IS, Purwoko BS. 2012. Anther culture to accelerate rice breeding in Indonesia. *J AgroBiogen* 8 (2): 78-88. DOI: 10.21082/jbio.v8n2.2012.p78-88. [Indonesian]
- Dewi IS, Syafii M, Purwoko BS, Suwarno WB. 2017. Efficient indica rice anther culture derived from three-way crosses. *SABRAO J Breed Genet* 49 (4): 336-345.
- Elrod S, Stansfield W. 2010. *Schaum's Easy Outline of Genetics*. Fifth Edition. McGraw-Hill, New York.
- Ferrater JB, de Jong PW, Dicke M, Chen YH, Horgan FG. 2013. Symbiont-mediated adaptation by planthoppers and leafhoppers to resistant rice varieties. *Arthropod-Plant Interact* 7: 591-605. DOI: 10.1007/s11829-013-9277-9.
- Hickey LT, Hafeez AN, Robinson H, Jackson SA, Leal-Bertioli SCM, Tester M, Gao C, Godwin ID, Hayes BJ, Wulff BBH. 2019. Breeding crops to feed 10 billion. *Nat Biotechnol* 37: 744-754. DOI: 10.1038/s41587-019-0152-9.
- Hidayatullah A, Purwoko BS, Dewi IS, Suwarno WB. 2018. Agronomic performance and yield of doubled haploid rice lines in advanced yield trial. *SABRAO J Breed Genet* 50 (3): 242-253.
- International Rice Research Institute [IRRI]. 2013. *Standard Evaluation System for Rice*. INGER-IRRI, Manila.
- Jolliffe IT, Cadima J. 2016. Principal component analysis: A review and recent developments. *Phil Trans R Soc A* 374: 20150202. DOI: 10.1098/rsta.2015.0202.
- Kang MS. 2015. Efficient SAS programs for computing path coefficients and index weights for selection indices. *J Crop Improv* 29 (1): 6-22. DOI: 10.1080/15427528.2014.959628.
- Kato Y, Katsura K. 2014. Rice adaptation to aerobic soils: physiological considerations and implications for agronomy. *Plant Prod Sci* 17: 1-12. DOI: 10.1626/pp.s.17.1.
- Kumar V, Koshta N, Sohaura N, Koutu GK. 2014. Genetic evaluation of RILs population for yield and quality attributing traits in rice (*Oryza sativa* L.). *J Agric Technol* 1 (1): 43-51.
- Mishra AK, Mottaleb KA, Khanal AR, Mohanty S. 2014. Abiotic stress and its impact on production efficiency: The case of rice farming in Bangladesh. *Agric Ecosys Environ* 199: 146-153. DOI: 10.1016/j.agee.2014.09.006.
- Mishra R, Rao GJ, Rao RN, Kaushal P. 2015. Development and characterization of elite doubled haploid lines from two indica rice hybrids. *Rice Sci* 22 (6): 290-299. DOI: 10.1016/j.rsci.2015.07.002.
- Oladosu Y, Rafii MY, Magaji U, Abdullah N, Miah G, Chukwu SC, Hussin G, Ramli A, Kareem I. 2018. Genotypic and phenotypic relationship among yield components in rice under tropical conditions. *BioMed Res Intl*. DOI: 10.1155/2018/8936767.
- Ortiz AMD, Charlotte L, Outhwaite CL, Dalin C, Newbold T. 2021. A review of the interactions between biodiversity, agriculture, climate change, and international trade: Research and policy priorities. *One Earth* 4 (1): 88-101. DOI: 10.1016/j.oneear.2020.12.008.
- Purwoko BS. 2018. Development of anther culture technique and its application in accelerating rice breeding in Indonesia. In: Sudarsono, Purwoko BS (eds). *Theoretical Basis, Genetics Application, and Environmental Manipulation to Increase Crop Production*. IPB Press, Bogor. [Indonesian]
- Rafii MY, Zakiah MZ, Asfaliza R, Haifa MDI, Latif MA, Malek MA. 2014. Grain quality performance and heritability estimation in selected F1 rice genotypes. *Sains Malays* 43: 1-7. DOI: 10.1155/2014/308042.
- Ramos HCC, Pereira MG, Viana AP, Luz LN, Cardoso DL, Ferregueti GA. 2014. Combined selection in backcross population of papaya (*Carica papaya* L.) by the mixed model methodology. *Am J Plant Sci* 5: 2973-2983. DOI: 10.4236/ajps.2014.520314.
- Sabouri A, Dadras AR, Azari M, Kouchesfahani AS, Taslimi M, Jalalifar R. 2022. Screening of rice drought tolerant lines by introducing a new composite selection index and competitive with multivariate methods. *Sci Rep* 12: 2163. DOI: 10.1038/s41598-022-06123-9.
- Sadimantara GR, Yusuf DN, Febrianti E, Leomo S, Muhidin. 2021. The performance of agronomic traits, genetic variability, and correlation studies for yield and its components in some red rice (*Oryza sativa*) promising lines. *Biodiversitas* 22 (9): 3994-4001. DOI: 10.13057/biodiv/d220947
- Smiderle EC, Furtini IV, da Silva CSC, Botelho FBS, Resende MPM, Botelho RTC, Filho JMC, de Castro AP, Utumi MM. 2019. Index selection for multiple traits in upland rice progenies. *Rev Ciênc Agrár* 2 (1): 4-12. DOI: 10.19084/RCA18059.
- Syukur M, Sujiprihati S, Yunianti R. 2015. *Plant Breeding Techniques* (revised). Penebar Swadaya, Jakarta. [Indonesian]
- Tripathy SK, Lenka D, Prusti AM, Mishra D, Swain D, Behera SK. 2019. Anther culture in rice: progress and breeding perspective. *Appl Biol Res* 21 (2): 87-104. DOI: 10.5958/0974-4517.2019.00012.0
- Venmuhil R, Sassikumar D, Vanniarajan C, Indirani R. 2020. Selection indices for improving the selection efficiency of rice genotypes using grain quality traits. *Electron J Plant Breed* 11 (2): 543-549. DOI: 10.37992/2020.1102.09.