



## Yield Trial of Doubled-Haploid Rice Lines with Multiple Abiotic Stress Tolerance

Hayu Widi Yuana<sup>1</sup>, Bambang Sapta Purwoko<sup>1\*</sup>, Willy Bayuardi Suwarno<sup>1</sup>,  
Iswari Saraswati Dewi<sup>2</sup> and Cucu Gunarsih<sup>3</sup>

<sup>1</sup>Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia;

<sup>2</sup>Research Center of Food Crops, National Research and Innovation Agency (BRIN), Bogor, Indonesia;

<sup>3</sup>Indonesian Center for Rice Instrument Standard Testing (ICRIST), Subang, Indonesia

\*Corresponding author: [bspurwoko@apps.ipb.ac.id](mailto:bspurwoko@apps.ipb.ac.id)

### Abstract

Rice production is facing a decline due to climate change and land conversion. The development of high-yielding and multi-tolerant abiotic stress rice varieties (such as drought, salinity, and flooding) is needed for adaptation to a changing climate. This research aimed to identify potential doubled-haploid (DH) rice lines with good agronomic performance and high yield through index selection. The study was conducted in the rainy season of 2024, and used a randomized complete block design with a single genotype factor and three replications. The genetic material evaluated consisted of 56 DH rice lines and 5 check varieties, i.e., Inpari 18, Inpari 30, Inpari 34, Inpari 35, and Ciherang. Observation was conducted on plant height (PH), number of vegetative tillers (NVT), number of productive tillers (NPT), days to 50% flowering (DF), days to harvesting (DTH), number of filled grains (NFG), total grains per panicle (TG), panicle length (PL), and productivity. The results showed high heritability in all observed variables, indicating that the variables could be used as selection criteria. The weighted index selection involving PH, NPT, DTH, and productivity resulted in 30 DH rice lines with good agronomic traits such as medium PH, medium NPT, medium maturity, and productivity above the check varieties. Those selected DH lines can be evaluated further in more diverse environments to study the effects of genotype, environment, and G×E interactions.

**Keywords:** anther culture; climate change; heritability; index selection; productivity

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### INTRODUCTION

Rice (*Oryza sativa* L.) provides 20% of the daily caloric needs of more than half of the world's population, especially in Asia, the Middle East, India, and Latin America (Fukagawa and Ziska, 2019). Rice is a source of fiber, protein, minerals, vitamins, and other important biomolecules (Burlando and Cornara, 2014; Nur et al., 2015). The importance of rice makes it the 3<sup>rd</sup> most produced commodity in the world (FAO, 2023). Indonesia's national rice production decreased by 1.6% from 2019 (31.3 million tons) to 2023 (30.8 million tons), while rice

consumption continued to increase by 6.7% from 2019 (20.9 million tons year<sup>-1</sup>) to 2022 (22.3 million tons year<sup>-1</sup>) (Statistics Indonesia, 2020, 2023). Increasing rice production needs to continue to meet the needs of rice consumption, which continues to increase along with the increase in population.

Climate change and land conversion are challenges to increasing national rice production. The area of rice fields decreased by 7.4% from 2015 (8.1 million ha) to 2019 (7.5 million ha) (Indonesian Ministry of Agriculture, 2023).

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The decrease in the area of rice fields is caused by the conversion of agricultural land to other uses. Increased air temperature and changes in rainfall patterns are the impacts of climate change that can cause abiotic stress on the growing environment (Hasegawa et al., 2011; Eckardt et al., 2023). Abiotic stresses such as drought (Oladosu et al., 2019), flooding (Panda and Barik, 2021), and salinity (Reddy et al., 2017) can lead to yield loss and crop failure. Flooding can harm rice plants because it causes slow gas exchange rates, shading by turbid water, and inhibits growth due to flow rates (Neog et al., 2016). Drought conditions during floral development and anthesis are critical points that can cause pollen sterility (Kumar et al., 2014). Drought also reduces panicle initiation and biomass production (Sandhu and Kumar, 2017). Salinity stress inhibits germination and reduces leaf area, plant height, and plant biomass (Ghosh et al., 2016).

Generally, plant mechanisms against abiotic stress are detoxification, homeostasis, and growth regulation. Reactive oxygen species (ROS) resulting from abiotic stress need to be detoxified by the production of antioxidants and osmolytes. After ROS inactivation, cell turgidity needs to be maintained to prevent damage through the production of osmoprotectants. Under abiotic stress, plants need to conserve resources by suppressing unnecessary growth (Sarma et al., 2023). In 2020, 39,247 ha of rice fields in Indonesia were affected by floods and 90,656 ha by drought (Indonesian Ministry of Agriculture Republic Indonesia, 2021). Sea level rise due to global warming needs to be considered as it leads to coastal erosion and increased salinity stress on rice fields, especially in nearshore areas (Rumanti et al., 2018).

High-input cultivation techniques such as chemical fertilization and pesticides are commonly used to increase rice yields but have an impact on reducing soil quality and the environment (Mauro et al., 2022). High-yielding and multi-tolerant abiotic stress rice varieties can increase national rice production while maintaining environmental sustainability with low-input cultivation. Varieties with multi-tolerant abiotic stress characters can be planted in marginal lands, so they have the potential to have wide land adoption and be more affordable for farmers than cultural methods with increasingly high inputs of fertilizers and pesticides. Therefore, developing abiotic stress-tolerant and high-yielding rice varieties should be conducted as one of the efforts to overcome the

challenges of growing environmental stress for rice fields caused by land conversion and climate change.

Previously, the development of new varieties with anther culture techniques successfully produced high-yielding doubled-haploid (DH) lines with characters tolerant of abiotic stresses such as salinity (Anshori et al., 2022), drought (Akbar et al., 2018; Hadiananto et al., 2023b), and flash flooding (Anshori et al., 2023). The anther culture technique also successfully obtained DH rice lines with moderate resistance to biotic stresses of bacterial leaf blight pathotypes III, IV, and VIII (Putri et al., 2023; Nurhidayah et al., 2024). Anther culture, as one of the DH technologies, has been widely used, especially in rice breeding programs to shorten the time to obtain homozygous lines. Selection can be done directly on spontaneous DH or pure lines obtained in the first generation (Mayakaduwa and Silva, 2023). Recently, rice breeding programs at IPB University and the Indonesian Ministry of Agriculture released superior rice varieties developed by anther culture, i.e., IPB 11S Bepe (2023), IPB 10G Tanimar (2022), Bioemas Agritan (2021), and Bioprime Agritan (2021).

Rice varieties with multiple abiotic stress tolerance are not yet available in Indonesia. The research using anther culture by Gunarsih (2022) successfully obtained DH lines that had a multi-tolerance to abiotic stresses such as drought, flash flooding, and salinity (Gunarsih, 2022; Gunarsih et al., 2022). Those DH rice lines can be further evaluated through yield trials to select potential high-yield lines with good agronomic traits. Although high-yielding genotypes can be directly selected based on their yield, direct selection is not very effective because crop yield is a quantitative trait with a complex genetic control mechanism, thus, traits that affect yield must also be considered (Islam et al., 2016). The index selection method has been widely used in rice to select rice lines based on several selection criteria that affect yield and are weighted according to their economic value (Alsabah et al., 2019; Htwe et al., 2020; Akbar et al., 2021; Karima et al., 2021; Hadiananto et al., 2023a). Based on the need to develop rice varieties adapted to climate change, while anticipating the impact of land conversion on the rice fields from fertile to marginal land, this study aimed to identify potential DH rice lines with good agronomic performance and high yield through index selection.

## MATERIALS AND METHOD

### Plant materials and growing conditions

The research was conducted in the rainy season of 2024 at the Sawah Baru Experimental Station, IPB University in Bogor, Indonesia at an altitude of 220 m above sea level (m asl) with latosol soil type and soil characteristics medium total N (0.21%), low P (5.42 ppm), low K (0.19 cmol kg<sup>-1</sup>), medium C-organic (2.10%), and soil pH moderately acidic (5.19). Climatic conditions during the experiment had an average temperature of 27 °C, relative humidity of 85%, rainfall of 531 mm month<sup>-1</sup>, and length of irradiation of 5 hours day<sup>-1</sup>. The experimental design used was a randomized complete block design with three replications.

The genetic material used was 61 genotypes, i.e., 56 DH lines and 5 check commercial varieties (Inpari 18, Inpari 30, Inpari 34, Inpari 35, and Ciherang). DH rice lines were obtained from anther cultures of 8 combinations of crossing parents with abiotic stress tolerant traits, i.e., CGH1 (GN01-GN14) = HS4-11-1-2/B1396E-KA-23, CGH2 (GN17-GN34) = HS4-11-1-2/CG8-93-1-1, CGH3 (GN35b) = DR5-83-1-3/IR86384-46-3-1-B, CGH4 (GN36-GN50) = DR5-83-1-3/B13926E-KA-23, CGH5 (GN52-GN60) = FL478/B13926E-KA-23, CGH6 (GN61-GN65) = Inpari 30 Ciherang Sub1/DR5-83-1-3, CGH7 (GN67-GN74) = IR64 Dro1/IR86384-46-3-1-B, and CGH8 (GN78-GN90) = IR64 Dro1/HS4-11-1-2. The DH lines have been pre-screened for multi-tolerance to abiotic stresses such as drought, flash flooding, and salinity (Gunarsih, 2022).

### Procedures and observations

Twenty-one-day-old seedlings were transplanted into 1 m x 4 m plots with plant spacing of 25 cm x 25 cm. Fertilization was carried out based on the recommended dose of Dramaga site-specific fertilization with urea 350 kg ha<sup>-1</sup>, SP36 100 kg ha<sup>-1</sup>, and KCl 100 kg ha<sup>-1</sup> (Regulation of the Ministry of Agriculture of the Republic of Indonesia Number 13 of 2022). Maintenance in the form of weeding was done manually at 3 and 6 weeks after transplanting. Pest and disease control was adjusted to the attack level to reduce the use of excessive pesticides. Fish nets were installed to control bird pests when the plants started flowering. Pesticides with the active ingredient pymetrozine were applied for managing brown planthoppers when they reached the economic threshold. The grain was harvested when the rice was fully

ripe, as characterized by 90% of the grain turning yellow and hard. Observations were made on five samples per experimental unit. Observations on plant growth include plant height (PH), number of vegetative tillers (NVT), number of productive tillers (NPT), days to 50% flowering (DF), and days to harvesting (DTH). Observations of yield and yield components include panicle length (PL), number of filled grains (NFG), total grains per panicle (TG), and productivity.

### Data analysis

Data were analyzed using the F test followed by the t-Dunnett test at a 5% level. Genetic analyses involved the estimation of genetic variance ( $\sigma_g^2$ ), environmental variance ( $\sigma_e^2$ ), phenotypic variance ( $\sigma_p^2$ ), broad-sense heritability ( $h_{bs}^2$ ) (Stansfield, 1991), and genetic coefficient of variation (Kumar et al., 2018). The weighted index selection (Falconer, 1989) using multiple traits was performed. The weighting of selection criteria was determined based on breeding direction and economic value. The weighting was adapted from a modification of Hadianoto et al. (2023a), considering the NPT (+1), PH (-1), DTH (-1), and productivity (+5). The index values were ranked in descending order to select the best lines. Phenotypic values were standardized before index calculation using the formula by Walpole (1982). Genetic analysis and selection index were carried out using SAS OnDemand for Academics and Microsoft Excel.

The estimation  $h_{bs}^2$  on an entry-mean basis was calculated using Equation 1.

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2} \quad (1)$$

The estimates of  $h_{bs}^2$  were then categorized according to Stansfield (1991): high ( $0.50 < h_{bs}^2 < 1.00$ ), moderate ( $0.20 < h_{bs}^2 < 0.50$ ), and low ( $h_{bs}^2 < 0.20$ ).

The genotypic coefficient of variation (GCV) was calculated using Equation 2.

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \quad (2)$$

Grouping of GCV was done according to Kumar et al. (2018): low ( $< 0.10$ ), medium (0.10 to 0.20), and high ( $> 0.20$ ).

The standardized phenotype mean of each genotype was calculated based on Walpole (1982) using Equation 3.

$$p_i = \frac{(x_i - \bar{x})}{s} \quad (3)$$

Where,  $p_i$  = the standardized phenotypic mean of genotype- $i$ ,  $x_i$  = the original phenotypic mean of genotype- $i$ ,  $\bar{x}$  = the mean of the variable,  $s$  = the standard deviation of the variable.

The selection index formula based on Falconer (1989) is presented in Equation 4.

$$I = b_1X_1 + b_2X_2 + b_3X_3 + \dots + b_nX_n. \quad (4)$$

Where,  $I$  = the selection index,  $b_n$  = the weight of the selection variable- $n$ ,  $X_n$  = the selection variable- $n$ .

## RESULTS AND DISCUSSION

### Analysis of variance and heritability

Variance analysis showed that genotype significantly affected NVT, NPT, PH, DF, DTH, PL, NFG, TG, and productivity (Table 1). The coefficient of variation (CV) obtained ranged from 2.02 to 16.27%. The coefficient of variation indicates the level of error in the experiment, and a lower CV value suggests higher reliability (Gomez and Gomez, 1984). The tested genotypes originated from 8 cross combinations, and each cross would produce unique DH lines due to gametoclonal variation (Forster et al., 2007; Pham et al., 2022). Genetic variation, as the basis for plant breeding, made differences in the genetic makeup of individuals in a population and provided genetic material for selection (Tuhina-Khatun et al., 2015). Selected genotypes in the population are expected to have the desired traits in the direction of selection.

Genetic variation in agronomic traits is a crucial element in plant breeding programs aimed at expanding the gene pool of rice plants, and heritability ( $h^2_{bs}$ ) estimation is necessary for more efficient breeding program planning (Shrestha et al., 2021). The relationship between  $\sigma^2_g$  and  $\sigma^2_p$  showed that  $\sigma^2_p$  in all traits was slightly greater than  $\sigma^2_g$  (Table 1). For traits with high heritability, the difference among genotypes is caused more by genetic factors than environmental factors (Hidayatullah et al., 2018). Therefore, high heritability values could lead to effective selection, whereas low heritability values may imply less response to selection (Akbar et al., 2021; Shrestha et al., 2021). The results showed that the observed traits had high heritability with  $h^2_{bs}$  values ranging from 0.59 to 0.97, which would make selection quite effective to be carried out in the population (Table 1). Alsabah et al. (2019) also found high heritability values in growth traits, yield components, and productivity of DH black rice lines.

The GCV obtained in this study was classified as low to medium. GCV value  $< 0.10$  is low,  $0.10$  to  $0.20$  is medium, and  $> 0.20$  is high (Kumar et al., 2018). The GCV value shows the level of diversity between individuals in a population. In this study, NVT, PH, NFG, TG, and productivity were the characters with a relatively high GCV ( $0.10$  to  $0.18$ ). Estimation of heritability combined with the coefficient of GCV could be better in estimating the genetic influence than just the heritability value alone (Akbar et al., 2021). The traits with a relatively high GCV value indicated an opportunity to improve the traits through selection (Abebe et al.,

Table 1. Mean squares, variance components, and heritability of several traits of DH rice lines and check varieties

Traits	MS	CV (%)	$\sigma^2_g$	$\sigma^2_e$	$\sigma^2_p$	$h^2_{bs}$	GCV
NVT	23.71**	10.84	6.40	4.50	7.90	0.81	0.129
NPT	11.45**	13.80	2.23	4.75	3.82	0.59	0.095
PH	519.65**	3.53	167.35	17.59	173.22	0.97	0.109
DF	99.98**	3.50	30.05	9.82	33.33	0.90	0.061
DTH	52.92**	2.02	15.65	5.96	17.64	0.89	0.033
PL	7.94**	4.28	2.15	1.49	2.65	0.81	0.051
NFG	2,043.83**	16.27	490.38	572.70	681.28	0.72	0.151
TG	5,645.45**	12.63	1,629.37	757.33	1,881.82	0.87	0.185
Productivity	1.56**	14.37	0.32	0.60	0.52	0.62	0.105

Note: NVT = Number of vegetative tillers, NPT = Number of productive tillers, PH = Plant height, DF = Days to 50% flowering, DTH = Days to harvesting, PL = Panicle length, NFG = Number of filled grains, TG = Total grains per panicle, MS = Mean square of genotype, CV = Coefficient of variation,  $\sigma^2_g$  = Genetic variance,  $\sigma^2_e$  = Environmental variance,  $\sigma^2_p$  = Phenotypic variance,  $h^2_{bs}$  = Broad sense heritability, GCV = Genetic coefficient of variation, \*\*significant at  $\alpha = 1\%$

2017). The breeding conducted by Rohaeni et al. (2023) also used high heritability estimates and moderate GCV values to determine strategies for selecting rice lines.

### Agronomic performance

Agronomic traits are presented in Table 2. The NVT ranged from 13.4 to 26.6 tillers. The mean NVT of the observed lines was 19.4 tillers, while the mean NVT of the check varieties was 21.8 tillers. The NVT affected the NPT, where a higher NVT leads to a higher NPT. The NPT ranged from 11.1 to 20.1 tillers. The NPT is classified into several groups consisting of very low (< 5 tillers), low (5 to 9 tillers), medium (10 to 19 tillers), good (20 to 25 tillers), and very high (> 25 tillers) (International Rice Research

Institute, 2002). The observed DH rice lines were classified as having a medium number of tillers from 11.1 to 19.9. Tiller formation is also affected by air temperature, with temperatures below 16 °C and above 38 °C inhibiting or causing abnormalities in rice tillers (Yan et al., 2023). The research location has an average temperature of 27 °C during the study, which is considered suitable for tiller formation.

The average PH of the observed lines was 118.97 cm, while the mean PH of the check varieties was 115.22 cm (Table 2). According to the International Rice Research Institute (2002), PH is categorized as short (< 110 cm), medium (110 to 130 cm), and tall (> 130 cm). In this study, 15 lines and 1 check variety were classified as

Table 2. Means of plant growth traits of DH rice lines and check varieties

Genotype	NVT (tillers)	NPT (tillers)	PH (cm)	Genotype	NVT (tillers)	NPT (tillers)	PH (cm)
GN01	19.5 <sup>d</sup>	15.4	115.27 <sup>A</sup>	GN44	17.5 <sup>d</sup>	14.8	152.13 <sup>ABCDE</sup>
GN02	17.7 <sup>d</sup>	15.3	108.37 <sup>Ac</sup>	GN46	20.7 <sup>d</sup>	14.6	107.03 <sup>Abcd</sup>
GN04	22.9	17.7	126.09 <sup>A</sup>	GN50	21.6	16.1	130.43 <sup>ABE</sup>
GN06	16.9 <sup>cde</sup>	15.4	120.10 <sup>A</sup>	GN52	21.1	18.4	111.27 <sup>Ac</sup>
GN08	18.9 <sup>d</sup>	14.9	133.40 <sup>ABDE</sup>	GN56	22.1	17.0	120.43 <sup>A</sup>
GN10	20.5 <sup>d</sup>	17.6	130.20 <sup>ABE</sup>	GN57	15.3 <sup>cde</sup>	12.9 <sup>d</sup>	125.77 <sup>A</sup>
GN12	17.8 <sup>d</sup>	14.2 <sup>d</sup>	116.37 <sup>A</sup>	GN58	17.1 <sup>cde</sup>	14.5	125.37 <sup>A</sup>
GN14	20.8 <sup>d</sup>	14.8	117.57 <sup>A</sup>	GN59	17.5 <sup>d</sup>	14.7	131.73 <sup>ABDE</sup>
GN17	21.4	18.3	96.08 <sup>bcde</sup>	GN60	23.5	17.8	123.93 <sup>A</sup>
GN18	22.8	17.9	112.37 <sup>Ac</sup>	GN61	22.9	15.7	112.23 <sup>Ac</sup>
GN20	17.9 <sup>d</sup>	14.5	113.00 <sup>Ac</sup>	GN63	18.9 <sup>d</sup>	15.1	132.80 <sup>ABDE</sup>
GN21	20.8 <sup>d</sup>	16.6	103.02 <sup>bcde</sup>	GN64	21.4	15.9	109.03 <sup>Ac</sup>
GN22	14.5 <sup>cde</sup>	12.7 <sup>d</sup>	103.90 <sup>bcde</sup>	GN65	16.1 <sup>cde</sup>	13.7 <sup>d</sup>	127.13 <sup>A</sup>
GN23	19.9 <sup>d</sup>	14.3 <sup>d</sup>	97.08 <sup>bcde</sup>	GN67	22.7	18.5	105.53 <sup>bcde</sup>
GN24	20.9 <sup>d</sup>	16.5	103.78 <sup>bcde</sup>	GN69	20.6 <sup>d</sup>	16.8	107.03 <sup>Abcd</sup>
GN25	17.1 <sup>cde</sup>	15.3	139.57 <sup>ABCDE</sup>	GN70	22.1	17.1	97.97 <sup>bcde</sup>
GN26	21.5	14.6	101.27 <sup>bcde</sup>	GN73	21.9	16.0	131.87 <sup>ABDE</sup>
GN27	16.1 <sup>cde</sup>	13.5 <sup>d</sup>	115.20 <sup>A</sup>	GN74	22.2	15.9	136.55 <sup>ABCDE</sup>
GN28	19.5 <sup>d</sup>	15.7	108.57 <sup>Ac</sup>	GN78	21.6	18.6	114.67 <sup>A</sup>
GN29	19.5 <sup>d</sup>	15.3	106.67 <sup>bcd</sup>	GN79	21.1	17.0	123.20 <sup>A</sup>
GN30	17.1 <sup>cde</sup>	15.0	108.33 <sup>Ac</sup>	GN83	21.7	19.1	123.65 <sup>A</sup>
GN31	20.9 <sup>d</sup>	14.4	110.47 <sup>Ac</sup>	GN85	21.9	19.9	112.75 <sup>Ac</sup>
GN32	19.8 <sup>d</sup>	16.5	111.90 <sup>Ac</sup>	GN87	21.3	18.2	111.23 <sup>Ac</sup>
GN33	13.4 <sup>cde</sup>	11.1 <sup>acd</sup>	137.40 <sup>ABCDE</sup>	GN88	18.3 <sup>d</sup>	15.6	111.93 <sup>Ac</sup>
GN34	13.7 <sup>cde</sup>	12.2 <sup>d</sup>	133.20 <sup>ABDE</sup>	GN90	21.7	18.6	120.27 <sup>A</sup>
GN35b	14.4 <sup>cde</sup>	13.0 <sup>d</sup>	138.67 <sup>ABCDE</sup>	Inpari 18	17.9 <sup>d</sup>	17.3	95.65 <sup>bcde</sup>
GN36	14.7 <sup>cde</sup>	12.8 <sup>d</sup>	135.33 <sup>ABDE</sup>	Inpari 30	18.6 <sup>d</sup>	14.3 <sup>d</sup>	118.62 <sup>A</sup>
GN37	15.9 <sup>cde</sup>	13.3 <sup>d</sup>	133.53 <sup>ABDE</sup>	Inpari 34	23.0	17.0	125.40 <sup>A</sup>
GN39	21.1	17.8	119.43 <sup>A</sup>	Inpari 35	26.6 <sup>AB</sup>	20.1 <sup>B</sup>	119.44 <sup>A</sup>
GN41	17.5 <sup>d</sup>	13.9 <sup>d</sup>	107.43 <sup>Abcd</sup>	Ciherang	22.9	16.2	117.00 <sup>A</sup>
GN43	17.0 <sup>cde</sup>	15.5	152.80 <sup>ABCDE</sup>				

Note: NVT = The number of vegetative tillers, NPT = The number of productive tillers, and PH = Plant height. A/a = Significantly higher/lower than Inpari 18, B/b = Significantly higher/lower than Inpari 30, C/c = Significantly higher/lower than Inpari 34, D/d = Significantly higher/lower than Inpari 35, E/e = Significantly higher/lower than Ciherang

short, 24 DH rice lines and 4 check varieties were classified as medium, and 15 DH rice lines were classified as tall (Table 2). However, farmers preferred to plant short to medium-tall rice varieties because excessively tall rice plants would be prone to lodging (Khan et al., 2015; Wang et al., 2024).

### Plant age

Variety development is also widely directed to obtain early maturing varieties (Fatimah et al., 2014). Unpredictable climate change can lead to yield loss. Early maturing varieties can reduce the impact of climate change and shorten breeding cycles, promoting food availability and security for a rapidly growing population (Kim et al., 2024). In this study, there were 18 DH rice lines with shorter days

to flowering than the check varieties, while the DTH ranged from 105 to 128.7 days after sowing (DAS). Based on the International Rice Research Institute (2002) classification, the crop age is classified into very early ( $\leq 110$  DAS), early (111 to 115 DAS), medium (116 to 125 DAS), and long (126 to 150 DAS). DTH of rice genotypes showed 1 very early maturing check variety, 2 early maturing lines, 48 medium maturing lines, 3 medium maturing check varieties, 6 long maturing lines, and 1 long maturing check variety. Some of the lines had significantly earlier DTH than the check varieties. Two DH rice lines were earlier than Inpari 30 (122 DAS), 25 lines were earlier than Inpari 35 (126.7 DAS), and 12 lines were earlier than Ciherang (124 DAS) (Table 3).

Table 3. Means of DF and DTH of DH rice lines and check varieties

Genotype	DF (DAS)	DTH (DAS)	Genotype	DF (DAS)	DTH (DAS)
GN01	90.3 <sup>A</sup>	118.0 <sup>Ad</sup>	GN44	97.0 <sup>AC</sup>	126.0 <sup>AC</sup>
GN02	78.7 <sup>bde</sup>	117.3 <sup>Ade</sup>	GN46	88.0 <sup>A</sup>	117.7 <sup>Ad</sup>
GN04	86.3 <sup>A</sup>	119.3 <sup>Ad</sup>	GN50	94.7 <sup>AC</sup>	123.3 <sup>AC</sup>
GN06	89.0 <sup>A</sup>	124.0 <sup>AC</sup>	GN52	82.0 <sup>b</sup>	117.0 <sup>Ade</sup>
GN08	94.7 <sup>AC</sup>	124.7 <sup>AC</sup>	GN56	91.3 <sup>A</sup>	121.0 <sup>A</sup>
GN10	99.3 <sup>ACDE</sup>	128.7 <sup>ABC</sup>	GN57	87.0 <sup>A</sup>	118.3 <sup>Ad</sup>
GN12	89.7 <sup>A</sup>	121.0 <sup>A</sup>	GN58	87.7 <sup>A</sup>	119.7 <sup>Ad</sup>
GN14	91.7 <sup>A</sup>	121.0 <sup>A</sup>	GN59	84.0	118.0 <sup>Ad</sup>
GN17	83.0 <sup>b</sup>	116.7 <sup>Ade</sup>	GN60	94.3 <sup>AC</sup>	121.0 <sup>A</sup>
GN18	92.3 <sup>A</sup>	125.3 <sup>AC</sup>	GN61	100.7 <sup>ABCDE</sup>	126.7 <sup>AC</sup>
GN20	84.0	117.3 <sup>Ade</sup>	GN63	105.7 <sup>ABCDE</sup>	128.3 <sup>AC</sup>
GN21	86.7 <sup>A</sup>	119.0 <sup>Ad</sup>	GN64	90.7 <sup>A</sup>	117.3 <sup>Ade</sup>
GN22	84.0	123.0 <sup>AC</sup>	GN65	85.7	119.7 <sup>Ad</sup>
GN23	84.0	120.7 <sup>A</sup>	GN67	92.0 <sup>A</sup>	120.0 <sup>Ad</sup>
GN24	88.0 <sup>A</sup>	123.0 <sup>AC</sup>	GN69	87.7 <sup>A</sup>	120.3 <sup>A</sup>
GN25	91.3 <sup>A</sup>	123.7 <sup>AC</sup>	GN70	83.3 <sup>b</sup>	118.3 <sup>Ad</sup>
GN26	85.7	119.3 <sup>Ad</sup>	GN73	94.0 <sup>AC</sup>	122.3 <sup>A</sup>
GN27	90.3 <sup>A</sup>	121.7 <sup>A</sup>	GN74	103.0 <sup>ABCDE</sup>	125.3 <sup>AC</sup>
GN28	83.0 <sup>b</sup>	117.0 <sup>Ade</sup>	GN78	96.0 <sup>AC</sup>	124.0 <sup>AC</sup>
GN29	82.3 <sup>b</sup>	116.7 <sup>Ade</sup>	GN79	92.3 <sup>A</sup>	124.3 <sup>AC</sup>
GN30	87.3 <sup>A</sup>	125.3 <sup>AC</sup>	GN83	93.7 <sup>AC</sup>	124.0 <sup>AC</sup>
GN31	88.0 <sup>A</sup>	126.3 <sup>AC</sup>	GN85	89.7 <sup>A</sup>	125.0 <sup>AC</sup>
GN32	95.3 <sup>AC</sup>	125.0 <sup>AC</sup>	GN87	95.3 <sup>AC</sup>	121.0 <sup>A</sup>
GN33	82.7 <sup>b</sup>	116.3 <sup>Ade</sup>	GN88	89.3 <sup>A</sup>	119.0 <sup>Ad</sup>
GN34	82.7 <sup>b</sup>	114.7 <sup>Abde</sup>	GN90	99.0 <sup>ACDE</sup>	126.0 <sup>AC</sup>
GN35b	80.3 <sup>bde</sup>	112.7 <sup>Abde</sup>	Inpari 18	77.7 <sup>bde</sup>	105.0 <sup>bde</sup>
GN36	87.0 <sup>A</sup>	116.0 <sup>Ade</sup>	Inpari 30	92.0 <sup>A</sup>	122.0 <sup>A</sup>
GN37	86.0 <sup>A</sup>	117.3 <sup>Ade</sup>	Inpari 34	85.0	116.3 <sup>Ade</sup>
GN39	85.3	118.0 <sup>Ad</sup>	Inpari 35	90.0 <sup>A</sup>	126.7 <sup>AC</sup>
GN41	89.7 <sup>A</sup>	120.7 <sup>A</sup>	Ciherang	89.7 <sup>A</sup>	124.0 <sup>AC</sup>
GN43	92.3 <sup>A</sup>	123.0 <sup>AC</sup>			

Note: DF = Days to 50% flowering, DTH = Days to harvesting, DAS = Days after sowing. A/a = Significantly higher/lower than Inpari 18, B/b = Significantly higher/lower than Inpari 30, C/c = Significantly higher/lower than Inpari 34, D/d = Significantly higher/lower than Inpari 35, E/e = Significantly higher/lower than Ciherang

### Yield components

The panicle is a crucial part of the plant for yield component characters. PL determines the number of grains that can be covered in one panicle (Agata et al., 2020). The PL is an important factor affecting productivity. The DH lines had an average PL of 28.69 cm, compared to 26.95 cm for the check varieties. PL is directly related to the number of grains, as longer panicles tend to have more grains. PL and number of grains are linked to the efficient allocation of nutrients in the panicle. Nutrient allocation and seed filling may become ineffective due to high sinks within a plant, leading to unfilled seeds (Das et al., 2018; Dongling et al., 2023).

The NFG ranged from 84 to 216 grains (Table 4). The mean of NFG of the DH lines was 150 grains, while the mean of the check varieties was 120 grains. The NFG of more than 150 grains is categorized as good. A total of 29 DH lines had more than 150 grains, while 5 check varieties had less than 150 grains. The number of grains varied greatly among genotypes. The proportion of  $\sigma^2_g$  in the character of the NFG and TG tends to be greater than the  $\sigma^2_e$  (Table 1), so the  $\sigma^2_p$  of the number of grains is considered to be influenced by genetic factors. The TG ranged from 145 to 318 grains. The DH rice lines had a higher TG compared to the check varieties. The mean of TG of the DH lines was 222 grains, while 167 grains were for the check varieties

Table 4. Means of yield components of DH rice lines and check varieties

Genotype	PL (cm)	NFG (grains)	TG (grains)	Genotype	PL (cm)	NFG (grains)	TG (grains)
GN01	28.33	110.0	175.8	GN44	29.86 <sup>AD</sup>	142.1	215.6
GN02	26.77	129.9	176.6	GN46	27.78	163.1 <sup>D</sup>	233.9 <sup>AD</sup>
GN04	32.20 <sup>ABDE</sup>	164.9 <sup>D</sup>	216.9	GN50	28.16	156.1 <sup>D</sup>	206.7
GN06	28.91	148.3 <sup>D</sup>	224.2 <sup>D</sup>	GN52	26.94	133.9	178.1
GN08	30.92 <sup>ABDE</sup>	150.7 <sup>D</sup>	209.5	GN56	29.36 <sup>AD</sup>	156.4 <sup>D</sup>	248.9 <sup>ACDE</sup>
GN10	30.93 <sup>ABDE</sup>	180.3 <sup>AD</sup>	246.8 <sup>AD</sup>	GN57	30.25 <sup>ADE</sup>	215.9 <sup>ABCDE</sup>	317.5 <sup>ABCDE</sup>
GN12	27.52	166.3 <sup>D</sup>	212.5	GN58	29.71 <sup>AD</sup>	204.3 <sup>ABCDE</sup>	308.9 <sup>ABCDE</sup>
GN14	28.64	119.5	169.6	GN59	30.28 <sup>ADE</sup>	173.1 <sup>D</sup>	292.5 <sup>ABCDE</sup>
GN17	26.15	101.5	152.6	GN60	27.53	168.6 <sup>D</sup>	226.3 <sup>D</sup>
GN18	27.61	111.6	167.7	GN61	27.09	162.7 <sup>D</sup>	244.7 <sup>AD</sup>
GN20	27.52	102.8	155.9	GN63	30.37 <sup>ADE</sup>	150.3 <sup>D</sup>	209.7
GN21	26.57	120.9	174.3	GN64	26.57	133.7	188.7
GN22	30.31 <sup>ADE</sup>	162.9 <sup>D</sup>	229.5 <sup>D</sup>	GN65	30.71 <sup>ADE</sup>	178.0 <sup>AD</sup>	257.3 <sup>ABCDE</sup>
GN23	27.56	157.1 <sup>D</sup>	266.2 <sup>ABCDE</sup>	GN67	25.18 <sup>c</sup>	120.1	176.3
GN24	27.53	146.9	238.4 <sup>AD</sup>	GN69	26.05	135.5	173.5
GN25	31.61 <sup>ABDE</sup>	187.4 <sup>AD</sup>	281.9 <sup>ABCDE</sup>	GN70	26.49	115.1	165.5
GN26	27.01	151.7 <sup>D</sup>	261.1 <sup>ABCDE</sup>	GN73	28.55	169.7 <sup>D</sup>	225.5 <sup>D</sup>
GN27	27.41	142.5	208.9	GN74	31.77 <sup>ABDE</sup>	143.3	229.3 <sup>D</sup>
GN28	28.58	137.3	213.3	GN78	28.21	118.5	159.7
GN29	28.69	146.9	221.1 <sup>D</sup>	GN79	29.30 <sup>AD</sup>	180.1 <sup>AD</sup>	253.1 <sup>ACDE</sup>
GN30	29.33 <sup>AD</sup>	150.7 <sup>D</sup>	223.7 <sup>D</sup>	GN83	29.47 <sup>AD</sup>	149.6 <sup>D</sup>	189
GN31	30.24 <sup>ADE</sup>	165.9 <sup>D</sup>	251.8 <sup>ACDE</sup>	GN85	28.89	122.3	177.7
GN32	30.00 <sup>ADE</sup>	172.7 <sup>D</sup>	247.1 <sup>ACD</sup>	GN87	27.99	115.6	174.2
GN33	27.58	162.9 <sup>D</sup>	278.1 <sup>ABCDE</sup>	GN88	27.69	148.6 <sup>D</sup>	186.4
GN34	28.87	155.3 <sup>D</sup>	278.1 <sup>ABCDE</sup>	GN90	31.00 <sup>ABDE</sup>	135.4	212.4
GN35b	28.91	129.0	243.3 <sup>AD</sup>	Inpari 18	25.81	109.9	161.1
GN36	29.75 <sup>AD</sup>	162.7 <sup>D</sup>	279.3 <sup>ABCDE</sup>	Inpari 30	27.50	135.4	180.5
GN37	28.67	190.9 <sup>AD</sup>	302.7 <sup>ABCDE</sup>	Inpari 34	28.99 <sup>D</sup>	129.2	174.1
GN39	28.45	120.0	186.7	Inpari 35	25.74 <sup>c</sup>	83.7	144.9
GN41	28.62	158.9 <sup>D</sup>	263.3 <sup>ABCDE</sup>	Ciherang	26.73	139.8	176.1
GN43	30.25 <sup>ADE</sup>	173.7 <sup>AD</sup>	239.6 <sup>AD</sup>				

Note: NFG = Number of filled grains, TG = Total grains per panicle, and PL = Panicle length. A/a = Significantly higher/lower than Inpari 18, B/b = Significantly higher/lower than Inpari 30, C/c = Significantly higher/lower than Inpari 34, D/d = Significantly higher/lower than Inpari 35, E/e = Significantly higher/lower than Ciherang

(Table 4). Generally, TG of DH rice lines was higher than the check varieties.

### Yield

Productivity is a key character in yield trials. The productivity of the DH lines ranged from 4.02 to 7.57 tons ha<sup>-1</sup>, and the productivity of check varieties ranged from 4.03 to 5.29 tons ha<sup>-1</sup>. The means of productivity of the DH rice lines (5.48 tons ha<sup>-1</sup>) was higher than that of the check varieties (4.70 tons ha<sup>-1</sup>). The check variety with the highest productivity is Inpari 30 (5.29 tons ha<sup>-1</sup>), followed by Ciherang (5.10 tons ha<sup>-1</sup>). One DH rice line (GN60) had significantly higher productivity than Inpari 30, while 2 DH rice lines (GN60 and GN73) had significantly higher productivity than Ciherang (Table 5). Generally, the productivity of observed lines tends to be higher than check varieties. Direct selection based on productivity should be avoided because productivity is a polygenic trait and highly influenced by the environment, thus causing a complex chain of relationships between characters.

### Index selection

The results of the weighted index selection are presented in Table 6. Thirty DH rice lines were selected because of their positive index

selection value. Selection is important in plant breeding programs. The index selection was one of the selection methods that uses several traits and combines all information into an index as the basis of the selection process (Falconer, 1989). In this study, the selection traits as selection criteria were determined based on the breeding direction. The proposed breeding direction was to obtain high-yielding lines as the main character with many productive tillers, medium PH, and early maturity. The NPT is an important factor in determining the success of plant breeding programs (Oladosu et al., 2018). Short to medium PH is crucial to consider in the selection process. Reduced PH and reduced maturity duration can increase yield because the assimilates used for increasing PH and maturity duration can be transferred to grains (Sao et al., 2022). Therefore, the traits selected as selection criteria should have high heritability values to increase the effectiveness of selection. The selection criteria suitable for this study are yield, NPT, PH, and DTH. Those selected traits had high heritability values with a range of 0.59 to 0.97 (Table 1). The weighting used to construct the selection index model was a modification of Hadiananto et al. (2023a), i.e., PH (-1), NPT (+1), DTH (-1), and productivity (+5). Productivity was given the

Table 5. Means of yield of DH rice lines and check varieties

Genotype	Yield (tons ha <sup>-1</sup> )	Genotype	Yield (tons ha <sup>-1</sup> )	Genotype	Yield (tons ha <sup>-1</sup> )
GN01	5.81	GN31	5.95	GN64	5.18
GN02	4.83	GN32	5.44	GN65	5.57
GN04	6.19 <sup>A</sup>	GN33	4.98	GN67	4.58
GN06	5.23	GN34	5.52	GN69	5.37
GN08	5.57	GN35b	5.23	GN70	5.05
GN10	6.31 <sup>A</sup>	GN36	6.45 <sup>AC</sup>	GN73	7.26 <sup>ACDE</sup>
GN12	5.87	GN37	5.79	GN74	6.36 <sup>AC</sup>
GN14	6.32 <sup>A</sup>	GN39	6.29 <sup>A</sup>	GN78	4.02
GN17	4.72	GN41	5.02	GN79	5.83
GN18	5.87	GN43	5.93	GN83	5.91
GN20	4.65	GN44	5.41	GN85	5.34
GN21	5.13	GN46	6.71 <sup>AC</sup>	GN87	4.85
GN22	4.35	GN50	6.32 <sup>A</sup>	GN88	4.95
GN23	4.61	GN52	5.70	GN90	5.62
GN24	5.00	GN56	5.66	Inpari 18	4.03
GN25	5.18	GN57	5.63	Inpari 30	5.29
GN26	4.98	GN58	5.93	Inpari 34	4.27
GN27	5.28	GN59	4.67	Inpari 35	4.80
GN28	4.67	GN60	7.57 <sup>ABCDE</sup>	Ciherang	5.10
GN29	4.95	GN61	5.43		
GN30	5.03	GN63	4.59		

Note: A/a = Significantly higher/lower than Inpari 18, B/b = Significantly higher/lower than Inpari 30, C/c = Significantly higher/lower than Inpari 34, D/d = Significantly higher/lower than Inpari 35, E/e = Significantly higher/lower than Ciherang



highest and positive weight because productivity was the main selection trait in the breeding program, in which high-yielding genotypes are desired. PH and DTH were given negative weighting because the direction of selection was to obtain lines with medium PH and early maturity.

Thirty lines with the highest index values will be continued for advanced yield trials in several different environments to study the effect

of genetic, environmental, and G×E interactions. There was an improvement in the means of the selected traits in those DH lines (Table 6). The means of NPT of the lines before selection was 15.7 tillers, then after selection, it increased to 16.2 tillers and was almost close to the average number of tillers of the check varieties (17.0 tillers). The mean productivity of the selected lines increased by about 8% from 5.48 to 5.92 tons ha<sup>-1</sup>, and also higher than the means of the check

Table 6. Weighted selection index and means of the selection traits of selected lines and five check varieties

Rank	Genotype	Weighted index	Productivity (tons ha <sup>-1</sup> )	PH (cm)	NPT (tillers)	DTH (DAS)
1	GN60	10.65	7.57	123.93	17.8	121.0
2	GN73	8.37	7.26	131.87	16.0	122.3
3	GN46	8.14	6.71	107.03	14.6	117.7
4	GN39	5.82	6.29	119.43	17.8	118.0
5	GN14	4.91	6.32	117.57	14.8	121.0
6	GN36	4.44	6.45	135.33	12.8	116.0
7	GN04	4.14	6.19	126.09	17.7	119.3
8	GN52	4.07	5.70	111.27	18.4	117.0
9	GN50	3.45	6.32	130.43	16.1	123.3
10	GN01	2.98	5.81	115.27	15.4	118.0
11	GN18	2.97	5.87	112.37	17.9	125.3
12	GN83	2.67	5.91	123.65	19.1	124.0
13	GN10	2.57	6.31	130.20	17.6	128.7
14	GN74	2.50	6.36	136.55	15.9	125.3
15	GN12	1.98	5.87	116.37	14.2	121.0
16	GN58	1.98	5.93	125.37	14.5	119.7
17	GN31	1.73	5.95	110.47	14.4	126.3
18	GN79	1.32	5.83	123.20	17.0	124.3
19	GN56	1.25	5.66	120.43	17.0	121.0
20	GN69	1.01	5.37	107.03	16.8	120.3
21	GN37	0.85	5.79	133.53	13.3	117.3
22	GN90	0.41	5.62	120.27	18.6	126.0
23	GN34	0.38	5.52	133.20	12.2	114.7
24	GN70	0.35	5.05	97.97	17.1	118.3
25	GN21	0.32	5.13	103.02	16.6	119.0
26	GN65	0.30	5.57	127.13	13.7	119.7
27	GN57	0.16	5.63	125.77	12.9	118.3
28	GN85	0.05	5.34	112.75	19.9	125.0
29	GN17	-0.12	4.72	96.08	18.3	116.7
30	GN32	-0.28	5.44	111.90	16.5	125.0
31	Inpari 18	-1.16	4.03	95.65	17.3	105.0
32	Inpari 30	-1.34	5.29	118.62	14.3	122.0
33	Ciherang	-2.69	5.10	117.00	16.2	124.0
34	Inpari 35	-2.81	4.80	119.44	20.1	126.7
35	Inpari 34	-4.60	4.27	125.40	17.0	116.3
Means of all DH lines			5.48	118.70	15.7	121.0
Means of selected DH lines			5.92	119.52	16.2	121.0
Means of the check varieties			4.70	115.22	17.0	118.8

Note: PH = Plant height, NPT = Number of productive tillers, DTH = Days to harvesting, DAS = Days after sowing

varieties. The means of PH and DTH of the selected lines were almost close to the means of the check varieties with medium height and medium DTH. The selected DH rice lines have the potential to be further developed into new varieties. Improved agronomic characters and high yield of the selected lines are expected to align with farmers' preferences. The selected DH rice lines are the result of crossing superior parents with good agronomic traits, high yield, and tolerance to abiotic stress (Gunarsih, 2022). The selected DH rice lines have great potential to obtain high-yielding and multi-tolerant abiotic stress characters. The development of new varieties is directed to be able to face climate change, which causes abiotic stress and changes in pest and disease dynamics. The DH rice lines with multiple abiotic stress tolerance characters can utilize marginal lands, so they have the potential to have wide land adoption and be more affordable for farmers than increasing inputs on fertilizers and pesticides. Development of rice varieties also requires genotypes with wide adaptability, but selected DH rice lines have only been studied in one environment. Further trials in more diverse environments, both in optimum environments and environments with biotic or abiotic stress, are needed to study the effects of genotype, environment, and G×E interactions.

## CONCLUSIONS

Thirty DH rice lines with good agronomic traits were obtained based on the weighted selection index, with medium PH (an average of 119.52 cm), medium NPT (16.2 tillers), medium maturity (121 DAS), and productivity above the check varieties (5.92 tons ha<sup>-1</sup>). The selected DH lines need to be tested in an advanced yield trial under optimal conditions to study the effect of genetic, environmental, and G×E interactions on yield. The future research direction is to test DH rice lines in abiotic stress environments and test resistance to major rice pests and diseases. Rice plants with multiple abiotic stress tolerance, high yield, and resistance to major pests and diseases can increase farmers' adoption rate.

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