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Agronomic performance and selection of green super rice doubled haploid lines from anther culture

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Abstract. *Nurhidayah S, Purwoko BS, Dewi IS, Suwarno WB, Lubis I. 2023. Agronomic performance and selection of green super rice doubled haploid lines from anther culture. Biodiversitas 24: 819-826.* Rice is the staple food of most Asian, including Indonesian people. Plant breeding plays an important role in producing high-yielding varieties of rice to meet national carbohydrate sufficiency. Green Super Rice (GSR) is an alternative for rice variety development that has the advantages of high yield, good quality, pest resistance, and efficient fertilizer use to support sustainable agriculture. This study aimed to evaluate the agronomic character of GSR doubled haploid (DH) lines derived from anther culture and determine the best GSR rice lines based on index selection. The treatment was 65 genotypes consisting of 60 DH of GSR lines from anther culture of 5 combinations of crosses and 5 check varieties. The study used a randomized complete block design with three replications. The results showed that most of the tested lines had similar agronomic performances to the check variety Inpari 42 Agritan GSR. Almost all agronomic characters of the GSR DH lines had high genetic variability. Most characters had a high category of broad-sense heritability except panicle length. Line selection using index selection for economically important agronomic characters yielded 27 GSR DH lines having high productivity, number of productive tillers per hill, number of total grains per panicle, and percentage of filled grains per panicle.

Keywords: Heritability, high-yielding varieties, selection index, sustainable agriculture

INTRODUCTION

Rice is consumed by more than half of the world's population (Zhao et al. 2020) and is used as a source of carbohydrates and protein (Wing et al. 2018; Ziegler 2018). Nearly 90% of rice is produced and consumed by the Asian population (Venmuhil et al. 2020). Fulfillment of the adequacy of carbohydrates will continue to increase as the population increases. Indonesia is a country with the fourth largest population in the world, around 270.2 million people with a population growth rate of 1.25% (BPS 2021a). Based on BPS data (2021b), Indonesia has still faced the challenge of rice imports in the last ten years with a fluctuating trend from 2.7 million tons in 2011, then decreased in 2020 to 356,286.3 tons, and increased to 407,741.4 tons in 2021.

The supply of rice stocks is an important issue for the Indonesian government and research institutions to meet the people’s need for rice. Various efforts have been made by plant breeders to continuously breed new high-yielding varieties. One of them is the use of germplasm and high-yielding varieties as parents in crossbreeding in plant breeding programs. Currently, a breakthrough has been made through the development of Green Super Rice (GSR). GSR has advantages, such as high yield and good quality at low input conditions (Huang et al. 2018), pest resistance, fertilizer efficiency, resource-saving, and environmentally friendly for agricultural sustainability (Li and Ali 2017; Jewel et al. 2018; Yu et al. 2020).

Rice genetic improvement can be achieved by developing and selecting pure lines based on the desired phenotype. Line development takes about 6-9 selfing cycles followed by 3-5 years of evaluation in the field (Tripathy 2018). One of the obstacles in conventional rice breeding is the time needed to obtain pure lines. Therefore, it is necessary to have the technology to produce rice lines in a shorter period of time. Among the method is the use of doubled haploid (DH) technology. According to Mishra and Rao (2016), DH can be obtained from chromosomal duplication or induction of haploid cells. Anshori et al. (2022) suggested that DH technology could accelerate the release of new varieties of rice. Several elite lines have been obtained from the results of DH development including 28 black rice lines with good agronomic characters (Alsabah et al. 2019), 15 DH rice lines that are tolerant to salinity stress (Anshori et al. 2022), 2 lines superior rice widely adaptable in all locations (Akbar et al. 2021a), 9 lines DH that have the best performance based on the character of grain yield, number of grain content per panicle, percentage of grain content, and panicle length

(Chitanda et al. 2022), rice being injected with genes for resistance to blast and bacterial leaf blight (Chauhan et al. 2021), 4 varieties of hybrid rice (Sharma et al. 2021), and 129 DH plants from biparental crosses (Tripathy 2022).

Information on genetic variance and phenotypic variance as well as heritability, genetic and phenotypic correlation, and prediction of selection response are important steps as a selection consideration in plant breeding programs. Each parameter measured can be important information to be used for the selection index using several traits simultaneously (Smiderle et al. 2019). The use of selection index in rice has been reported by Venmuhil et al. (2020). The selection index on grain quality and selection index on upland rice by Smiderle et al. (2019), and combining selection index with genomic estimated breeding values (GEBV) by Chung and Liao (2022).

The first GSR variety in Indonesia was released namely the Inpari 42 Agritan GSR. It was obtained from the selection of GSR lines from IRRI (International Rice Research Institute) in 2016. The variety has an average productivity of 7.1 tons ha^{-1} and a potential yield of 10.58 tons ha^{-1}. Currently, we have 60 DH lines of GSR that need to be evaluated. This study aimed to evaluate the agronomic characteristics of GSR DH lines derived from anther culture and to select the best GSR rice lines based on index selection.

MATERIALS AND METHODS

Study area

The research was carried out from May to August 2022. The research location was at the experimental research station of Sawah Baru, Babakan, Dramaga, Bogor, Indonesia, at an altitude of 192 m above sea level at coordinates 6º33'50"S, 106º44'09"E.

Plant materials

The research materials used were 65 genotypes consising of 60 GSR DH lines from anther culture of 5 F_1 populations, and 5 check varieties (Inpari 42 Agritan GSR, Inpari 46, Inpari Nutri Zinc, B-22-1, and Bionil6-3). The check varieties were also performed as parental materials for the crossing. Urea, SP-36, and KCl fertilizers were applied at 200 kg ha^{-1}, 150 kg ha^{-1}, and 100 kg ha^{-1}, respectively. The genotypes used included SN1-SN2 from the F_1 of Inpari 42 Agritan GSR × B22-1, SN3-SN8 from the F_1 of B-22-1 × Inpari 42 GSR, SN9-SN21 from the F_1 of Inpari 42 Agritan GSR × Inpari 46 GSR, SN22-SN56 from the F1 of Inpari 42 Agritan GSR × Inpari IR Nutri Zinc, SN57-SN60 from the F_1 of Inpari42 Agritan GSR × Bionil 6-3, SN61: Inpari 42 Agritan GSR, SN62: B-22-1, SN63: Inpari 46 GSR, SN64: Inpari IR Nutri Zinc, and SN65: Bionil 6-3. The plant materials were obtained from previous collaboration research between IPB University and ICABIOGRAD (Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development), Indonesia.

Procedures

The study used a randomized complete block design. The treatment was 65 genotypes, as previously mentioned. Each treatment was replicated 3 times. Each experimental unit was a plot of 1 x 3.75 m. The seedlings were planted 21 days after sowing (DAS) with a spacing of 25 x 25 cm. Observations of agronomic characters included plant height (cm), flag leaf length (cm), number of productive tillers (tillers per hill), days to flowering (DAS), days to harvest (DAS), panicle length (cm), the weight of 1000 grains (g), number of total grains per panicle, percentage of filled grain per panicle (%), dry grain at harvest (ton ha^{-1}), and productivity (ton ha^{-1}).

Data analysis

The data were tested for normality using Shapiro-Wilk α= 5%. The data having normal distribution were then analyzed for analysis of variance (ANOVA) at α= 5%. If there is a significant effect of the treatment, then a further Dunnett's t-test at α= 5% is carried out using SAS software. If the data has a non-normal distribution, then the log transformation was applied prior to ANOVA. The linear model used follows the formula of Mattjik and Sumertajaya (2013) as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where:
Y_{ij} : observation at genotype i, block j
μ : general average
α_i : effect of genotype i, where i =1,2,3,..,65
β_j : effect of block j, where j=1,2,3
ε_{ij} : experimental error on genotype i, block j

Heritability and index selection were analyzed using Microsoft Excel. The calculation of broad-sense heritability (h^2_{bs}) was estimated for each trait following Hidayatullah et al. (2018) with the formula:

$$h^2bs = \frac{\sigma 2g}{\sigma 2p}$$

Where:
h^2bs : broad-sense heritability
σ^2g : genetic variance
σ^2p : phenotypic variance

Heritability is categorized into 3 classes according to Elrod and Stansfield (2010) as follows: Low = $h^2bs < 20\%$; Moderate = $20\% < h^2bs \leq 50\%$; High = $h^2bs > 50\%$.

The selection used several important agronomic characteristics related to the properties of yield and yield components of rice plants simultaneously with the consideration of broad-sense heritability values in the high category. The economically important agronomic character of rice refers to Kumar et al. (2014), Oladosu et al. (2018), Alsabah et al. (2019), and Sreedhar and Reddy (2019). The selected target characters, i.e. productivity given a weight of +3, the number of productive tillers given a weight of +1, the percentage of filled grain given a weight of +1, and the number of total grains given a weight of +1 (modified from Hidayatullah et al. 2018). The selection index was determined according to Falconer and Mackay (1996) by the following formula:

$$SI = A_1Z_1 + A_2Z_2 + A_3Z_3 + ... + A_nZ_n$$

Where:
SI : selection index;
An : weight of the variable n;
Zn : the standardized phenotype value of Z

RESULTS AND DISCUSSION

Analysis of variance on agronomic characters of Green Super Rice (GSR) doubled haploid lines

All tested genotypes had a significant effect on all observed agronomic characters (Table 1). The coefficient of variance (CV) is in the range of 1.58-44.05%. Characters of plant height, flag leaf length, number of productive tillers, days to flowering, days to harvest, panicle length, number of total grains, percentage of filled grains, and weight of 1000 grains had a low CV <20% while the dry grain at harvest and productivity has CV > 20%. Gomez and Gomez (1984) stated that the magnitude of the CV value indicates the reliability of the trial. The results of the analysis of variance, which showed a significant effect on the agronomic character were then analyzed further by Dunnet's t-test at α=5% as presented in Table 2 and Table 3.

Performance of agronomic characters of GSR DH lines at the vegetative stage

The mean value of plant height of the tested genotypes was in the range of 71.55-112.11 cm, while the check varieties or the parents were in the range of 97.22-109.78 cm (Table 2). The analyzed data were compared with the Inpari 42 Agritan GSR which was used as the parent in all cross combinations. Forty genotypes had significantly shorter plants and one genotype (SN8) showed significantly higher plant than Inpari 42 Agritan GSR (102.67 cm), while the rest had similarities with Inpari 42 Agritan GSR with a range of 95.78-106 cm. Based on the SES for rice guideline (IRRI 2013), rice plants that have a height of <110 cm are classified in the semidwarf category. The SN8 line was an intermediate type with plant height >110 cm. According to Kawamura et al. (2020) and Wu et al. (2022), plant height is an important agronomic trait of rice that can directly affect yield. The short plant height is beneficial in preventing rice lodging, but if the plants are too short, it will lead to insufficient growth and ultimately affect the yield of rice.

A total of 3 genotypes (SN25, SN52, and SN53) had a flag leaf length ranging from 23.56-24.22 cm shorter than the Inpari 42 Agritan GSR which reached 31.67 cm. Meanwhile, 57 genotypes had no different flag leaves compared to the Inpari 42 Agritan GSR variety. Tang et al. (2018) stated that leaf size, in this case, the length of the flag leaf is closely related to the increase in rice leaf architecture and the photosynthetic capacity of rice.

Performance of agronomic characters of GSR DH lines at the generative stage

The productive tiller of the tested lines had a range of 13.89-26.11 tillers per hill, while the parents had a range of 13.67-17.34 tillers per hill (Table 3). Based on the SES for rice guideline (IRRI 2013), the tillering ability of rice is classified as very low (<5 tillers/plant), low (5-9 tillers/plant), medium (10-19 tillers/plant), high (20-25 tillers/plant), and very high (>25 tillers/plant). Almost all DH lines (59 lines) had the same number of productive tillers as Inpari 42 Agritan GSR variety which is grouped in the medium category. Only the SN41 line had significantly more productive tillers (26.11 tillers) than Inpari 42 Agritan GSR variety (16.89 tillers).

The tested lines showed significant differences in the character of days to flowering. A total of 18 genotypes flowered earlier (70.00-82.33 DAS) and one line (SN55) flowered longer (96.33 DAS) compared to Inpari 42 Agritan GSR (88 DAS). Therefore, a total of 40 DH lines had days to flower that was not significantly different from the Inpari 42 Agritan GSR. The average of the days to the flower of all genotypes is around 83.59 DAS (Table 3).

The days to harvest of the tested DH lines were not significantly different from that of the check varieties, including Inpari 42 Agritan GSR. The average days to harvest of the tested genotypes was 123.26 DAS. Only one DH line, i.e. the SN55 line had a longer day to harvest (135 DAS) than Inpari 42 Agritan GSR and the other check varieties.

The panicle length of the tested lines was in the range of 21.55-26.22 cm, while all the parents or check varieties were in the range of 22.56-25.06 cm (Table 3). None of the DH lines had longer or shorter panicles compared to the Inpari 42 Agritan GSR variety based on the t-Dunnett test. In several studies, panicle size is associated with an increase in sink size and will affect rice yields (Huang et al. 2019), panicle size had the most consistent and closest positive correlation with grain yield (Laza et al. 2004). Panicle length plays an important role in grain yield (Sun et al. 2016) and panicle length is related to yield trait (Wang et al. 2019; Agata et al. 2020).

Table 1. Effects of genotypes on the agronomic characters of GSR doubled haploid lines from anther culture

Variables	Mean square genotype	Mean S.E.	Sig.	CV (%)
Plant height	251.89	8.62	*	3.21
Flag leaf length	22.47	6.57	*	8.68
Number of productive tillers	0.011	0.004	*	5.16^
Days to flowering	89.60	4.55	*	2.55
Days to harvest	12.30	4.16	*	1.65
Panicle length	2.34	1.41	*	5.05
Number total grains	0.011	0.004	*	5.19^
Percentage of filled grain	0.205	0.059	*	13.29^
Dry grain at harvest	3.12	1.11	*	22.64
Productivity	3.35	1.42	*	44.05
Weight of 1000 grains	0.003	0.001	*	1.58^

Note: *: significant at P < 0.05; ^ : data was transformed using log Y

Table 2. Means of plant height and flag leaf length of GSR DH lines from anther culture

Genotype	PH	FLL
SN1	89.00a	27.00
SN2	96.00	33.56
SN3	95.78	30.67
SN4	92.34a	28.22
SN5	93.33a	34.33
SN6	80.44a	31.33
SN7	85.11a	33.11
SN8	112.11a	31.22
SN9	97.89	32.56
SN10	100.00	28.56
SN11	100.22	29.22
SN12	99.33	29.22
SN13	94.56a	29.89
SN14	97.89	34.45
SN15	98.66	31.67
SN16	93.78a	33.89
SN17	92.67a	31.78
SN18	96.11	32.22
SN19	98.11	31.67
SN20	92.67a	30.22
SN21	92.78a	31.33
SN22	94.33a	28.44
SN23	91.22a	26.66
SN24	86.78a	25.89
SN25	87.56a	24.11a
SN26	82.00a	28.89
SN27	81.33a	33.00
SN28	95.78	30.00
SN29	106.00	30.00
SN30	101.11	26.56
SN31	87.56a	31.11
SN32	82.89a	29.33
SN33	80.22a	28.00
SN34	88.11a	31.11
SN35	85.22a	29.67
SN36	85.00a	30.56
SN37	86.22a	28.33
SN38	87.45a	28.44
SN39	83.78a	29.56
SN40	91.00a	25.33
SN41	82.33a	25.22
SN42	83.55a	25.00
SN43	97.34	29.78
SN44	74.67a	31.11
SN45	91.22a	27.33
SN46	76.45a	31.22
SN47	71.55a	29.67
SN48	72.22a	34.22
SN49	78.56a	29.89
SN50	90.33a	27.55
SN51	80.55a	25.78
SN52	78.22a	24.22a
SN53	80.89a	23.56a
SN54	85.44a	27.11
SN55	95.67	35.11
SN56	101.00	27.11
SN57	102.44	28.11
SN58	102.11	29.55
SN59	99.11	29.78
SN60	98.33	28.00
SN61	102.67	31.67
SN62	109.78	32.33
SN63	100.33	30.56
SN64	97.22	25.89
SN65	107.22	27.67
Mean	91.41	29.52

Note: PH: plant height (cm), FLL: flag leaf length (cm); Numbers followed by the lowercase letters 'a' in the same trait were significantly smaller, and numbers followed by the capital letter 'A' in the same trait were significantly larger than SN61 (Inpari 42 Agritan GSR variety) based on the Dunnett's t-test at α=5%. *Check varieties: SN61: Inpari 42 Agritan GSR, SN62: B-22-1, SN63: Inpari 46 GSR, SN64: Inpari IR Nutri Zinc, and SN65: Bionil 6-3.

The total grain per panicle character in most of the DH lines was not significantly different from Inpari 42 Agritan GSR (Table 4). Only 7 DH lines (SN6, SN26, SN41, SN42, SN48, SN52, and SN54) significantly had fewer total grains per panicle than Inpari 42 Agritan GSR. Sadimantara et al. (2018) reported that the amount of grain per panicle would affect rice productivity and was greatly influenced by genetic and environmental factors.

The percentage of filled grain in the tested lines ranged from 4.09-92.58%, while the check variety ranged from 64.79-91.46% (Table 4). A total of 27% DH lines had a percentage of filled grain <75%, while the rest of those lines had a percentage of filled grain >75%. The SN47 and SN48 lines had a significantly lower percentage of filled grain than Inpari 42 Agritan GSR. According to Kim et al. (2021), the percentage of filled grain might be affected by solar radiation during the grain ripening process. Meanwhile, Sadimantara et al. (2018) stated that the grain yield was determined by the number of panicles per unit area, the fertility of the grains, the number of grains per panicle, and the weight of the grain. Each growth stage contributes to the rice yield.

The weight of 1000 grains (WG) tested was in the range of 17.18-25.30 g, while the check variety was in the range of 22.00-25.87 g (Table 4). Thus they appeared to be similar to the check varieties. Two lines, i.e. SN46 and SN47 had WG significantly lower while two DH lines, namely SN59 and SN60 had WG significantly heavier than the Inpari 42 Agritan GSR. According to Kim et al. (2021), the weight of 1000 grains was affected by solar radiation. A lot of solar radiation during the ripening stage can increase the yield of rice.

The dry grain at harvest (DGH) of the tested lines ranged from 2.54-7.09 ton ha^{-1} , while the check variety ranged from 4.85 to 6.07 ton ha^{-1} (Table 4). Harvested dry grain in the tested lines appeared to have a wider distribution among the parents used. The SN48 line had significantly lower dry grain at harvest than Inpari 42 Agritan GSR. As many as 98% of the tested lines were not significantly different from the Inpari 42 Agritan GSR on the characteristics of dry grain at harvest.

In order to calculate the productivity of each genotype, the harvested grains were dried under the sun for 3-5 days until they reached a moisture content of about 14%. The tested lines have productivity in the range of 0.29-5.42 tons ha-1, while the check varieties ranged from 2.64 to 4.30 tons ha-1 (Table 4). All of the tested lines did not show significant differences compared to the check variety Inpari 42 Agritan GSR based on the t-Dunnet test.

Genetic variance, phenotypic variance, and broad-sense heritability

Breeders need to understand the genetic diversity in the population being tested. According to Suvi et al. (2020), genetic improvement for yield and other economically important traits in rice depends on the genetic diversity available within the plant species. Estimates that need to be considered include phenotype variance and genetic variance to estimate the selection gain (Guimarães et al. 2021). The values of genetic variance and phenotypic variance were used to calculate broad-sense heritability for all observed characters. A total of 10 characters had broad-sense heritability in the high category of 57.61-96.58%, except for the panicle length character, which had a broad-sense heritability of 37.50% (Table 5). This is in line with Nirmaladevi et al. (2015) in which the character of the weight of 1000 grains is classified as high-category broad-sense heritability. The genetic variance and heritability estimates presented herein, however, may be overestimated due to the genotype by environment variance if significant. Akbar et al. (2021b) reported that all of the measured characters had high category broad-sense heritability except for the number of productive tillers.

Selection of GSR DH lines using index selection

The selection index is determined based on the broad-sense heritability values in Table 5. The broad-sense heritability estimate of each character was in the high category. According to Hidayatullah et al. (2018), selection criteria of rice genotypes can be based on important agronomic characteristics, including days to harvesting, productivity, number of tillers, number of panicles, number of filled grains per panicle, percentage of filled grain per panicle, and 1000 grains weight. Four characters were selected and weighted in this study, i.e. the productivity character is weighted +3, the number of productive tillers is weighted +1, the total grain number is weighted +1, and the percentage of filled grain per panicle is weighted +1 (Table 6).

Table 3. Means of the number of tillers per hill, days to flowering, days to harvest, and panicle length of GSR DH lines from anther culture

No.	Genotype	NPT (tiller)	DTF (DAS)	DTH (DAS)	PL (cm)	No.	Genotype	NPT (tillers)	DTF (DAS)	DTH (DAS)	PL (cm)
1	SN1	15.78	88.00	121.33	23.44	35	SN35	15.78	83.67	119.67	23.83
2	SN2	14.22	87.67	124.00	23.67	36	SN36	18.11	81.00a	122.00	23.89
3	SN3	18.78	84.33	122.33	25.22	37	SN37	15.67	80.00a	122.33	22.56
4	SN4	18.67	86.00	121.67	24.11	38	SN38	14.11	82.33a	121.67	23.00
5	SN5	18.11	84.67	119.67	24.33	39	SN39	18.11	82.00a	122.00	23.78
6	SN6	12.89	75.33a	124.33	23.11	40	SN40	18.56	84.33	124.33	23.11
7	SN7	15.67	73.67a	124.33	22.22	41	SN41	26.11A	74.33a	124.67	22.56
8	SN8	14.11	82.67	120.33	24.00	42	SN42	20.67	73.00a	122.67	23.11
9	SN9	15.00	89.00	124.33	23.78	43	SN43	17.44	76.00a	122.33	22.17
10	SN10	15.11	87.00	122.67	23.34	44	SN44	23.11	78.33a	124.00	23.33
11	SN11	15.45	87.00	123.00	23.56	45	SN45	16.56	83.00	123.00	24.44
12	SN12	17.00	87.67	123.33	23.55	46	SN46	19.11	80.67a	123.33	22.33
13	SN13	21.56	87.33	125.33	23.11	47	SN47	21.22	74.33a	125.33	22.55
14	SN14	16.67	87.67	122.00	23.50	48	SN48	22.11	74.00a	124.67	22.67
15	SN15	17.44	86.67	121.67	23.44	49	SN49	16.22	74.67a	124.00	23.72
16	SN16	19.78	87.00	122.67	23.89	50	SN50	20.00	90.67	124.33	24.44
17	SN17	16.55	89.33	122.67	24.56	51	SN51	19.78	83.00	123.33	22.78
18	SN18	18.00	88.00	123.33	22.89	52	SN52	22.45	85.00	124.00	22.56
19	SN19	16.78	88.33	123.33	23.33	53	SN53	17.67	84.67	123.00	23.22
20	SN20	17.11	88.67	121.00	24.50	54	SN54	15.33	81.33a	122.33	21.55
21	SN21	17.33	87.67	123.00	23.33	55	SN55	18.22	96.33A	135.00A	25.37
22	SN22	17.45	85.67	124.33	23.78	56	SN56	16.00	90.00	125.33	23.00
23	SN23	18.22	85.67	119.33	23.11	57	SN57	16.00	85.00	122.67	22.67
24	SN24	18.11	83.67	123.33	22.78	58	SN58	15.44	87.67	122.33	24.78
25	SN25	17.56	85.00	123.00	23.89	59	SN59	13.89	87.67	123.00	23.33
26	SN26	19.56	70.00a	125.67	24.11	60	SN60	17.22	87.00	122.33	23.00
27	SN27	18.00	70.33a	124.67	26.22	***61**	**SN61**	**16.89**	**88.00**	**123.00**	**25.06**
28	SN28	16.78	85.33	125.33	22.78	*62	SN62	13.67	85.00	123.67	23.89
29	SN29	14.67	84.33	123.33	24.78	*63	SN63	17.34	86.67	123.33	23.78
30	SN30	14.11	87.33	122.00	25.22	*64	SN64	16.45	83.33	123.67	22.56
31	SN31	17.44	71.67a	125.00	23.56	*65	SN65	14.22	87.33	121.67	22.78
32	SN32	19.33	85.00	123.33	23.00	**Mean**		**17.41**	**83.59**	**123.26**	**23.49**
33	SN33	19.89	83.00	124.00	22.33	**Critical Value of Dunnett's t**					**3.23**
34	SN34	15.11	82.33a	123.33	22.78	**Min. Sig. different**		**7.08**	**5.42**	**5.40**	**3.15**

Note: NPT: number of productive tillers, DTF: days to flowering, DTH: days to harvest, PL: panicle length; Numbers followed by the lowercase letter 'a' were significantly lower, numbers followed by the capital letter 'A' are significantly higher than SN61 (Inpari 42 Agritan GSR variety); based on the results of Dunnett's t-test at α=5%.*Check varieties: SN61: Inpari 42 Agritan GSR, SN62: B-22-1, SN63: Inpari 46 GSR, SN64: Inpari IR Nutri Zinc, and SN65: Bionil 6-3

Table 4. Means of total grain number, percentage of filled grain, the weight of 1000 grains, dry grain at harvest, and productivity of GSR DH lines

No.	Genotype	NTG (gain)	PFG (%)	WG (g)	DGH (ton. ha^{-1})	PROD (ton. ha^{-1})	No.	Genotype	NTG (gain)	PFG (%)	WG (g)	DGH (ton. ha^{-1})	PROD (ton. ha^{-1})
1	SN1	160.89	91.55	23.76	4.48	2.55	35	SN35	143.22	87.12	24.38	5.04	2.61
2	SN2	195.00	84.33	22.64	5.86	3.83	36	SN36	148.89	62.20	22.27	4.53	2.24
3	SN3	179.89	85.47	21.17	5.44	3.14	37	SN37	132.44	90.61	23.29	4.19	2.28
4	SN4	158.78	75.09	21.74	3.92	1.85	38	SN38	144.78	66.12	22.77	4.97	3.17
5	SN5	199.11	86.64	21.53	5.54	3.65	39	SN39	146.22	91.41	23.36	4.65	2.70
6	SN6	126.67a	91.87	20.83	3.57	3.32	40	SN40	147.89	87.51	21.90	4.82	3.16
7	SN7	152.44	60.80	21.06	2.87	1.10	41	SN41	128.44a	88.96	21.60	3.13	1.44
8	SN8	164.67	91.18	23.74	4.97	2.55	42	SN42	121.00a	77.00	21.58	3.97	1.40
9	SN9	208.22	80.05	21.43	4.57	2.49	43	SN43	155.89	69.36	21.26	4.05	1.40
10	SN10	163.00	92.00	23.36	4.80	3.00	44	SN44	148.67	63.95	20.20	3.09	2.56
11	SN11	161.67	88.27	22.90	5.91	3.93	45	SN45	157.67	82.04	20.84	4.30	2.38
12	SN12	173.11	92.16	24.67	7.09	5.42	46	SN46	135.45	76.92	17.18a	2.85	0.94
13	SN13	154.11	82.83	22.55	4.81	2.75	47	SN47	143.11	11.58a	18.83a	2.81	0.37
14	SN14	173.66	89.34	22.88	7.32	4.86	48	SN48	126.67a	4.09a	19.79	2.54a	0.47
15	SN15	168.22	91.11	23.17	5.88	3.79	49	SN49	181.33	31.25	20.73	3.06	0.91
16	SN16	143.22	60.80	21.45	4.91	3.01	50	SN50	146.56	87.06	22.04	4.29	2.38
17	SN17	150.00	88.95	22.31	5.64	3.38	51	SN51	170.78	87.24	20.21	4.89	2.82
18	SN18	175.11	89.55	23.02	6.45	4.23	52	SN52	125.78a	90.80	22.88	4.66	3.07
19	SN19	145.11	84.99	21.47	5.37	2.96	53	SN53	135.55	82.82	21.09	5.00	2.71
20	SN20	160.89	82.56	22.60	6.04	3.84	54	SN54	114.89a	65.00	22.04	3.62	2.00
21	SN21	150.44	88.92	20.13	4.27	2.21	55	SN55	201.67	50.70	21.48	2.64	0.29
22	SN22	145.11	89.26	21.88	4.72	2.60	56	SN56	166.89	78.69	21.19	4.44	2.55
23	SN23	153.67	81.09	22.57	5.30	2.73	57	SN57	156.89	90.43	23.16	5.31	4.01
24	SN24	131.33	82.43	21.89	3.74	1.95	58	SN58	172.78	83.26	23.02	4.48	2.89
25	SN25	138.00	88.43	23.02	4.59	3.01	59	SN59	139.11	89.25	25.30A	4.87	3.70
26	SN26	120.89a	61.55	21.85	3.12	0.73	60	SN60	143.11	92.81	25.21A	5.47	3.93
27	SN27	170.11	58.02	20.89	4.42	3.02	***61**	**SN61**	**203.67**	**80.65**	**22.00**	**5.29**	**3.28**
28	SN28	151.89	66.76	22.28	5.55	3.47	*62	SN62	146.44	64.79	24.92a	5.29	3.38
29	SN29	168.89	70.48	23.36	4.87	2.83	*63	SN63	146.33	91.46	23.13	5.69	4.11
30	SN30	196.11	85.16	23.96	4.62	2.52	*64	SN64	162.00	80.63	21.97	4.85	2.64
31	SN31	157.22	63.59	21.47	4.02	1.41	*65	SN65	160.44	85.89	25.87A	6.07	4.30
32	SN32	159.67	91.70	22.40	4.46	2.73	**Mean**		**155.29**	**78.60**	**22.21**	**4.66**	**2.70**
33	SN33	140.22	79.69	22.22	4.65	2.49	**Critical value of Dunnett's t**				**3.23**		
34	SN34	141.78	92.58	22.21	4.22	2.25	**Min. sign. different**		**74.77**	**49.7**	**2.84**	**2.74**	**3.12**

Note: NTG: number of total grains, PFG: percentage of filled grains, WG: weight of 1000 grains, DGH: dry grain at harvest, PROD: productivity; Numbers followed by the lowercase letter 'a' were significantly lower, numbers followed by the capital letter 'A' are significantly higher than SN61 (Inpari 42 Agritan GSR variety); based on the results of Dunnett's t-test at α=5%.*Check varieties: SN61: Inpari 42 Agritan GSR, SN62: B-22-1, SN63: Inpari 46 GSR, SN64: Inpari IR Nutri Zinc, and SN65: Bionil 6-3

Table 5. Estimate of the genetic variance, phenotypic variance, and broad-sense heritability of the agronomic characters of GSR DH lines from anther culture

Variables	σ^2g	σ^2p	h^{2}bs (%)	Criteria of heritability
Plant height	81.090	83.960	96.58	High
Flag leaf length	5.300	7.490	70.76	High
Number of productive tillers	0.002	0.004	62.26	High
Days to flowering	28.35	29.870	94.92	High
Days to harvest	2.71	4.100	66.18	High
Panicle length	0.000	0.000	37.50	Moderate
Number total grains	0.002	0.004	62.39	High
Percentage of filled grains	0.049	0.068	71.26	High
Dry grain at harvest	0.67	1.040	64.42	High
Productivity	0.64	1.120	57.61	High
Weight of 1000 grains	0.001	0.001	81.48	High

Note: σ^2g: genetic variance, σ^2p: phenotypic variance; h^{2}bs: broad-sense heritability

Table 6. Selection index on 27 selected genotypes based on productivity, number of productive tillers per plant, number of total grains per panicle, and percentage of total grain per panicle GSR from anther culture

Genotype	PROD (ton.ha^{-1})	NPT	NTG	PFG (%)	Selection Index
SN2	3.83	14.22	195.00	84.33	4.33
SN3	3.14	18.78	179.89	85.47	3.45
SN5	3.65	18.11	199.11	86.64	5.67
SN9	2.49	15.00	208.22	80.05	1.13
SN10	3.00	15.11	163.00	92.00	1.12
SN11	3.93	15.45	161.67	88.27	3.69
SN12	5.42	17.00	173.11	92.16	9.44
SN13	2.75	21.56	154.11	82.83	1.99
SN14	4.86	16.67	173.66	89.34	7.54
SN15	3.79	17.44	168.22	91.11	4.54
SN17	3.38	16.55	150.00	88.95	1.97
SN18	4.23	18.00	175.11	89.55	6.31
SN19	2.96	16.78	145.11	84.99	0.37
SN20	3.84	17.11	160.89	82.56	3.71
SN25	3.01	17.56	138.00	88.43	0.68
SN27	3.02	18.00	170.11	58.02	0.72
SN28	3.47	16.78	151.89	66.76	1.14
SN32	2.73	19.33	159.67	91.70	1.81
SN39	2.70	18.11	146.22	91.41	0.57
SN40	3.16	18.56	147.89	87.51	1.95
SN44	2.56	23.11	148.67	63.95	0.68
SN51	2.82	19.78	170.78	87.24	2.55
SN52	3.07	22.45	125.78	90.80	2.33
SN57	4.01	16.00	156.89	90.43	4.02
SN58	2.89	15.44	172.78	83.26	0.92
SN59	3.70	13.89	139.11	89.25	1.33
SN60	3.93	17.22	143.11	92.81	3.74

Note: PROD: productivity, NPT: number of productive tillers per hill, NTG: number of total grains per panicle, PFG: percentage of filled grain per panicle

Based on the calculation of the simultaneous selection index, 27 GSR DH lines had a positive selection index for the four selected characters (Table 6). The average productivity based on the selection index ranged 2.49-5.42 ton ha^{-1}, the total number of tillers was 13.89-23.11 tillers per hill, the number of grains was 126-208 grains per panicle, and the percentage of filled grain was 58.02-92.81% with a positive selection index value in the range of 0.37 to 9.44. From the selected lines, it can be seen that they are represented by each cross combination used, namely FS1, FS2, FS5, FS6, and FS8. These selected lines will be further evaluated in several locations.

Kumar et al. (2014) reported that yield and yield components are needed for selection efficiency in developing varieties with high economic value. Alsabah et al. (2019) explained that the selection index was calculated using standard coefficients based on the nature of relative necessity, heritability estimates, and the correlation between genetics and phenotypes with different traits. Oladasu et al. (2018) reported that selection for increasing grain yield could be efficient if it is based on grain weight per hill and the number of tillers per hill because it contributes directly to grain yield. Sreedhar and Reddy (2019) stated that the number of productive tillers per m^2 showed a positive correlation with yield. Htwe et al. (2020) reported that the selection index based on the combination of the number of effective tillers per hill, the number of spikelets per panicle, filled grain percentage, and seed yield per plant has the highest genetic advance and relative efficiency.

In conclusion, almost all the agronomic characters of the GSR DH lines have high genetic variability. Most characters showed a high category of broad-sense heritability. Line selection using index selection yielded 27 GSR DH lines having high productivity characters, number of productive tillers per hill, number of total grains per panicle, and percentage of filled grains per panicle.

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