



Study of Black Rice Parents Performance and the Crossing Ability

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Abstract

Black rice generally has problems in cultivation such as relatively long plant life, high habitus and low productivity. Plant breeders use several methods in hybridization activities, such as backcross method that can lead to the development towards emphasizing the superiority of each parent. This research aimed to study and determined the success rate of the black rice lines F1 backcross and the parents' performance. This research was conducted using F1 of promising lines, parental lines and the Jeliteng variety with 9 sets of crosses. Each line and variety were repeated 3 times, in total there were 57 experimental units. The observation parameters of this research were plant height, crossing success, weight of seeds, length and width of grain, flowering age, harvesting age also number of grains and unfilled spikelet. The results of this study showed that parents plant height was positively correlated with the number of productive tillers. The taller the plant, the more productive tillers and the more flowers can be crossed. The success of the cross can increase with the number of flowers crossed. The success of crosses between F1 black rice promising lines and their parents has a success rate range of 10.82% to 33.75%. The findings imply that F1 crossbreeding of black rice promising lines with their parents can be carried out to produce backcross offspring.

Keywords: backcross; black rice breeding; cross success rate; rice improvement; rice variety

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INTRODUCTION

Aside from white rice and brown rice, black rice is the most abundant type of rice in the world. Kristamtini et al. (2014) stated that recently black rice has become a popular food ingredient used by some people. Black rice naturally or through a certain process contains one or more compounds that are considered to have physiological functions which are beneficial to health (Pratiwi and Purwestri, 2017). Consumption of black rice is also increasing due to an increase in people's living standards and increased awareness of health (Aryana et al., 2018). Black rice has a high anthocyanin content in the seed coat so it is dark purple in color

(Nurlaili, 2020). This rice's bran is extremely rich in fiber and phytochemicals such tocopherols, tocotrienols, oryzanol, vitamin B complex and other phenolic substances (Francavilla and Joye, 2020). Additionally, the rice bran in purple and black varieties contains proanthocyanin and anthocyanin. These phytochemicals are referred to as bioactive substances, which can enhance immune and physical well-being in people (Das et al., 2017).

Black rice generally has problems in cultivation such as relatively long plant life, high habitus and low productivity (Patmi et al., 2020). Plant breeding is carried out to improve the weakness of black rice, one of which is by means of gamma-ray radiation. Universitas

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Sebelas Maret develop Cempo Ireng variety, named Intan Semar, in the rice fields of Ngijo Wetan Village, Tasikmadu, Karanganyar. Intan Semar black rice has been subjected to gamma radiation with doses of 200 Gy and 300 Gy at Center for Application of Technology of Isotope and Radiation (PAIR), National Nuclear Energy Agency (BATAN), South Jakarta and obtained six promising lines, namely GH numbers 8, 13, 44, 46, 51 and 52 (Nandariyah et al., 2021). Gamma ray irradiation can cause mutations in chromosomes so that individuals with different traits are obtained as expected (Hariyati et al., 2019). Intan Semar black rice must go through several generations of selection and yield testing before it can be released as a new variety. The results were tested on the best lines produced in a certain generation (Setiawan et al., 2019). Yield test is one of the breeding activities to study the nature and yield potential of each genotype (Pujiasmanto et al., 2021b). In the study of black rice mutants GH 8, GH 13, GH 44, GH 46, GH 51, GH 52, the results of gamma ray irradiation had a life cycle as expected, namely short life and high productivity (Nandariyah et al., 2021).

Warman et al. (2016) stated that the age of black rice can reach more than 145 days, therefore, black rice is not widely cultivated by farmers. Local black rice is known to be large, chronic, low yielding and susceptible to pests and diseases (Abdullah, 2017). The mutagenesis plant breeding program succeeded in producing superior short-lived black rice lines. This line is very susceptible to brown planthopper pests. In 2019, Indonesian Agency of Agricultural Research and Development launched black Jeliteng rice with a new superior variety. This variety is ± 113 days old, has a potential yield of ± 9.87 Mg ha⁻¹, plant height is ± 106 cm, resistant to brown planthopper (BP) type 1 and suitable as a parent/parent for a breeding program (BBPADI, 2019).

Research on the formation of black rice cultivars with superior characteristics of high productivity, early ripening age, low plant habitus, high anthocyanin content and good taste in Indonesia is still ongoing. Efforts to improve black rice varieties that have been carried out include mutation induction through radiation and crosses. In a previous study Pujiasmanto et al. (2021a), it was concluded that the yield and anthocyanin content of the black rice strain irradiated by gamma rays Cempo Ireng M7 was superior to the strain that was not irradiated (control). GH 8 and GH 51 have

the shortest harvest period and are classified as early maturing. The line with the highest productivity (per hectare) was GH 51, which was 8.45 ton ha⁻¹. GH 44 has the highest anthocyanin content, with an average of 75.10 ppm (Pujiasmanto et al., 2021a).

Hybridization is one of the conventional plant breeding methods and new varieties of interest can be obtained through transfer of genetic material. Hybridization is the process of inheritance from parents through the combination of pollen and pistil (Taryono, 2018). In the process of crossing, it is expected that the traits or genes from the parents will unite and be passed on to the offspring (Patil, 2021). Plant breeders use several methods in hybridization activities, one of which is the backcross method. In a previous study regarding the parents of the promising black rice line, based on the value of heritability, genetic variability and genetic progress, the characteristics that can be used as selection criteria and are superior traits are plant height, flowering age, harvest age, rice color and anthocyanin content (Sofian et al., 2019).

Backcrossing aims to emphasize the superiority of each parent. From the previous study, the assembling of high-yielding variety (HYVs) is an effective effort to obtain the potential lines which resistant to brown planthoppers amid these uncertain climate change problems (Nandariyah et al., 2022). The female parent showed that the resistance character was controlled by a gene outside the nucleus which was cytoplasmic inheritance (Napitupulu and Damanhuri, 2016). Therefore, to improve the performance of the superior traits of the parents, a backcross was carried out between F1 and each parent (Jeliteng varieties and promising lines) and the success of the crosses was observed. Improved yield and yield stability are essential to provide more sustainable food supplies and services in agricultural production by breeding program (Wang et al., 2021). In this study, observations were also made on the performance of the crossbreeding parents which had never been done in other studies. This research was conducted to study and determined the success of the backcross F1 black rice lines and the parents' performance.

MATERIALS AND METHOD

This research was conducted from April to December 2021 at the Screen House Experimental Laboratory of the Faculty of Agriculture Universitas Sebelas Maret, Jumantono Sub-

district, Central Java, Indonesia (coordinates 7°37'8"S and 100°56'52"E, altitude 196 m above sea level).

Research material

Seeds came from the offspring of potential lines of black rice crossed with Jeliteng variety (Nandariyah et al., 2022). Rice was planted in polybags with a diameter of 30 cm and a height of 40 cm, which contained a mixture of Alluvial soil and manure in a ratio of 2:1 to three quarters of the polybag height (Haryanta et al., 2017; Azalika et al., 2018). Black rice was grown with seeds from crosses, F1 GH, GH elders and Jeliteng varieties with 9 sets of crosses (Figure 1). The first six crossbreeding sets were carried out between F1 black rice GH number 8, 13, 44, 46, 51 and 52 (female parent) with black rice GH Cempo Ireng number 8, 13, 44, 46, 51 and 52 (male parent). Whereas the next three crosses sets were of F1 black rice GH crosses 44, 51 and 52 (male elders) with Jeliteng varieties (female elders). Each line and variety were replicated 3 times, so in total there were 57 experimental units.

Experimental design and parameters

The observation parameters were plant height (measured from the base of the stem to the highest panicle, measured at the end of the vegetative period before flowering). Measurements conducted at 50 days after sowing (DAS), flowering age (calculated when rice starts to germinate the panicle development phase and flowers form), harvest age (criteria for the age of rice plants are divided into 3, namely early (< 100 to 125 days), medium (125 to 145 days) and deep (> 145 days) (Sukamandi and Barat, 2019), success of crossing, number of grain and unfilled spikelet from crosses, the length and width of the grain (measurements were carried out on 10 samples of grain per line and variety using millimeter block), weight of the seeds from crossing (measurement using an analytical balance). Quantitative and qualitative data from the observations were analyzed by T-test, descriptive analysis and correlation test between parameters.

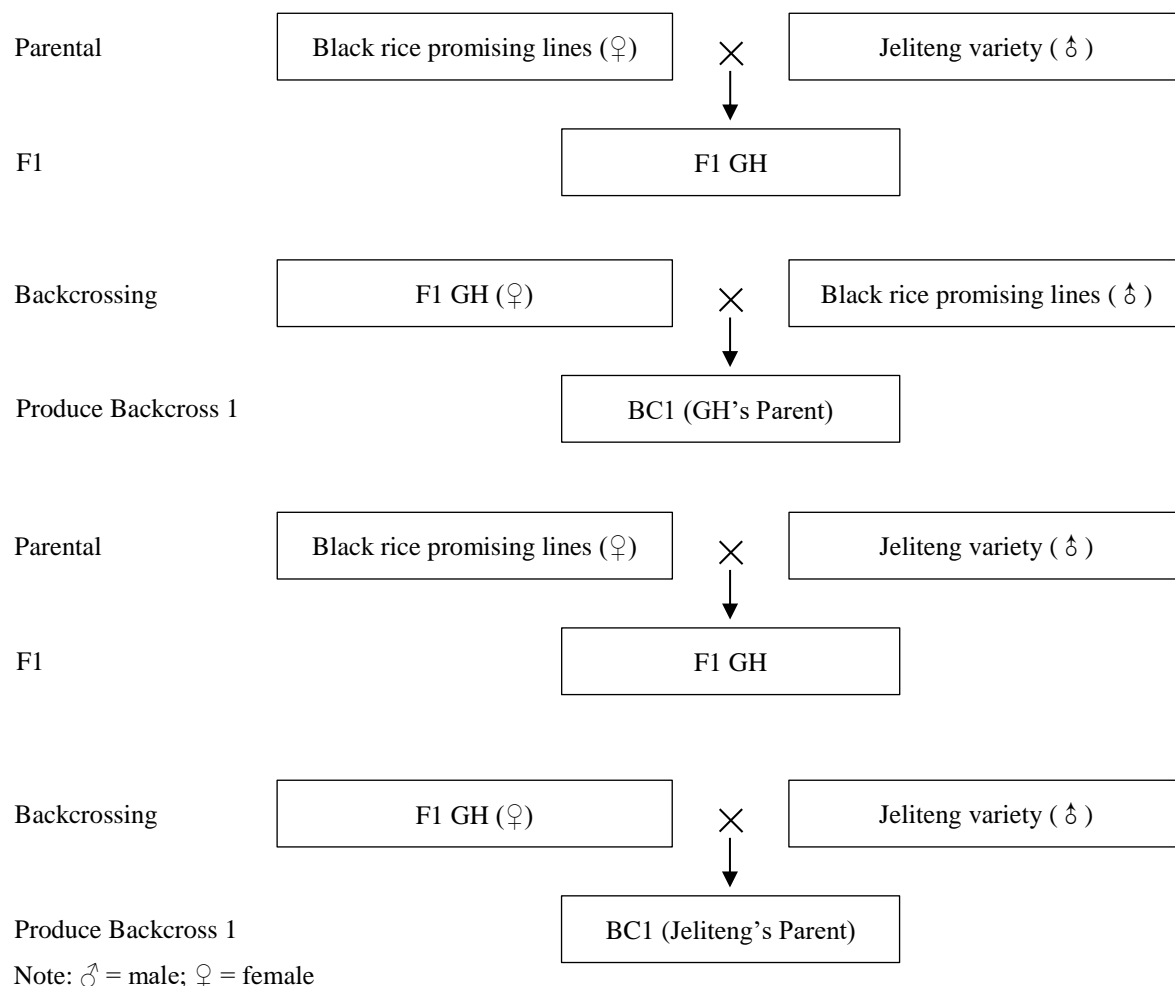


Figure 1. Cross set diagram

Crossing technique

The castration technique in this research was performed by cutting the top of the grain and removing the anther manually (hand emasculation) using tweezers (Suwignyo et al., 2021). The castration and hybridization process is carried out alternately for each flower from July 21st to September 7th 2021. The female parent flower is castrated, when the anther is in the middle of a flower bud, this can be seen with the help of sunlight or a flashlight. Castration is done the day before crossing after 03.00 p.m., so the pistils are ripe and ready to be pollinated the following day. The hybridization success factor requires understanding the parameters that affect the flowering period's duration, the pollination method and the formation of seeds. The process of anthesis (opening of flowers/spikelets) begins on the second or third day after the appearance of the panicles. Flowering takes place from the top to the bottom of the panicle. It takes about two weeks to complete flowering in one panicle. Anthesis occurs between 08.00 to 10.30 a.m. The entire anthesis process is completed within 2 to 5 minutes. Based on the research results of Thorat et al. (2020) in a pollen viability study, the results showed that pollen could be viable for 1 to 11 minutes after the anther bursts at the complete bloom stage. The percentage of pollen viability was observed from 90.79% to 94.92%. Stigma (pistil) receptivity is adequate for up to 7 days from flowering. It should be noted that the entire pollination process should be done before the midday sun becomes hot because it can increase the temperature around the rice. After making sure each female parent has been pollinated, please wrap the panicles using plastic clips thoroughly. According to Rice and McQuillan (2018), packaging (isolation) after hybridization aims to prevent the anther from coming out of the spikelet, not being contaminated by other pollen, and protected from water, wind and plant-disturbing organisms.

RESULTS AND DISCUSSION

Plant height

The average height of F1 GH8 to F1 GH52 was 97.4 to 103.8 cm with minimum height of 84 cm and maximum of 100 cm, shorter than the control's height. According to the International Plant Genetic Resources Institute (2007) rice plant height was classified as semi-dwarf (< 110 cm), medium (110 to 130 cm) and tall (> 130 cm).

The height of control, GH and Jeliteng rice was classified into the medium category; whereas for F1 GH rice it was in the semi-dwarf category. All of the F1 GH, GH and Jeliteng rice had an average height that was significantly different from the control. So F1 GH rice with GH and Jeliteng parents produced high semi-dwarf rice category. According to Zhang et al. (2017) the dwarf rice phenotype is useful for minimizing the rate of rice fall, but if the plant is too short it will cause insufficient growth and ultimately affect the potential for rice yields. Based on the correlation test, the plant height parameter was positively correlated at 0.01 significance with the number of productive tillers ($r = 1,000^{**}$). Based on these results, it can be said that the increase in plant height also resulted in an increase in the number of productive tillers. Based on research conducted by Nurhidayah and Umbara (2019), the average height of black rice is 124.15 cm, this can indicate the results of crosses between the black rice promising lines and the Jeliteng variety resulted in lower plant height.

Crossing success and weight of the seeds

The percentage of successful crosses above 30% were the cross of F1 GH13 x GH13 (33.75%), F1 GH44 x GH44 (32.61%) and F1 GH51 x GH51 (30.3%). While the lowest percentage of success was the cross of F1 GH51 x Jeliteng (12.34%) (Table 1 and 2). Based on the research results of Rahayu et al. (2022) the percentage of success of rice crosses has a range of 1.67% to 48.33%. The success of the cross was obtained from the percentage of seeds formed with the total number of flowers crossed (Yang et al., 2019; Guo et al., 2020). The seeds formed can be seen 5 days after crossing (Figure 2a) and the color of the seeds will begin to blacken starting from 7 days after crossing (Figure 2b). As rice is a self-pollinating plant where each flower contains male and female parts. Thus, each rice flower can self-pollinate without the need for flowers or other rice varieties. However, according to Hashim et al. (2021) the rate of natural crossing or cross-pollination in cultivated rice is relatively low (< 4%) and the success rate depends on the layout of the land and environmental conditions. There are various factors that affect the success of crosses such as temperature, weather, technicality and time. According to Takai et al. (2020) too high heat causes loss of anthers, impaired pollination and abnormal pollen germination and flowers become sterile.

Table 1. Percentage of successful crosses between F1 black rice promising lines (female) x black rice promising lines (male)

Cross set	Success percentage (%)	Weight of 21 seeds (g)
F1 GH8 x GH8	25.83	0.26
F1 GH13 x GH13	33.75	0.29
F1 GH44 x GH44	32.61	0.35
F1 GH46 x GH46	14.28	0.31
F1 GH51 x GH51	30.30	0.27
F1 GH52 x GH52	10.82	0.25

Table 2. Percentage of successful crosses between F1 black rice promising lines (male) x Jeliteng variety (female)

Cross set	Success percentage (%)	Weight of 21 seeds (g)
F1 GH44 x Jeliteng	19.16	0.30
F1 GH51 x Jeliteng	12.34	0.31
F1 GH52 x Jeliteng	25.85	0.34

Based on Table 1, the highest weight of the seeds was of the F1 GH44 x GH44 cross set, which was 0.35 g. Meanwhile, the lowest seed weight occurred in the F1 GH52 x GH52 cross set, which was 0.25 g. In all sets of crosses with Jeliteng the seed weight was above 0.3 g, with details of the crosses set for F1 GH44 x Jeliteng was 0.3 g, F1 GH51 x Jeliteng was 0.31 g and F1 GH52 x Jeliteng was 0.34 g (Table 2). However, overall seed weights were not much different among all sets of crosses. According to Zhao et al. (2018), grain weight as the most important factor that determines yield, is largely determined by the speed of filling and the length of time it takes to fill the rice. Based on research conducted by Nandariyah et al. (2022), the average weight of the overall seeds in this study was higher, this was because improvements had been made, namely the addition of cross samples and a higher success rate of crosses.

Length and width of grain

The average grain length range of F1 GH8 to F1 GH 52 were 7.66 to 8.11 mm. The average range of grain GH8 to GH 52 were 8.34 to

8.48 mm. The average grain range of control was 8.26 mm while Jeliteng was 8.37 mm. The average grain width range of F1 GH8 to F1 GH 52 is 2.08 to 2.84 mm. The average grain width range of GH8 to GH 52 is 2.1 to 2.38 mm. The average width range of control grain was 2.26 mm while Jeliteng was 2.31 mm (Table 3). Identification of the physical shape of the grain can facilitate the breeding of rice cultivars to obtain optimal grain shape and appearance quality. According to Zhao et al. (2018) differences in size can be caused by genetic variations in each line and variety. Rice F1 GH46, F1 GH51, GH8, GH13, GH44, GH46 and GH52 had significantly different seed lengths from the control. Rice F1 GH8, F1 GH13, F1 GH44, F1 GH52, GH8, GH51 and Jeliteng had significantly different seed widths from the control. Based on previous research conducted by Nandariyah et al. (2022), the average length and width of the grain in this study were higher, this indicated that crosses between the black rice promising lines and the Jeliteng variety could produce grain with a larger size.

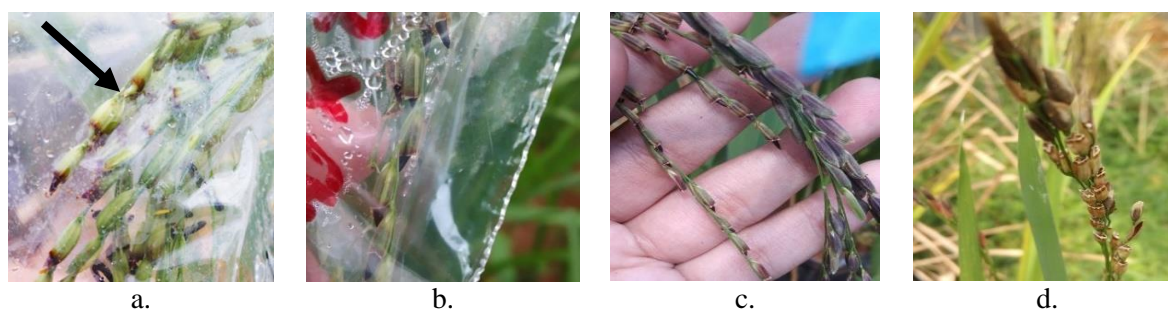


Figure 2. Condition of rice grains after crossing

Note: DAC = day after crossing; a) condition of seeds formed 5 DAC; b) condition of seeds formed 7 DAC, c) condition of seeds formed 10 DAC, d) condition of seeds that failed to form after crossing

Table 3. Length and width of grain promising lines and black rice varieties

Promising lines and variety	Grain length (mm)		Grain width (mm)	
	Range	Average	Range	Average
F1 GH8 x GH8	7.0–9.0	7.66	2.3–3.0	2.65
F1 GH13 x GH13	8.0–9.0	8.11	2.5–3.0	2.84
F1 GH44 x GH44	7.0–9.0	7.89	2.0–3.0	2.44
F1 GH46 x GH46	7.0–8.5	7.75	2.0–2.5	2.19
F1 GH51 x GH51	7.5–8.5	7.97	2.0–2.5	2.29
F1 GH52 x GH52	6.0–9.0	8.00	2.1–3.0	2.59
F1 GH44 x Jeliteng	7.5–8.0	7.87	2.0–2.3	2.17
F1 GH51 x Jeliteng	7.0–8.1	7.81	2.0–2.3	2.12
F1 GH52 x Jeliteng	7.5–8.0	7.83	2.0–2.3	2.08
Cempo Ireng	8.0–8.5	8.26	2.1–2.5	2.26
Jeliteng	8.0–9.0	8.37	2.0–2.7	2.31
F1 GH8	7.7–8.1	7.97	2.0–2.3	2.05
F1 GH13	7.5–8.3	7.94	2.1–2.5	2.20
F1 GH44	7.9–8.5	8.13	2.1–2.3	2.18
F1 GH46	7.9–9.0	8.24	2.0–3.0	2.22
F1 GH51	8.0–8.5	8.30	2.0–2.5	2.28
F1 GH52	7.9–8.0	7.95	2.0–2.3	2.05
GH8	7.9–8.1	8.00	2.0–2.3	2.10
GH13	8.1–8.5	8.42	2.1–2.5	2.38
GH44	8.1–8.5	8.40	2.0–2.5	2.27
GH46	8.1–8.5	8.34	2.0–2.5	2.19
GH51	8.1–8.5	8.40	2.1–2.5	2.28
GH52	8.3–8.5	8.48	2.1–2.5	2.32

Flowering and harvesting age

The flowering age of F1 GH was closer to Jeliteng, faster than GH and Control, so it can be said that F1 GH inherited the flowering age from the Jeliteng as one of its parents (Figure 3). The flowering age of F1 GH was not quite simultaneous, this is in accordance with the opinion of Guo et al. (2020). Biologically, unique local environmental conditions can change

flowering time in different directions and can reduce predictive accuracy and uniformity of flowering time (Li et al., 2021). According to Yang et al. (2019) the flowering stage is very important for rice yield and quality establishment. Flowering time was measured on the date when the first panicle sprouted from the flag leaf. At the time of crossing must carefully monitor which plants the flowers can be immediately crossed.

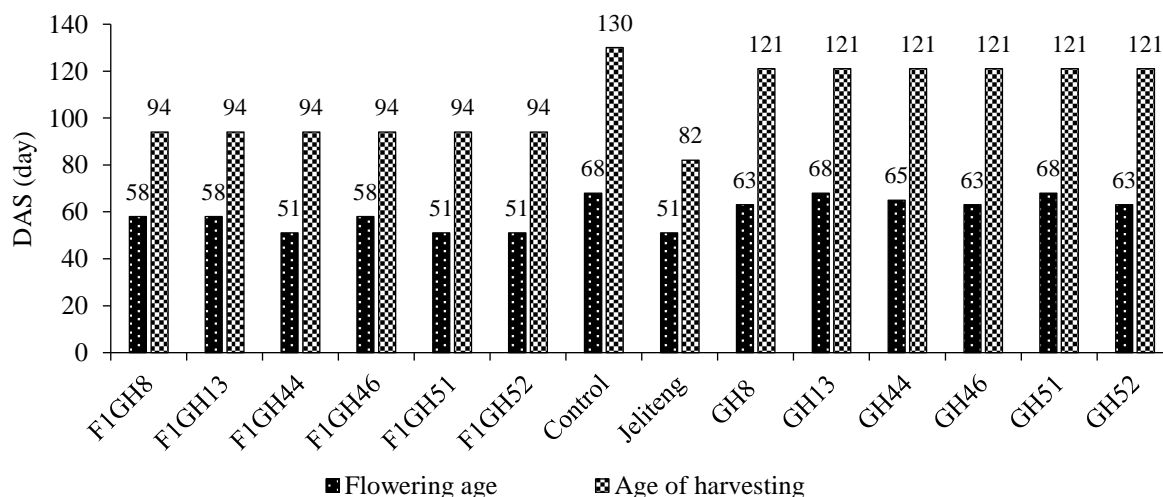


Figure 3. Flowering and harvesting age of promising lines and black rice varieties

Table 4. Number of filled and empty rice from crosses of promising lines and black rice varieties

Cross set	Amount of filled grain (seeds)	Amount of empty spikelet	Total of flower crossed
F1 GH8 x GH8	99	287	386
F1 GH13 x GH13	54	104	158
F1 GH44 x GH44	121	250	371
F1 GH46 x GH46	21	126	147
F1 GH51 x GH51	157	361	518
F1 GH52 x GH52	34	280	314
F1 GH44 x Jeliteng	32	135	167
F1 GH51 x Jeliteng	30	213	243
F1 GH52 x Jeliteng	38	109	147
Total	586	1,865	2,451

According to the classification of the Indonesian Institute for Rice Research (2016), the harvesting age of rice was categorized into; deep: > 151 DAS, moderate: 125 to 150 DAS, early: 105 to 124 DAS, very early: 90 to 104 DAS, ultra-early: < 90 DAS. Jeliteng age was included in the ultra-early harvesting category, F1 GH and GH were in the early harvesting category and control was in the medium harvesting category. It can be said that F1 GH inherited the harvesting age of the Jeliteng parental which was faster than the GH parental. Based on the opinion of Djoar and Nandariyah (2011), early maturing plants and early harvesting age are one of the plant mechanisms to avoid pests and diseases, because plants can complete their life cycle before a pest attacks and spreads.

Number of filled and empty grains from crosses

Predicting grain yield during the early to mid-growth stages is important for early diagnosis of the yield of rice that will form. The level of rice grain filling makes a major contribution to grain productivity and nutrient accumulation. Based on Table 4, the highest amount of filled grains (157 grains) obtained by the cross set F1 GH51 x GH51. This is also because the total number of flowers crossed was at the highest (518 flowers). The more flowers crossed can increase the probability of a successful cross. This is a development of the research Nandariyah et al. (2022) which explains that one of the obstacles and the reason why many crosses have not been successful is due to the lack of samples or crossed flowers. The results of the cross set of F1 GH46 x GH46 is 21 seeds, F1 GH52 x GH52 34 seeds, F1 GH44 x Jeliteng 32 seeds, F1 GH51 x Jeliteng 30 seeds and F1 GH52 x Jeliteng 38 seeds, tended to be low (below 50 seeds) because some panicles were

attacked by pests. Filled and empty spikelet are also influenced by various factors such as temperature (Wu et al., 2019; Liu et al., 2020). According to Ren et al. (2021), high temperatures (> 35 °C) often lead to failure of grain filling in rice which causes yield loss.

CONCLUSIONS

In this study, F1 crossbreeding of black rice promising lines with their parents can be carried out and produce backcross offspring. All observed parameters on parents' performance were significantly different from the control. The success of crosses between F1 black rice promising lines and their parents has a success rate range of 10.82% to 33.75%. The success of the cross can increase with the number of flowers crossed, the weight and number of seeds from the cross can also increase. It can be suggested to include follow-up backcross treatment that produces backcross 2 seeds to strengthen superior traits.

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