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Agronomic performance of F4 population of rice breeding lines derived from the cross of Black Rice and Mentik Wangi varieties

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Abstract. The cross between Black Rice and Mentik Wangi rice varieties was carried out to obtain black rice breeding lines with good palatability and aromatic fragrance. In this research, F4 breeding lines derived from the cross of Black Rice and Mentik Wangi varieties were evaluated for their agronomic performances and selected to be F5 generation. The research was conducted in paddy field of Mersi Village, East Purwokerto District, Banyumas Regency, Indonesia, from July-November 2016. Augmented Complete Randomized Block Design with three replicates was used to arrange the F4 breeding lines and the check varieties of Black Rice, Mentik Wangi, IR 36 and Red Rice varieties. The results showed that plant height, number of productive tillers, number of seeds per panicle and 1000 seed weight of F4 breeding lines are uniform, while the flowering date, total tillers, panicle length and seed weight per hill still transgressively segregate. Based on the segregating agronomic characters, six breeding lines were selected to be continued to become F5 generation, namely: PHMW 48211431, PHMW 482171867, PHMW 48291349, PHMW 48211447, PHMW 4829134102, PHMW 48724850. Analysis on amylose content showed that the selected breeding lines have low to moderate amylose content.

1. Introduction

The need of rice increases, both quantity and quality that include nutritional content, in line with the increase of human population. Improvement of nutritional quality of rice could be made through development of rice variety for functional food, a food that has an additional function related to health-promotion or disease prevention. Functional rice may apply to traits purposely breed into existing edible rice enriched with certain nutrition, such as anthocyanin. Enriched anthocyanin black rice may be designed to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, that similar in appearance to conventional rice and consumed as part of a regular diet.

Despite the beneficial nutritional content, commercially available black rice is mostly not favored as human diet due to its low palatability; so that it is used more for bird feed. The cross of Black Rice and Mentik Wangi rice varieties which was then followed by selection across generations has been carried out in the Faculty of Agriculture, Jenderal Soedirman University, to improve palatability, while also adding aromatic character, of black rice variety in order to release a new aromatic black rice



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variety [1]. This crossing is also aimed at obtain new back rive variety with short harvest date and shorter habitus, as local black rice variety usually has longer harvest date and taller habitus [2].

The F4 segregating population of the cross of Black Rice and Mentik Wangi rice varieties was evaluated for their agronomic characters. Selection on segregating generation is based on agronomic characters to obtained breeding lines which could be further grown as the next generation. The objectives of this research were: (1) to determine the diversity of agronomic characters of F4 population derived from the cross of Black Rice and Mentik Wangi rice varieties, and (2) to obtain individual F4 that could be further grown as F5 generation.

2. Methods

The research was carried out at the paddy field of Mersi Village, East Purwokerto District, Banyumas Regency, Indonesia, from July-November 2016. Augmented Complete Randomized Block Design with three replicates was used to arrange the F4 breeding lines of PHMW 482114, PHMW 48214, PHMW 487248, PHMW 4872363, PHMW 4821724, PHMW 4821718, PHMW 482177, PHMW 4829134 and PHMW 48211; and the check varieties of Black Rice, Mentik Wangi, IR 36 and Red Rice varieties. A total of 539 F4 individual was planted without replication as its individual plant represents its own genotype [3]. The experimental error was accounted from the replicated check varieties. The observed variables included plant height, total tiller number, number of productive tillers, flowering date, panicle length, number of seeds per panicle, thousand weed weight, seed weight per hill, and amylose content of the selected F4 individuals.

Data were analyzed using SAS version 9.0 [4]. The effect of block is accounted during analysis of variance which is shown as part of adjusted mean calculation. Differences among F4 individuals was analyzed using LSD pairwise comparison of adjusted mean ($P \leq 0.05$).

3. Results and discussions

3.1. Effect of block and check varieties

The result showed that block did not effect on the agronomic performance of the F4 population, except on seed weight per hill (Table 1), indicating the homogeneity of the experimental condition. Among the observed variables, Black Rice and Mentik Wangi varieties were phenotypically different only on flowering date, plant height, and panicle length (Table 2).

Table 1. Effect of block on the observed variables of the check varieties

Variable	Block
Flowering date	ns
Plant height	ns
Total tiller number	ns
Productive tiller number	ns
Seed number per panicle	ns
Thousand seed weight	ns
Panicle length	ns
Seed weight per hill	*

Note: (ns) not significant, (*) significant.

Table 2. Pairwise comparison on the observed variables among the check varieties

Check Varieties	FD	PH	TTN	PTN	SNPP	PL	TSW	SWPH
IR36	63.3 ^c	94.7 ^a	20 ^a	18.2 ^c	64.7 ^a	21.6 ^a	23.7 ^a	24.7 ^{ab}
Red Rice	62.7 ^c	142 ^d	16.1 ^a	13.4 ^{abc}	125 ^c	29.3 ^d	23.8 ^a	29.9 ^b
Black Rice	61.1 ^b	111 ^b	17.5 ^a	11.3 ^{ab}	69 ^{ab}	23.5 ^b	25.8 ^a	23.7 ^{ab}
Mentik Wangi	55.2 ^a	127 ^c	19.2 ^a	8.45 ^a	85.2 ^{ab}	27.1 ^c	28.6 ^a	20 ^a

Note: (FD) Flowering date, (PH) Plant Height, (TTN) Total Tiller Number, (PTN) Productive Tiller Number, (SNPP) Seed Number Per Panicle, (PL) Panicle Length, (TSW) Thousand Seed Weight, (SWPH) Seed Weight Per Hill.

3.2. Evaluation of agronomic characters of F4 population

Until F4 generation, the progenies derived from the cross of Black Rice and Mentik Wangi are already homogeneous on plant height, productive tiller numbers, thousand seed weight and seed weight per panicle, but are still segregating on flowering date, total tiller numbers, panicle length and seed weight per hill (Table 3). The agronomic diversity among the F4 population indicates the genetic divergence among the parents [5]. Selection is effective when carried out on a segregating population or on characters with high diversity [6].

Table 3. Result of analysis of variance on the observed variables among the F4 population

Variable	Block
Flowering date	*
Plant height	ns
Total tiller number	*
Productive tiller number	ns
Seed number per panicle	ns
Thousand seed weight	ns
Panicle length	*
Seed weight per hill	**

Note: (ns) not significant, (*) significant, (**) highly significant

3.2.1. Flowering date. The onset of flowering of rice plant terminates the vegetative phase and commences the generative phase. The shorter the generative phase, the shorter the harvest date. Rice growth phase could be divided into three phases, namely: vegetative phase (0-60 days after seeding [das]), generative phase (60-90 das), and maturity phase (90-120 das). The F4 population showed significant difference in flowering date (Table 3) with distribution pattern as shown in Figure 1. The range of flowering date varied among the individuals and showed a transgressive segregation. Transgressive segregation is a phenomenon of which the range of data distribution on the progenies goes beyond (smaller and/or greater) that of the parents [7].

Among the population, a total 319 genotypes may be categorized to have ideal flowering date (50-60 das). Flowering date positively correlates with maturing date [8]. Difference in flowering date is determined by difference in vegetative phase, and is under genetic control. Selection for next generation is based on the idio type of rice which in the range of 50-60 das for rice [9].

3.2.2. Plant height. Despite the difference between the parents Black Rice and Mentik Wangi on plant height (Table 2), no such difference could be observed in the F4 population (Table 3). It may indicate that this population has reached homogeneity for plant height. A total of 95 individual was selected as plant with moderate height based on Standard Evaluation System for Rice [10]. Tall habitus was not selected as they tend to get shattered [10].

3.2.3. Total tiller number. Significant differences were observed among F4 population on total tiller number (Table 3) indicating that this population is still segregating on total tiller number (Figure 1).

The graph of pattern of distribution, however, was truncated on the left side, indicating an intensive negative selection pressure during previous generation.

Selection for the next generation was carried out based on IRRI standard for high tiller number, i.e. greater than 25 tiller per hill [10]. Individuals with very high tiller number are not recommended to be selected as they tend to mature timely varied so that resulting in high percentage of empty grain. Based on figure 1, individuals with very high total tiller number may also be considered as outliers. In this breeding program, genetic improvement for tiller number could be likely gained. The selected individuals had greater tiller number than the parents. However, genetic improvement on total tiller number does not likely contribute to yield improvement as this population is fixed for productive tiller number (see point d).

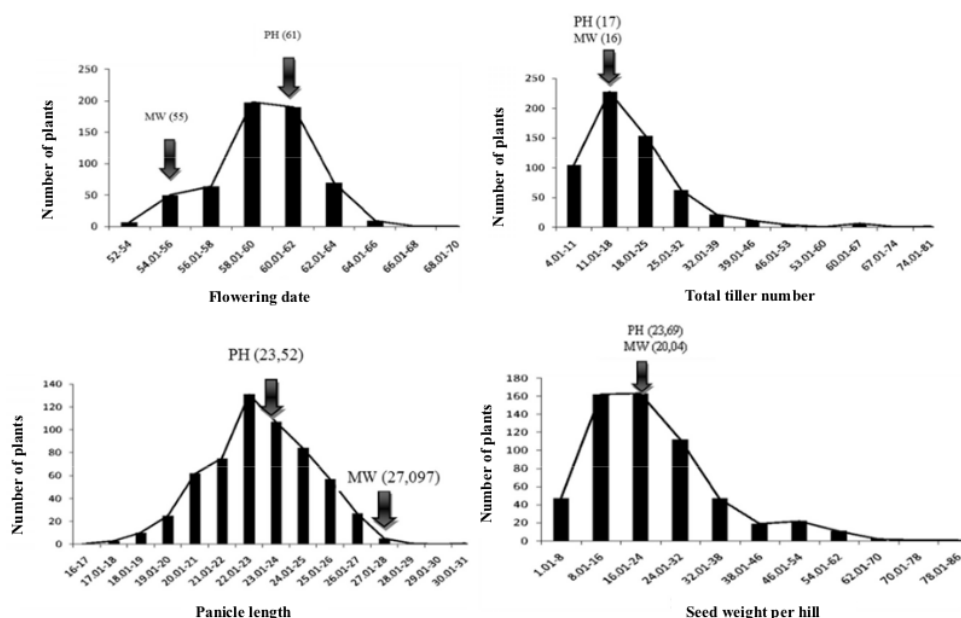


Figure 1. Distribution pattern of Flowering date (top left), total tiller number (top right), panicle length (bottom left) and seed weight per hill (bottom right) among the individuals in the F4 population of Black Rice and Mentik Wangi

3.2.4. Productive tiller number No difference could be observed on productive tiller number among the F4 population (Table 3). This could possibly mean that selection that has been carried out until this generation have made this population reached homogeneity for this trait. The average productive tiller number is ranging from 15 to 25 tillers per hill.

3.2.5. Panicle length and seed number per panicle Transgressive segregation was observed on panicle length, but no individuals had greater panicle length than the parent Mentik Wangi (Figure 1). Selection was carried out on individuals with panicle length of >30 cm [11] (Table 4). Panicle length usually correlates well with seed number [11] but it was not observed in this population. The seed number was not difference (Table 2 and 3) regardless of the panicle length.

With a range of 83-100, individual in this population meets the standard of Ministry of Agriculture for rice seed number per panicle [13]. Seed number per panicle directly correlate to panicle length and weed weight per hill, and contribute to yield [14].

3.2.6. Thousand seed weight No difference could be observed on thousand seeds weight among the individuals in the population (Table 3). This may indicate that the population has reach fixation for this trait. Regardless of the insignificant difference of thousand seed weight among the population, previous generation selection has likely ignored thousand seed weight while focusing much on other traits. Consequently, the average seed weight of the population (Table 4) is lower than that of the parents (Table 2).

3.2.7. Seed weight per hill Seed weight per hill of the population was varied significantly (Table 3) showing a transgressive segregation pattern as shown in Figure 1. Just like on tiller number, truncation on the left side was also seen, indicating an intensive negative selection pressure on seed weight per hill during previous generation (Figure 1). Selection for the next generation was carried out for individuals with seed weight per hill higher than 25 g.

3.3. Selection of F4 individuals for F5 generation

Selection of F4 individuals for F5 generation was based on the segregating traits i.e.: flowering date, total tiller number, panicle length and seed weight per hill. Based on ideal measures of these traits only six individuals (breeding lines) could be further released to be F5 generation, namely: PHMW 48211431, PHMW 482171867, PHMW 48291349, PHMW 48211447, PHMW 4829134102, PHMW 48724850. These lines have seed weight per hill of 26.7-35.3 gram, greater than the parents Black rice and Mentik Wangi (Table 2).

Table 4. The F4 individuals (breeding lines) selected for F5 generation and their agronomic characteristics.

Galur	FD	PH	TTN	PTN	SNPP	TSW	PL	SWPH
PHMW48211431	59,8908	112,09	26,8008	22,7892	94,7575	20,5008	22,5234	32,8324
PHMW48211447	59,8908	115,69	22,8008	20,7892	82,7575	22,8408	22,3234	34,5724
PHMW482171867	56,8908	108,19	27,8008	23,7892	100,76	20,5108	22,3234	35,3824
PHMW4829134102	61,7883	115,28	23,6783	15,0642	133,42	20,9933	24,4682	31,4215
PHMW48291349	61,7883	117,98	24,6783	16,0642	112,42	20,4133	26,6682	30,5115
PHMW48724850	60,3203	116,93	26,5208	16,1467	94,825	20,5058	20,1084	26,7161

Note: (FD) Flowering date, (PH) Plant Height, (TTN) Total Tiller Number, (PTN) Productive Tiller Number, (SNPP) Seed Number Per Panicle, (PL) Panicle Length, (TSW) Thousand Seed Weight, (SWPH) Seed Weight Per Hill.

Analysis on amylose content of the rice grain showed that the six selected lines had moderate amylose content. Similarly, a sample of the non-selected lines also showed moderate content of amylose (Table 5). Amylose content is under genetic control [15]. Rice grain amylose content correlates significantly with rice fluffiness. Based on amylose content, rice grain may be grouped into three categories, namely: (1) rice with a high amylose content (25-30%) tends to cook firm and dry, (2) rice with a intermediate amylose content (20-25%) tends to be softer and stickier, and (3) rice with a low amylose content (<20%) is generally quite soft and sticky. While waxy rice has a zero-amylose content and is often referred to as sticky rice [15].

Table 5. Amylose content (%) of the selected and the unselected lines

	Amylose content (%)	category
Selected plant		
PHMW 482-1-14-31	22.16	moderate
PHMW 482-17-18-67	22.75	moderate
PHMW 482-9-134-9	19.85	moderate
PHMW 482-1-14-47	22.48	moderate
PHMW 482-9-134-102	18.86	moderate
PHMW 487-24-8-50	19.79	moderate
Non-selected plant		
PHMW 482-17-18-42	22.66	moderate
PHMW 487-23-63-21	21.55	moderate
PHMW 482-17-7-74	20.82	moderate

4. Conclusion

Segregation on flowering date, total tiller number, panicle length and seed weight per hill still could be found in F4 population of Black Rice and Mentik Wangi. Breeding lines of: PHMW 48211431, PHMW 482171867, PHMW 48291349, PHMW 48211447, PHMW 4829134102, and PHMW 48724850 were selected for F5 generation. The selected lines have moderate amylose content so that this black rice breeding program may be expected to result in improvement of cook fluffiness and palatability.

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