IOP Conf. Series: Earth and Environmental Science **482** (2020) 012025 doi:10.1088/1755-1315/482/1/012025

Agronomic performance of several doubled-haploid lines derived from anther culture of black rice × white rice

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Abstract. Black rice is a well-known type of rice in Indonesia, but its availability is still limited. So far, only one black rice variety that has been released by rice breeders. Recently, public demand for black rice increases due to its health benefits. Therefore, the development of new variety of black rice is needed. Anther culture is often used to accelerate the process in obtaining homozygous lines in the form of doubled-haploid (DH) plants for rice breeding. This research aimed to evaluate the agronomic performance of several DH lines derived from anther culture. The experiment was conducted at Sukamandi, West Java, during the dry season of 2017. Plant materials used in this study were 13 DH lines derived from anther culture of F_1s and five improved varieties as control. The F_1s were obtained from crosses between local black rice Melik with two white rice varieties, Inpari 13 or Fatmawati. The experiment was conducted in a randomized complete block design with three replications. Observation was conducted on plant height, number of productive tiller, days to flower, days to harvest, panicle length, number of grain per panicle, number of filled and empty grain number per panicle, yield, and pericarp color. The results showed that plant height, number of tillers, yield and yield components of DH lines were significantly different compared to some of the control varieties. Ten DH lines were similar in yield compared to Inpari 13, but five of them produced higher yield than Inpari 13. Days to flower of DH lines and control varieties were similar (75–89 days). The pericarp color of DH lines varies from dark purple to reddish-brown (maroon).

Keywords: agronomic characters, doubled-haploid (DH) lines, anther culture, black rice.

1. Introduction

Black rice has more benefit compared to white rice, because it contains nutrients such as anthocyanin, various vitamins and minerals, including iron, vitamin A and B [1–3]. The pericarp layer of black rice accumulates anthocyanin, which is known as an antioxidant beneficial for human health. Black rice has a pericarp, aleurone, and endosperm with red, blue, or purple with deep red-blue-purple color, which indicate anthocyanin content [4]. Some antioxidants in black rice are reported to be soluble in fat, while anthocyanins dissolve in water and can be spread in the body [5]. It was reported that some health benefits from black rice due to its association with a reduced risk of heart disease, cancer and memory improvement [4,6].

Black rice can be planted on various lands, not only in lowlands but also in highlands. Anthocyanin content of black rice increases with the increasing altitude and the presence of a favorable environment [7]. Limited quantity of black rice is currently available in the market,but the price is quite expensive, up to 2–3 times of white rice price. Meanwhile, the demand for black rice tends to increase because of the better public awareness of the health benefit of black rice.

A number of local black rice exists in several rice areas in Indonesia. Local black rice cultivars such as Cibeusi was found in West Java; Aen Metan and Hare Kwa were found in East Nusa Tenggara; while Melik, Cempo Ireng, Jeliteng, Wulung and Sirampog were found in Central Java [2]. All these local varieties were tall (>150 cm) and have a low yielding ability [2,3,8], but can be used as the gene source(s) in rice breeding programs. Selection of doubled-haploid (DH) derived from anther culture can shorten the duration of the breeding process because the agronomic character of the DH lines is stable from generation to generation [9]. Maeda et al. [10] reported three loci on chromosomes 1, 3 and 4 which contained genes that control color (black/purple) in rice by assessing them with RM8129, RM15191 and RM2441 SSR markers, respectively. Two genes that control purple pericarp were found in chromosome 4 (*b* genes) and chromosome 1 (*a* genes), while those controlling black pigmentation in pericarp was located in a locus on chromosome 3.

This research aimed to evaluate agronomic performance of several DH lines derived from anther culture of black rice × white rice to obtain black rice lines that has good agronomic characters, such as dwarf (90–115 cm), high yield (>5 t/ha) and early maturing (90–120 days after sowing) which are equivalent to improved variety.

2. Materials and methods

The research was carried out at Sukamandi Experimental Station in dry season (DS) 2017. Fourth generation of doubled-haploid (DH4) lines were tested. These lines were obtained from anther culture of F_1s derived from the cross between the local black rice Melik with two improved varieties of white rice (Inpari 13 and Fatmawati), i.e. Melik/Inpari 13^2, $Melik^2$/Inpari 13, $Melik/Fatmawati^2$ and $Melik^2$/Fatmawati. A total of 13DH4 lines and five improved varieties (Inpari24, Aek Sibundong, Inpari 32, Inpari 13 and Fatmawati) as control were planted in a randomized complete block design (RCBD) with three replications. Each line was planted in a plot size of 2 × 5 m^2 at seedling stage of ±21 days after sowing (DAS) and plant spacing of 25 cm × 25 cm. Fertilizer used were 300 kg urea/ha, 100 kg TSP/ha and 100 kg KCl/ha. Observation was conducted on plant height, days to flower, days to harvest, number of productive tiller, yield per plot, panicle length, number of grain per panicle, number of filled grain, number of empty grain, weight of 1,000 grains and the color of pericarp.

3. Results and discussion

Variance analysis showed that there was a significant difference between doubled haploid (DH) lines and control varieties in all traits observed (Table 1).The yield of ten DH black rice lines were not significantly different from that of the control varieties, i.e. Inpari 13, Fatmawati, Inpari 24, Aek Sibundong and Inpari 32 varieties (Table 2). Unfortunately, the local black rice Melik was not included in this research due to its late-maturing trait which is more than 150 days [11].

Five DH lines derived from Melik/Inpari 13 had higher yield (7–7.3 t/ha) than the parent, Inpari 13 (6.9 t/ha). There were also two DH lines derived from $Melik/Fatmawati^2$ that had a yield of 6.9–7.2 t/ha, while Fatmawati only yielded 6.8 t/ha. Out of the five improved varieties tested, Inpari 24 (brown rice) had the highest yield (7.3 t/ha). In this research, seven DH lines were selected, i.e. YD1-61-1-1, YD1-71-1-1, YD1-51-2-1, YD1-51-2-2, YD1-48-1-2, YD6-1-1-1 and YD6-1-1-2, based on their higher or equivalent yields compared to the parental varieties and Aek Sibundong variety (7–7.2 t/ha). These lines can be tested further in advanced yield trials atseveral locations.

Table 1. Analysis of variance of agronomic characters of DH lines at Sukamandi in dry season of 2017.

Traits	CV (%)	Mean square	F value	Significance level
Plant height	8.9	292.04	16.94	**
Number of panicle	13.8	14.79	3.18	*
Days to flower	4.0	52.18	60.96	**
Panicle length	7.6	12.53	7.21	**
Number of grain per panicle	17.2	2914.90	4.52	**
Number of filled grain per panicle	18.3	2084.08	3.83	**
Number of empty grain per panicle	31.6	190.87	2.90	**
1,000 grain weight	4.5	4.00	3.05	*
Yield (t/ha)	11.7	2.06	1.94*	*

**Significantly different at α = 1%, *Significantly different at α = 5%.

Table 2. Yield of DH lines at Sukamandi Experimental Station in dry season of 2017.

Line no.	Genotype	Yield (t/ha)	Line no.	Genotype	Yield (t/ha)
1	YD1-61-1-1	7.13	10	YD5-37-1-2	6.4
2	YD1-71-1-1	7.13	11	YD5-37-1-3	6.17
3	YD1-51-2-1	7.13	12	YD6-1-1-1	7.17
4	YD1-51-2-2	7.13	13	YD6-1-1-2	6.87
5	YD1-51-2-3	6.07		Inpari 13	6.9
6	YD1-48-1-2	7.00		Fatmawati	6.8
7	YD2-29-2-1	4.80*		Inpari 24	7.3
8	YD5-10-1-2	5.33*		Aek Sibundong	7.05
9	YD5-37-1-1	5.16*		Inpari 32	6.3
	LSD 5%				1.7
	LSD 1%				2.3
	CV (%)				11.7

1–6 = lines derived from the crossof Melik/Inpari13^2, 7 = Melik^2/Inpari13, 8–11 = Melik/Fatmawati^2, 12–13 = Melik^2/Fatmawati.
*Significantly different at α = 5%.

The days to flower of DH lines ranged from 75 to 84 days, while the days to harvest ranged from 115 to 125 days (Table 3), which were similar to the control varieties Inpari 13 and Fatmawati. However, these lines had earlier harvesting time (30 days faster) when compared to Melik, which are harvested at 150 DAS [11]. For flowering time, there were three lines with late flowering time, i.e. YD1-61-1-1, YD1-71-1-1 and YD2-29-2-1, at 84, 83 and 90 DAS, respectively. The difference in flowering time to Inpari 13 and Fatmawati was 3–10 days. Flowering and harvesting time are positively correlated with the character of grain weight per plant [12]. In other words, higher grain weight per plant typically requires longer flowering and harvesting time. Therefore, it is difficult to obtain an early maturing variety with high yield. However, this theory contradicted with the results of this research. DH line YD2-29-2-1 showed late maturity trait (125 days) but had low yielding ability (4.8 t/ha), as shown by the low number of filled grains and productive tillers. Susanto et al. [13] also reported similar results, where longer harvesting days was not followed by yield increase. However, the DH lines in this research were still categorized as early-maturing rice varieties (105−124 DAS) according to the Indonesian Center for Rice Research [13] and they produced higher yield when compared to one of the parents, the local black rice Melik.

Six DH lines, i.e. YD1-61-1-1, YD1-71-1-1, YD1-51-2-1, YD1-51-2-2, YD1-51-2-3 and YD1-48-1-2 had 16-17 productive tillers per plant (Table 3). These lines were not significantly different from Inpari 13 and Fatmawati as well as the other three control varieties. The number of productive tiller is an important agronomic trait in rice plant[14]. The number of productive tiller per plant is positively correlated with the character of grain weight per plant, and hence high yield on rice is supported by the number of productive tillers per plant [12].

Table 3. The average value of agronomical traits of DH lines observed at Sukamandi Experimental Station in dry season of 2017.

Genotype	DTF (DAS)	DTH (DAS)	HT (cm)	NPT (tiller/hill)	NTG (tiller/hill)	NFG	NEG	PL (cm)	W1000 (g)
YD1-61-1-1	84**	119*	113.2	17	150.6	130.8	19.8	27.44	26.3
YD1-71-1-1	83*	118*	110.6	16.8	159.9	135.5	24.4	26.41**	26.7
YD1-51-2-1	80	115	106	15.9	153.9	139	14.9*	26.22**	25.3
YD1-51-2-2	80	115	106.8	17.2	161.32	140.21	21.11	26.72**	24.3*
YD1-51-2-3	80	115	102.8**	18.9	149.9	127.56	22.3	25.78**	25
YD1-48-1-2	81	116	96.6*	16.7	160.34	142.23	18.11	26.47**	25.3
YD2-29-2-1	90**	125 **	130.8**	12.6*	143	124.33*	18.7	23.11**	26
YD5-10-1-2	80	115	93.6*	17.2	118.2**	97.00**	21.2	26.20**	23.7*
YD5-37-1-1	75	110**	105.4	14.4	167.8	134.67	33.1	27.21**	25.3
YD5-37-1-2	79	114	107.2	13.7	188.3	148.44	39.9**	26.04**	25.7
YD5-37-1-3	77	112*	115.3	13.4*	204	170.69	33.3	28.3	25.3
YD6-1-1-1	80	115	126.8**	14.3	171.6	157.78	13.8	26.34**	26.3
YD6-1-1-2	79	114	127.0**	16.2	163.8	135.89	27.9	26.77**	25.3
Inpari 13	80	115	111.9	15.6	206.6	190.44	16.1	30.36	26.3
Fatmawati	80	115	117.2	13.7	246.9	216.44	30.4	32.06	28
Inpari 24	81	116	106.7	19.2	145.6	134.67	10.9	27.57	27.3
Aek Sibundong	80	115	108.4	19.8	165.7	148.11	17.6	26.79	27.7
Inpari 32	85	120	109.7	18.3	159.2	145.56	13.7	23.48	27.3
CV %	4	2.8	8.9	13.8	17.2	18.3	36.1	7.6	4.5
LSD 5%	2.7	2.7	6.9	6,193	40.7	66.95	23.3	2.18	3.11
LSD 1%	3.6	3.6	9.2	8,311	54.9	89.71	31.2	2.93	4.18

DTF = days to flower, DAS = days after sowing, DTH = days to harvest, HT = plant height, NPT = number of productive tiller, NTG = number of grain per panicle, NFG = number of filled grain per panicle, NEG = number of empty grain per panicle, PL = panicle length, W1000 = weight of 1000 grains.
**Significantly different at α = 1%, *Significantly different at α = 5%.

Plant height, days to flower, number of productive tiller, panicle length and 1,000 grains weight had the coefficient of variance (CV) of less than 10%, while the number of grain per panicle, number of filled grains per panicle and yield had CV that ranged from 17–30%. The number of empty grain per panicle had the highest CV, i.e. 36% (Table 1). This means that generally, DH lines derived from

Melik/Inpari 13 or Melik/Fatmawati had similar characteristics in terms of plant height, productive tillers number, panicle length and grain size.

The plant height of two DH lines, namely YD1-48-1-2 and YD5-10-1-2, were shorter (94-97 cm) than Inpari 13, whereas three lines, namely YD2-29-2-1, YD6-1-1-1 and YD6-1-1-2, were taller (127−131 cm) than Inpari 13. Inpari 13 and Fatmawati had 112 cm and 117 cm height, respectively. Plants with high postures tend to experience lodging in the grain filling and maturity phases. Higher posture plants also tend to produce smaller numbers of tillers, while shorter plants have a large number of tillers. Consistent with that, our results showed that YD1-48-1-2 and YD5-10-1-2 lines with the plant height of 94–97 cm, had 17 tillers, while YD2-29-2-1 line with a plant height of 131 cm had 13 tillers (Table 3). The height of rice plants is influenced by genetic factors. The plant height of irrigated rice is usually lower (<130 cm) than upland or rainfed rice. Local rice usually has higher plant height (150 cm) and it correlates with large culm character.

Seed size is related to the weight of 1,000 grains. DH lines tested in this research had an average 1,000 grains weight of 26 g (Table 3). The highest weight of 1,000 grains was recorded in Fatmawati (28 g) and Inpari 13 (26.3 g), while the lowest was observed in YD5-10-1-2 (23.7 g). The grain size of DH lines was not significantly different from that of Inpari 13 and Fatmawati, except for YD1-51-2-2 (24.3 g) and YD5-10-1-2 lines that were significantly different from Fatmawati.

The number of empty grain per panicle did not affect the overall yield of DH lines tested. The average number of empty grains was 22 per panicle and the highest number was 39.9 empty grains per panicle. In the control varieties, Fatmawati produced 30 empty grains per panicles, while Inpari 13 produced 16 empty grains per panicles. Fatmawati and Inpari 13 apparently had a high number of empty grain per panicle. However, the percentage of empty grain was only 12 and 8% for Fatmawati and Inpari 13, respectively. Fatmawati often have a high number of empty grain (>30%) and also less total number of panicle. Thus, the number of filled grain per panicle could be less as well. Besides the number of productive tillers, the yield of rice also depends on the number of grains per panicle.

The diversity of grain color of the DH lines was only observed from the color of rice pericarp and its anthocyanin levels were not measured. The grain color in the progeny of Melik/Inpari 13^2 was reddish-purple, while darker color grain was expressed in the progeny of Melik/Fatmawati (Figure 1). According to the Annual Activity Report in 2017, the anthocyanin level of Melik variety was 300 ppm when tested by Yogyakarta AIAT, but it was more than twofold level (780.6 ppm) when measured by ICABIOGRAD.

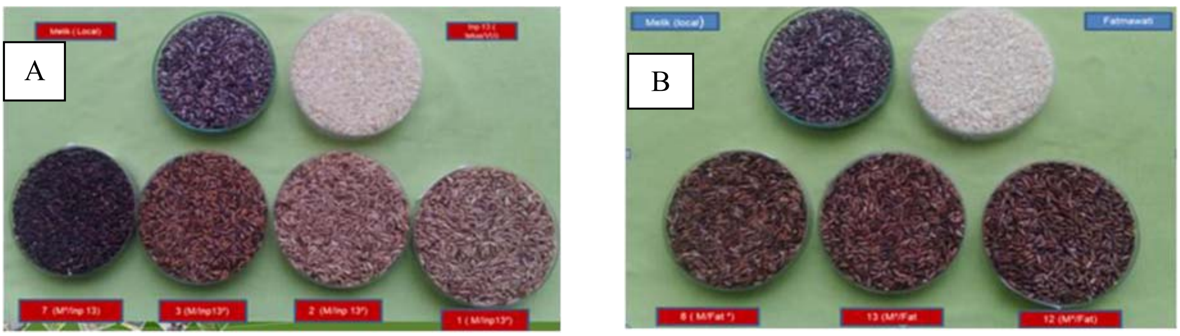

Figure 1. Diversity of rice color in DH lines progeny of Melik/Inpari 13^2 (A) and Melik/Fatmawati^2 (B).

4. Conclusions

Ten DH lines (YD1-61-1-1, YD1-71-1-1, YD1-51-2-1, YD1-51-2-2, YD1-51-2-3, YD1-48-1-2, YD5-37-1-2, YD5-37-1-3, YD6-1-1-1 and YD6-1-1-2) showed an early maturity trait (115 days), which was not significantly different to that of Fatmawati and Inpari 13. These lines can be categorized into two groups, one which had reddish-purple grain color (YD1-61-1-1, YD1-71-1-1, YD1-51-2-1, YD1-51-2-2, YD1-51-2-3 and YD1-48-1-2), and the other which had reddish-black grain color (YD5-37-1-

2, YD5-37-1-3, YD6-1-1-1 and YD6-1-1-2). The yield range of the first group, which derived from Melik/Inpari 13), was 6.1 to 7.13 t/ha. The second group, which was derived from Melik/Fatmawati[2], had yield in the range of 6.4 to 7.2 t/ha. Advanced yield trials in several locations need to be conducted to evaluate the agronomical performance and anthocyanin content, as well as their resistance to major pests and diseases in rice.

5. References

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