Indian J. Agric. Res., 52 (3) 2018 : 264-270
Print ISSN:0367-8245 / Online ISSN:0976-058X

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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Selection and field evaluation to increase yield and quality of several black rice accessions

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Received: 09-11-2017 Accepted: 21-04-2018 DOI: 10.18805/IJARe.A-306

ABSTRACT
Yield and quality of some local black rice cultivars were low as the panicle in the same clump do not reach maturity at the same time. This research program utilized plants of three local black rice cultivars of "Mariana", "Purwokerto", and "Toraja". The plants were planted in pots and placed under a plastic house. Some agronomic traits were measured, including number of productive tiller (NPT) and NPT with mature grains in the panicle>85% (NPT-85). Selected plants were determined by quadrant method of NPT-85 over NPT. After three selection cycles, the research reveals four potential accessions of Mariana-58.07.(10,17,21,28),Mariana-96.04 (11,15,23,33); Purwokerto-06.09(10,23,27), and Toraja-06.07(11,13,19). These four accessions called as Unsri P-1 to Unsri P-4, respectively. Field evaluation indicated, the accessions were able to produce high quality grain with NPT-85 over NPT ratio ranged from 87% to 95 %, and yield potential ranged from 6.16 to 8.81 dry un-hulled grain t/ha.

Key words: Black-rice, Quality, Selection, Yield.

INRODUCTION

Despite its color, black rice accessions apparently become popular, due to the fact that such rice accessions contain anthocyanin pigment fraction that has been proven as an antioxidant to cure several major health problems including coronary disease, hepatitis, high blood tension, and diabetes mellitus. Yodmanee, *et al.*, (2011) reported that anthocyanin content of black rice accessions of BWR-96025 and BWR-96044 (about 129 and 245 mg Cy-3-glc/100 g respectively), are very much higher than anthocyanin content of brown rice accession of RWR-96060 (16.69 mg Cy-3-glc/100 g). Scientists have proven that black-rice and its anthocyanin pigment fraction called as BRF could exert cardio-protective effect on coronary heart diseases (Wang *et al.*, 2007), reducing the risk of hepatic steatosis and blood glucose level (Jang *et al.*, 2012), enhancing stabilization of apolipoprotein inhibit low density lipoprotein (LDL) oxidation, and reducing blood glucose level (Xia *et. al.*, 2016).

Researchers said that the origin of black rice cultivars might be from Asian countries including China, Vietnam, India, and Japan (Mingwei *et. al.*, 1995; and Hoahua *et.al.*, 1996; Sastry, 1978; Natsumi and Noriko, 1994). While Chaudary and Tran (2001) reported the origin of black rice might be come from Sri Lanka, Bangladesh, the Philippine, Thailand, and Indonesia. Kristiani *et.al.* (2012) identified 11 local black rice cultivars collected from Yogyakarta and its surrounding area. Based on the average taxonomic distance, they conclude these black rice cultivars could be grouped into 5 genetic clusters which include cultivar of "Melik", "Jliteng", "Cempo Ireng", "Padi Ireng", and "Situ Bagendit".

Black rice is, indeed, getting more popular as a functional food. Unlike research about nutrition aspects, such as, physicochemical, content and effects of anthocyanin, efforts of scientists to develop a superior black rice cultivars, are still very little. This research therefore, was intended to evaluate growth, yield, and to initiate selection program to increase yield and quality of several local black rice cultivars.

MATERIALS AND METHODS

Genetic materials. : The research utilized genetic materials of three local black rice cultivars "Mariana", "Purwokerto", and "Toraja", which were considered as low-land rice types as initial populations for selection. Random sample of the seeds of "Mariana" cultivar were directly obtained from the farmer, while "Purwokerto" and "Toraja" cultivars were obtained from seed producers through on-line market. Viability test indicated these seed samples had viability of more than 95%.

Selection: Series of researches to obtained selected plants were conducted in 2014 to 2016. Selections were done by using plants grown in a 10-kg pots (Fig 1) at experimental station of Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Ogan Ilir (Haryadi, 2015; Kholiq, 2016; and Saputra, 2016). For the first part of selection, about

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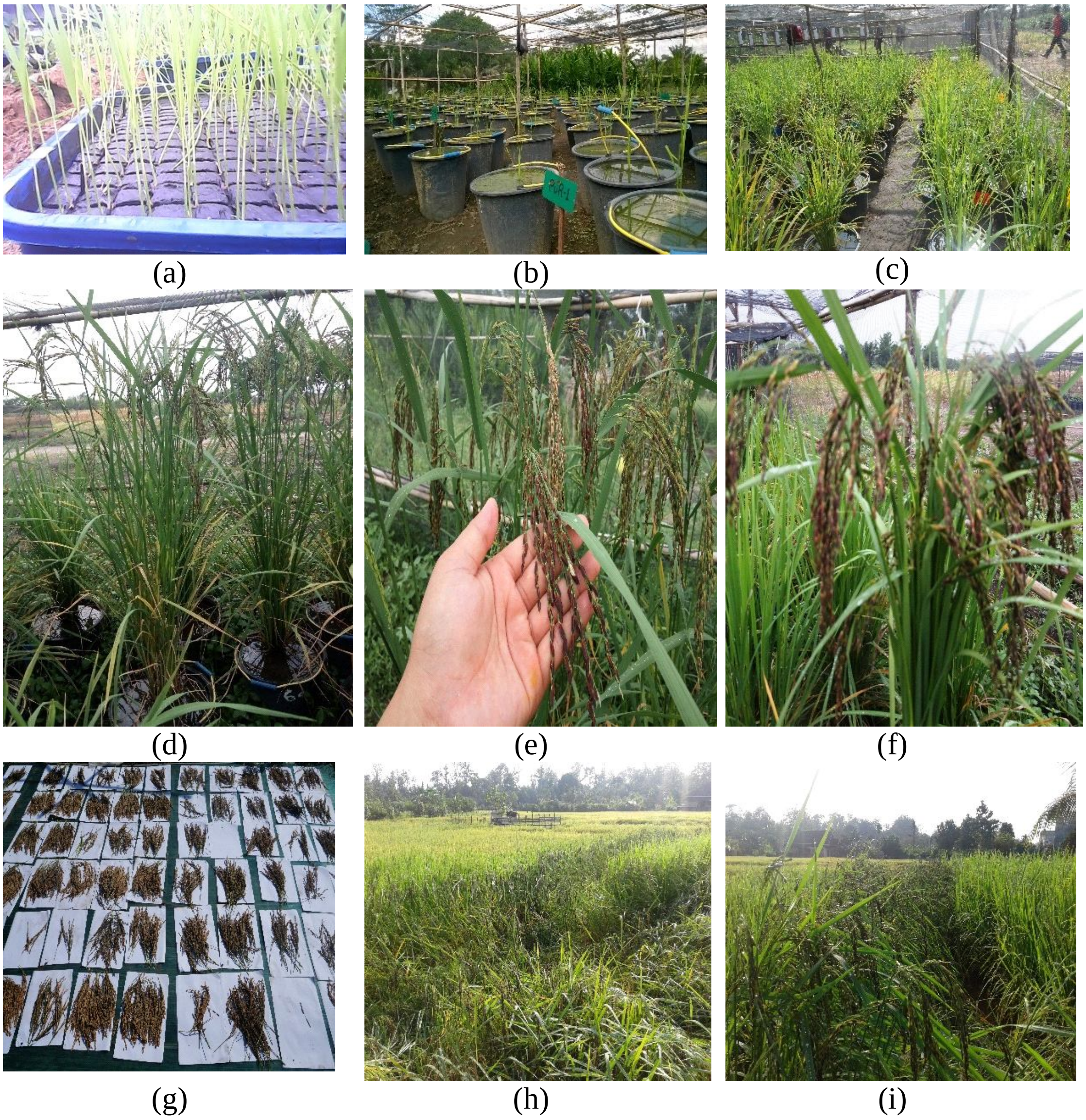

Fig 1: Research materials of 7-days old seedling (a); A single seedling planted in 10 kg pot (b); 8-weeks old black rice plants (c); and 18-weeks old black rice plants with panicles grown by using pots (d,e,f); harvested panicle of each entry (g) and some black rice accession plants grown in the field (h,i)

five hundreds seed samples of each cultivar separately soaked on petri dish for 24 hours and then germinated in the media to produce 7-days old plants. A single plant of these 7-days old plant, then planted in 10-kg pots (Fig 1). Each cultivar consisted of 200 pots that placed under a plastic house, according to the completely randomized design (CRD). The media to be used in this study consisted of regular organic soil and chicken manure with 50:50 ratio(v/v). This media had been prepared for about a month before experiment was started. Furthermore, standard practices for weeding, pest control, and NPK fertilizer were applied (20 g/pot). Level of water surface was control by making 3-5 holes corresponding to the media surface in each pot. Observation and measurement were done on each individual plant in a pot. Observed variables consisted of plant height (PH) in cm; harvesting time (HT) in day; total number of tiller (TNT); number of productive tiller per clump (NPT); tiller number of productive tiller per clump with mature seed in the panicle > 85% (NPT-85); weight of wet un-hulled grain (WWG) in gram/clump; weight of dry un-hulled grain (WDG) in gram/clump, weight of 100 dry un-hulled grain (WDG-100) in gram, predicted yield potential (PYP) in dry unhusked grain t/ha. These observed variables were alike with a research done by Banumathy et.al. (2018). For simplicity in analyzing and selecting superior plants, collected data were plotted as shown in Fig 2.

About similar procedure was applied for the second and the third part of selection which was performed by using “Early Generation Testing” as described by Fehr (1987) along with “Directed selection Method” as out lined by Lerner (1958). The differences with the first part of selection, was in number of plants observed that depend on the number of selected plants to be used in selection. On the second part of selection, random sample of seed of selected plants

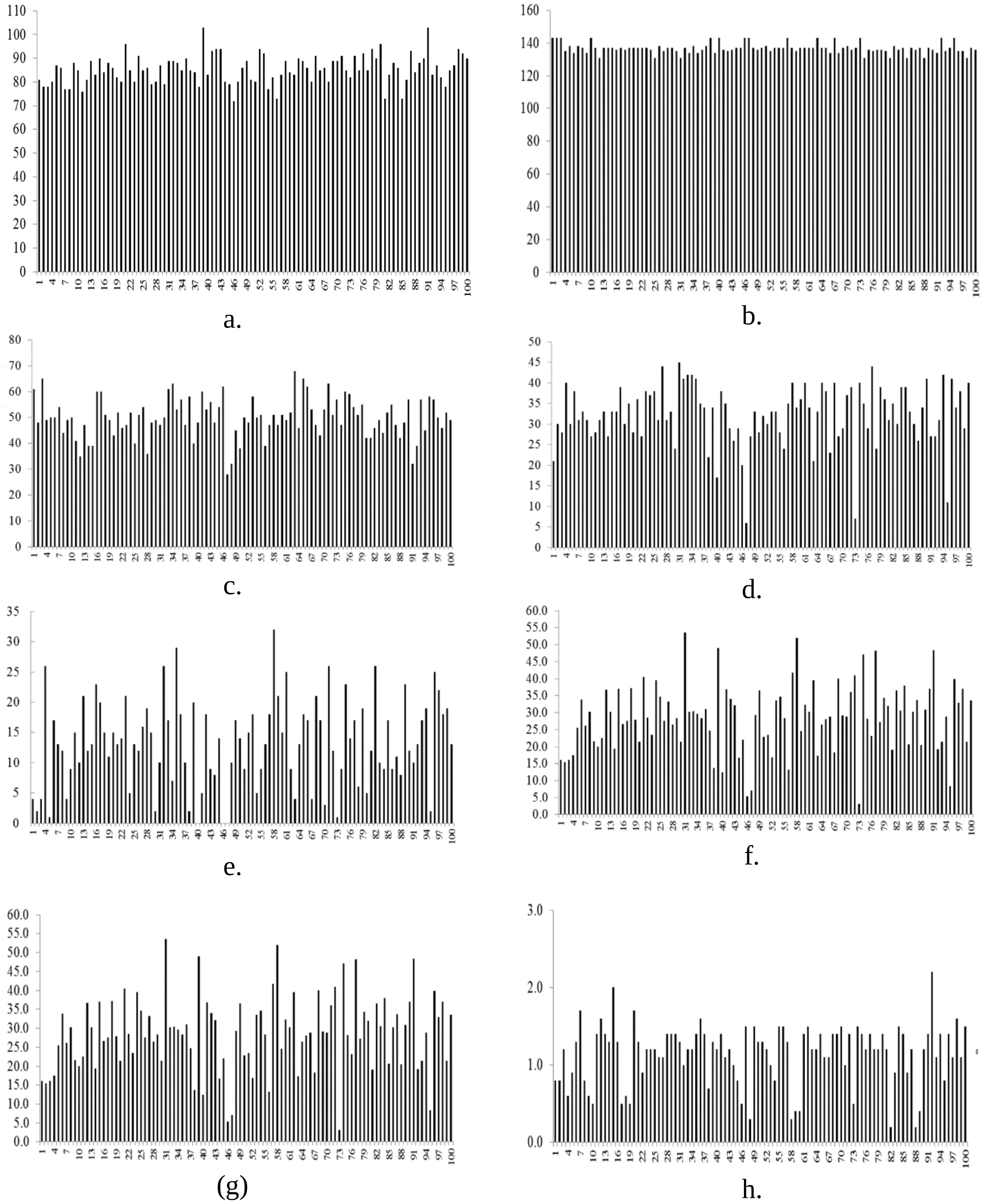

Fig 2: Example of the graph describing collected data of plant height (PH) in cm (a); harvesting time (HT) in day (b); total number of tiller (TNT) per clump (c); number of productive tiller per clump (NPT) (d); e. number of productive tiller per clump with mature seed in the panicle > 85% (NPT-85) (e); weight of wet un-hulled grain (WWG) in gram/clump (f); weight of dry un-hulled grain (WDG) in gram/clump (g); and weight of 100-dry un-hulled grains (WDG-100) in gram of 100-plant number (h) at initial populations for selection of cultivars “Mariana”. The “X” axis is plant numbers of 1 to 100, and the “Y” axis is a respected collected data.

(15-20 Plants) in each cultivars were grown using 10-kg pots, observed and measured to determine superior-parental plants to be used in the next selection cycles. While on the third part of selection, bulking seeds of selected plants (3 -4 plants) were grown in the field. The comparison between cultivars of certain variables was done by using Fisher's LSD test following Analysis of Variance (ANOVA) at α=0.05. (Milliken and Johnson, 1992). Data calculation was done by using computer application of Statistical Analysis System (SAS-Institute, 1985). Furthermore, selection for superior plants was done by using the "Quadrant Method" of certain variable, especially variables of NPT and NPT-85. Example of this method was shown in Fig 3.

Field evaluation: The activities were carried out at low-land farm area in cooperation with local farmer at Muara Enim, South Sumatra in December 2015 to March 2016. Plants of each accessions were grown by using spaced-planted method of 20 cm x 20 cm in randomized complete block (RCB) pattern, consisted of three equal blocks with total area about 300 m^2 (Fig 1). Measurements on several variables as stated above were taken on 20 clumps per experimental plot. Data were analyzed by using computer application of Statistical Analysis System (SAS-Institute, 1985).

RESULTS AND DISCUSSION

Initial population and selection.: Values of mean and standard deviations of each observed variable that was measured on initial populations were shown in Tabel 1. Test statistic by using Fisher's LSD test at alpha=0.05 indicated significant difference for most observed variables. The plant height (PH) ranged from 85 to 100 cm and harvesting time (HT) range from 120 to 136 days. Although, variable of number of productive tiller (NPT) was not significantly different, total number of tiller (TNT) per clump and number of productive tiller with mature grains in a panicle > 85% (NPT-85) were significant. The calculated values of NPT-85 over NPT were only 59.95 for "Mariana", 90.45% for "Purwokerto" and 83.01 % for "Toraja", with potential yield of 5.63, 7.48. and 7.31 tons dry un-hulled grains per ha, respectively.

This research indicated that growth and productivity of initial populations of local black rice cultivars varied greatly either between cultivars or among plants in the cultivar that can be shown as significant differences of the mean value and large value of standard deviation on the variable observed (Table 1). As stated by Lerner (1958), the presence of large amount in variation value in any cultivars will essentially contributes for low quality of the product, and for that reason, a directed selection methods should be applied to increase or decrease mean value and decrease variability.

As shown on Table 1, plant height (PH) was significantly different, in which cultivar "Mariana" having lower height of 85.47 cm, than "Purwokerto" cultivar (100.30 cm). The standard deviation also large, ranged from 6 to 9 cm. Due to lodging sensitivity problem, rice plants with lower plant height are more desirable for further

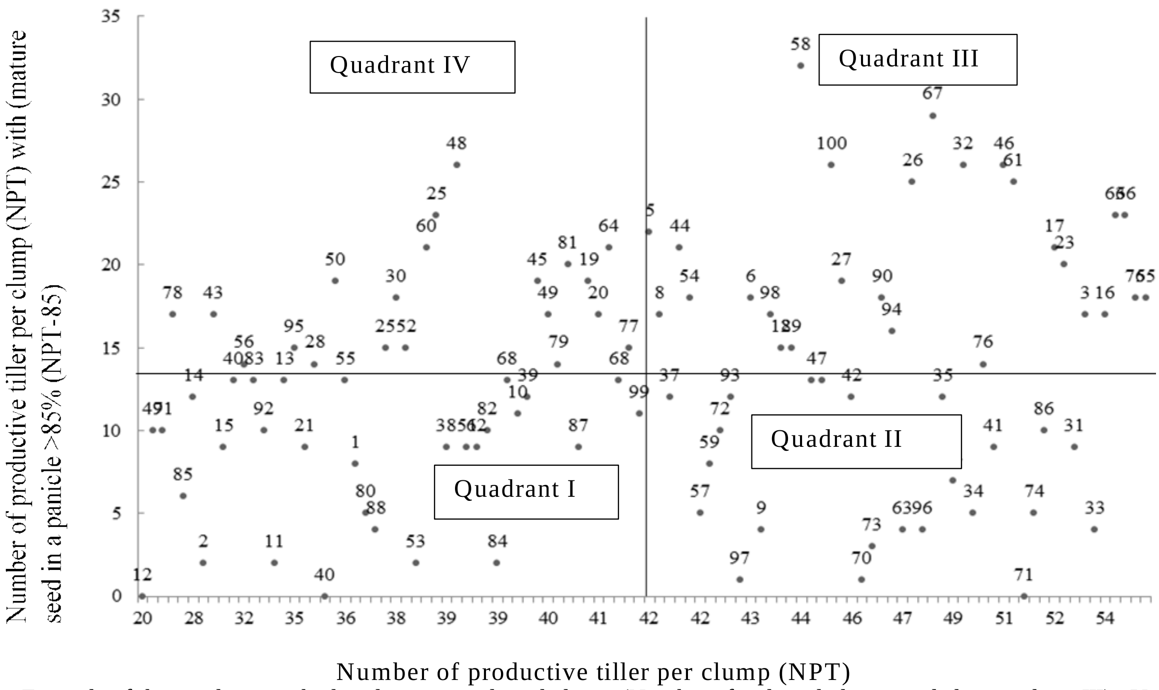

Fig 3: Example of the quadrant method to determine selected plants. (Number of selected plants resided in quadrant III). Vertical and horizontal lines indicated an average value of NPT and NPT-85, respectively.

Table 1: Values of mean ± standard deviation of each variable measured at original population of local black rice cultivar of "Mariana", "Purwokerto", and "Toraja" at initial selections in 2014.

Variables	Unit	Values of mean ± standard deviation of variable in the cultivars		
		"Mariana"	"Purwokerto"	"Toraja"
Plant height (PH)	cm	85.47[a] ±8.78	100.30[b]±9.13	90.5[a]±6.43
Harvesting time (HT)	day after planting	136.32±13.50	120.44±12.10	125.35±12.62
Total number of tiller (TNT)	tiller per clump	37.26[c]±9.61	26.30[b]±6.20	21.03[a]±5.72
Number of productive tiller (NPT);	tiller per clump	21.30±8.20	20.53±5.91	20.42±5.25
Number of productive tiller with mature grains in the panicle > 85% (NPT-85);	tiller per clump	12.77 [a]±6.10	18.57[b] ±6.82	16.95[b]±5.91
Weight of wet un-hulled grain (WWG)	g per clump	28.53 [a]±8.63	37.65[b]±9.12	29.24[a]±7.32
Weight of dry un-hulled grain (WDG)	g per clump	22.51[a] ±9.30	29.92[b]±10.31	24.84[a]±9.10
Weight of 100-dry un-hulled grain (WDG-100)	g	1.94±0.53	2.52±0.72	2.31±0.23
Percent of NPT-85 over NPT (calculated)	%	59.95	90.45	83.01
Potential yield (dry un-hulled grains) **	t/ha	5.63	7.48	7.31

Values of the means in the same row, followed by the same letter mean non-significantly different according to Fisher's LSD test at alpha=0.05. **=Assumed 250.000 plants per ha.

development. Other variable of harvesting time (HT) ranged 120 to 136 days after planting, which was not statistically different. The harvesting time consider very long, of more than 120 days and having larger standard deviation of more than 12 days (almost two weeks). Further selection program of these cultivars, therefore, need special attention to decrease harvesting time and standard deviation value, so the plants in the cultivar become more homogenous.

Furthermore, most variables for yield-components showed differences, except for weight of dry un-hulled grain (WDG) per clump (Table 1). In general, cultivar of "Purwokerto", performed better yield as compared to others because of having greater mean values of NPT-85, WWG, and WDG, of 18.57 tillers; 37.65 gram, and 29.92 gram per clump , respectively. The cultivar of "Mariana", however seemed to have higher potential for further development. Initial population of cultivar "Mariana" had the lowest mean value of NPT-85 (12.77 tillers per clump), but produced very high TNT and NPT with mean value of 37.26, and 21.30 tillers per clump that significantly greater than other cultivars. The NPT-85 variable created in this research, was considered as very important variable for selecting high quality grain of rice. The NPT-85 measures the number of productive tillers having mature grains in a panicle more than 85%. This value, therefore, clearly describes the amount of mature grains to harvest, that ultimately will contribute to physical quality of rice grain. The lower value of NPT-85 will indicate greater portion of broken grain in the product, while ratio of NPT-85 over NPT values will count the amount of broken-grains in the product. As shown in Table 1, the mean value of NPT-85 of "Mariana" cultivar was considered very low of 12.77 tiller/clump (about 59.95.04%) as compared to "Purwokerto" and "Toraja" cultivars with NPT-85 values of 18.57 tiller per clump (about 90.45.5%) and 16.95 tiller per clump (about 83.01%), respectively. By selection program, called as "directed selection method" (Lerner, 1987), this value could be increased to certain desirable level. The selection, basically, was known as "Pedigree Selection Technique" and it has been proven to be effective, especially, to breed self- pollinated crops such as rice (Fehr, 1987). As shown on Table 2. after three cycles of selection, this research come up with four black rice accessions. For the purpose of institutional branded, the resulted accessions called as Unsri-P1, Unsri-P2, Unsri-P3 and Unsri- P4. These accessions derived from bulking seeds of parental ID number of Mariana 58.07(10,17,21,28); Mariana 96.04(11,15,23,30); Purwokerto 06.09(10,23,27); and Toraja 06.07(11,,13,19), respectively.

Field Evaluation: Field evaluation, in general, indicated significant improvement on growth, yield and quality of black rice accessions. The plant height (PH) decreased to be 75 to 93 cm, and harvesting time (HT) range from 120 to 130 days. The number productive tiller (NPT) increase with range 20 to 23 tillers per clump, as well as productive tiller with mature seed in a panicle > 85% (NPT-85) increased to be 17 to 22 tillers per clumps. There was a huge increase in calculated values of ratio NPT-85 over NPT, especially on accessions derived from "Mariana" cultivar, Unsri-P1 and Unsri P-2, with values of 95.17 % and 87.73 %. Along with that, the yield potential of those accessions also increased to be 6 to almost 9 tons dry un-hulled grains per ha (Tabel 3).

Field evaluation was carried out at low-land farm area in Muara Enim, South Sumatra in December 2015 to March 2016. It was a rainy season and this area considered as a centre for rice production in South Sumatera Indonesia. Result of field evaluation (Table 3), in general, showed better performance and even improvement of those accessions. Number of productive tiller with mature grains in the panicle > 85% (NPT-85) values, were not significantly different among the accessions, but indeed, there were huge improvement, especially, for Unsri-P1 and Unsri-P2. The

Table 2: Selected plants on each cycle of selection program to increase yield and quality of several local black rice accessions

Breeding item and identity	Cultivars			
	"Mariana"		"Purwokerto"	"Toraja"
Selected plant number on 1st cycle of selection	58	96	06	06
Selected plant number on 2nd cycle of selection	07	04	09	07
Selected plant number bulked on 3rd cycle of selection	10,17,21,28	11,15,23,30	10,23,27	11,13,19
Parental ID Number	Mariana 58.07 (10,17, 21,28)	Mariana 96.04 (11,15,23,30)	Purwokerto 06.09 (10,23,27)	Toraja 06.07 (11,13,19)
Breeding ID code	C1:Mariana-58	C2:Mariana-96	C3:Purwokerto-06	C4:Toraja-06
Accession name	Unsri-P1	Unsri-P2	Unsri-P3	Unsri-P4

Table 3: Values of mean ± standard deviation of each variable measured on black rice accessions of Unsri-P1, Unsri-P2, and Unsri- P3 derived from selections program of local black rice cultivars planted in the field at 2016.

Variable measured accession	Values of mean ± standard deviation of variable in the			
	Unsri-P1	Unsri-P2	Unsri-P3	Unsri-P4
Plant height (PH) in cm	75.81^{a}±3.73	75.52^{a}±2.34	93.21^{c} ±7.70	85.30^{b}±6.32
Harvesting time (HT) in day after planting.	130.44±10.70	127.50±9.21	120.32±8.62	120.43±7.31
Total number of tiller (TNT) per clump	28.34^{b}±7.95	27.82^{b}±7.13	26.40^{b}±4.42	21.72^{a}±5.62
Number of productive tiller per clump (NPT);	22.80^{b}±6.91	23.71^{b}±4.82	20.62^{a}±4.80	20.53^{a}±5.24
Number of productive tiller per clump with mature grains in the panicle > 85% (NPT-85);	21.70±5.93	20.80±4.93	19.76±4.11	17.97±7.62
Weight of wet un-hulled grain (WWG) in gram/clump	28.72^{a}±7.64	29.53^{a}±6.82	38.24^{b}±6.31	29.90^{a}±7.22
Weight of dry un-hulled grain (WDG) in gram/clump	24.64^{a}±6.01	25.42^{a}±5.12	35.26^{b}±7.13	26.83^{a}±7.80
Weight of 100-dry un-hulled grain (WDG-100) in gram.	2.62±0.13	2.84±0.31	3.06±0.24	2.93±0.26
Percent of NPT-85 over NPT (%)	95.17	87.73	95.83	87.53
Potential yield (dry un-hulled grain t/ha) **	6.16	6.35	8.81	6.71

NPT-85 values of these accessions reached over 20 tillers per clump (about 38% to 63 % increase). The calculated ratio of the NPT-85 over NPT values of the accessions has also increase greatly of 95.17% for Unsri-P1, 87.73% for Unsri-P2, 95.83% for Unsri-P3, and 87.53% for Unsri-P4. Furthermore, field evaluation also showed progressive increase in yield potential that reached 6.16; 6.35, 8.81, and 6.71 tons dry un-hulled grains per ha, respectively. With great values of the NPT-85 over NPT, these four black rice accessions will be able to produce high yield and quality, since they will have less portion of broken grain in the product.

CONCLUSION

This research concludes that local black rice cultivars of "Mariana", "Purwokerto", and "Toraja", at initial populations for selection, exhibited a large variability in growth and yield either between cultivars or among the plants in the cultivar. The quality, as described by the value number of productive tiller per clump with mature grains in the panicle > 85% over number of productive tiller per clump (NPT-85 over NPT) were also low. Further selection program on these populations resulted in four black rice accessions, namely Unsri-P1, Unsri-P2, Unsri- P3, and Unsri-P4. In the field, these accessions showed progressive increase in the value of NPT-85 over NPT that ranged from 87% to 95 %. The yield potential was also progressively increased from 6.16 to 8.81 tons dry un-hulled grain per hectare. These four black rice accessions, therefore, will be able to produce high yield with high-quality grain as they might have less portion of broken grains in the product.

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