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Identification of drought tolerant markers, *DREB2A* and *BADH2* genes, and yield potential from single-crossing varieties of rice in Bengkulu, Indonesia

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Abstract. *Herawati R, Alnopri, Masdar, Simarmata M, Sipriyadi, Sutrawati M. 2021. Identification of drought-tolerant markers, DREB2A and BADH2 genes, and yield potential from single-crossing varieties of rice in Bengkulu, Indonesia. Biodiversitas 22: 785-793.* This study aimed to identify drought-tolerance and molecular characteristics of *DREB2A* and *BADH2* genes, as well as yield potential from single-crossing varieties of rice in Bengkulu. The drought-sensitive varieties of IR20 and Salumpikit (as the control plants) and 39 F6 progeny lines were used in the screening at seedling stages in the greenhouse. The Standard Evaluation System (SES) developed by IRRI was used to assess the recovery ability of tested varieties/lines. The molecular analysis used to detect the presence of the *DREB2A* gene was carried out by PCR amplification in the genomic DNA using the forward and reverse oligonucleotide primers consisting of CCTCATTGGGTCAGGAAGAA and GGATCTCAGCCACCCACTTA, respectively. Meanwhile, for the *BADH2* gene, the forward and reverse oligonucleotide primers of GGCCAAGTACCTCAAGGCGA and TGTCCCCAGCTGCTTCATCC were used, respectively. Molecular markers of *DREB2A* and *BADH2* genes were identified in 39 tested lines with approximately 250 and 2300 bp lengths. This result showed that the progeny of F6 lines generated from crossing the local varieties of IR7858 and IR148 are potential drought-tolerant upland rice varieties. Line numbers BKL2 B-2-264-6 and BKL4 B-1-268-10 have a potential yield of more than 12 t ha^{1} and can be developed on rain-fed, lowland, or dry land due to its drought tolerance.

Keywords: *BADH2, DREB2A,* drought tolerance, gene identification, yield potential

INTRODUCTION

Upland rice cultivation is an alternative strategy to increase the annual rice production in Indonesia, which has been significantly decreasing during the last decade due to an increase in lowland conversion. According to the Center for Research and Development (2006), this is carried out by optimizing the use of uncultivated lands, which are potential for upland rice cultivation. The use of high-yielding superior varieties tolerant to various obstacles is urgently needed to support efforts to increase rice yield in the dry land. Furthermore, it is important to anticipate the impact of climate change on sustainable agricultural systems by producing technological innovations that can overcome and suppress the impacts caused, such as by assembling the superior varieties of drought-tolerant rice. The genetic improvement to produce superior varieties that are adaptive to the drought stress conditions is an essential priority in rice breeding programs.

Crossbreeding is used to assemble drought-tolerant rice varieties, which combines the resistant traits of the parents with other high yield varieties. Molecular marker technology can be used to select the desirable traits more accurately through marker-assisted selection (MAS), and one of the markers related to drought tolerance is the QTL marker (quantitative trait locus) 12.1. Furthermore, the International Rice Research Institute (IRRI) crossed the Vandana variety of Indian rice using Way Rarem from Indonesia, which generated the filial with crossing number IR148+, derived from IR crossing 79971-B-369-B-B (Mulyaningsih et al. 2010). The crossing population contains QTL 12.1 markers, with the location on chromosome 12, as well as between SSR markers RM28048 and RM 511 (McCouch et al. 2002). The presence of these markers maintains yields before flowering and during severe drought stress at the reproductive stage. In normal conditions, the marker QTL 12.1 had an insignificant effect on some of the parameters observed (Bernier et al. 2007).

According to Matsukura et al. (2010), Srivastav et al. (2010), Akhtar et al. (2012), and Huang et al. (2018) *DREB2* gene controls the drought stress tolerance in plants. DREBs (Dehydration Responsive Element Bindings) are essential transcription factor proteins (TFs) used in regulating the expression of drought-responsive genes (Lata and Prasad 2011; Fujita et al. 2013). Sakuma et al. (2002) stated in rice that the *DREB2* gene is homolog to *DREB2A*. Some of the *DREB2A* target genes are *MT2A, At1g69870, At3g53990, At1g22985, RD29A, LEA14, At2g23120, RD29B, At1g52690, RD17* (Sakuma et al.

2006; Qin et al. 2011), *AtHsfA3*, *HSP18.2*, and *Hsp70* (Qin et al. 2011). *DREB2A* gene is important because it can be used as a regulator of various types of drought-responsive genes, making it a marker of drought stress-tolerance genes.

Osmotic adjustment in cells is the primary response of plants to drought (Zivcak et al. 2016; Blum 2017). Previous studies carried out by Sakamoto and Murata (2000) and Saxena et al. (2019) indicated that osmoprotectant substances, such as glycine betaine, play an essential role in cell stabilization by balancing the structure of the protein quaternary and membrane structure against the adverse effects of salinity. Besides, it facilitates osmotic adjustment by lowering the internal osmotic potential, which contributes to the ability to tolerate water pressure, stabilizing the PSII and RuBisCO complexes in the process of photosynthesis under seizure conditions (Holmström et al. 2000; Wang et al. 2019; Huang et al. 2020). The positive effects and exogenous application of glycine in plants grown under salinity stress conditions have been shown to protect plant cells (Demiral and Türkan 2004; Saxena et al. 2019).

Betaine aldehyde dehydrogenase (BADH) is a key enzyme for biosynthesis of glycine betaine. Several studies have reported the accumulation ability of glycine betaine and *BADH1*gene expression in tolerating salinity, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). The objective of this study was identification of drought-tolerance traits and molecular analysis of *DREB2A* and *BADH2* genes in the progeny of F6 lines derived from the crossing of local varieties (Sriwijaya and Bugis) with drought-tolerant varieties of IR7858 and IR148+ positive QTL on the chromosome.

MATERIALS AND METHODS

Plant materials

This research was carried out at the University of Bengkulu, Indonesia. The rice lines screening was conducted at the Greenhouse of Agricultural Faculty from February to April 2020, while molecular analysis was done in the laboratory of the Department of Biology from May to July 2020. The plant materials used were the progenies of 39 lines selected from F6 generations from single crossing of Bengkulu local varieties (Sriwijaya and Bugis) with drought-tolerant varieties of IR7858 and IR148+ QTL positive on chromosome 12.1 (Mulyaningsih et al. 2010). Table 1 shows the selected F6 lines for traits and molecular identification of drought-tolerant genes of *DREB2A* and *BADH2*, and var. Salumpikit and IR 20 as drought-tolerant and sensitive controls.

Drought tolerance screening

The standard Evaluation System (SES) developed by IRRI (2002) was used to screen the drought-tolerant rice from 39 F6 lines with the drought-susceptible variety (IR20), and local drought-tolerant variety (Salumpikit) used as control. Furthermore, the test was carried out in accordance with Kumar et al. (2015), Swain et al. (2017), and Herawati et al. (2017) methods with plastic trays of 40 cm x 25 cm x 20 cm size filled with soil. Each tub was planted using ten family lines and two control varieties, with each line sown for 20 seeds in a row. After planting, the seedlings were intensively watered for 2 weeks with the plants allowed to dry to determine their sensitivity. Drought tolerance assessment was carried out based on the SES methods, as shown in Table 2. Furthermore, the trait responses of the seedlings were recorded, followed by intensive watering for the next ten days. Recovery ability was recorded following the methods of SES, as shown in Table 2.

DNA extraction

Genomic DNA was isolated from fresh leaves 14 days after treatment (DAT) with a weight of 100 mg of rice leaf was added with liquid nitrogen and then ground using a mortar. The total DNA was isolated by modifying the protocols of Wizard's Genomic DNA Purification Kit, and the ground leaf was put into a 2 mL tube, added with 600 µL of Nuclei Lysis Solution, and shaken for 3 seconds. In addition, the solution was heated in a water bath at 65ºC for 15 minutes, then added with 3 µL RNase and incubated at 37 °C for 15 minutes. This was followed by the addition of 200 µL Precipitation Solution, and the microtubes containing the mixture were centrifuged for 3 minutes at 13,000 rpm. The supernatants were collected into a 1.5 mL tube, and then added with 600 µL of isopropanol. Furthermore, the microtubes were further centrifuged for 1 minute at room temperature, then the supernatant was discarded while the remaining DNA on the bottom of microtubes was air-dried for 15 minutes. DNA Rehydration Solution of 100 µL was added and further incubated at 65ºC for 1 hour or at 4ºC overnight. The total isolated DNA was used as a template for PCR amplification of *DREB2A* and *BADH2* genes.

DNA amplification and gel electrophoresis

PCR amplification of the DREB2A gene was determined using the forward and reverse oligonucleotide primers of CCTCATTGGGTCAGGAAGAA and GGATCTCAGCCACCCACTTA, respectively (Jadhao et al. 2014; Kumar 2016; Lathif et al. 2018). Meanwhile, the amplification of the BADH2 gene was carried out using forward- and reverse- oligonucleotide primers of GGCCAAGTACCTCAAGGCG and TGTCCCCAGCTGCTTCATCC (Robin et al. 2003). The PCR mixtures, which comprise the DNA template, forward and reverse primers, buffer, dNTP (nucleotide mix), and Taq polymerase, were developed during the thermocycling process. The program was carried out using a denaturation temperature at 94ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing at 59ºC for 2 minutes, and extension at 72ºC for 2 minutes, and the final extension at 72ºC for 10 minutes. PCR amplification products were subjected to electrophoresis in an agarose gel of 1% on TBE buffer to identify successful amplifications. The gel from electrophoresis was immersed in EtBr 1% for 10 minutes, rinsed with ddH2O for 5 minutes, and visualized under UV transilluminator light.

Table 1. Selected F6 lines for traits and molecular identification of drought-tolerant genes of *DREB2A* and *BADH2*

Lines number	Genotype	Initial crossing	Line number	Genotype	Initial crossing
1	262.A1.4-1	Bugis x IR148	22	259-6	Bugis x IR7858
2	260.A3.2	Bugis x IR7858	23	259-9	Bugis x IR7858
3	260.A3.2	Bugis x IR7858	24	259-15	Bugis x IR7858
4	262.A1.4-2	Bugis x IR148	25	260-21	Bugis x IR7858
5	262.A1.4-3	Bugis x IR148	26	260-26	Bugis x IR7858
6	260.A3.2	Bugis x IR7858	27	262-43	Bugis x IR148
7	262.A1.4-4	Bugis x IR148	28	262-48	Bugis x IR148
8	260.A3.2	Bugis x IR7858	29	255-59	Sriwijaya x IR148
9	262.A1.4-5	Bugis x IR148	30	253-2	Sriwijaya x IR148
10	262.A1.4-6	Bugis x IR148	31	259-17	Bugis x IR7858
11	251-17	Bugis x IR148	32	259-3	Bugis x IR7858
12	248-14-1	Bugis x IR7858	33	254-54	Sriwijaya x IR148
13	249-15-1	Bugis x IR7858	34	258-60	Sriwijaya x IR7858
14	250-16	Bugis x IR148	35	255-56	Sriwijaya x IR148
15	247-13	Bugis x IR7858	36	262-44	Bugis x IR148
16	269-11	Sriwijaya x IR7858	37	262-46	Bugis x IR148
17	248-14-2	Bugis x IR7858	38	259-18	Bugis x IR7858
18	249-15-2	Bugis x IR7858	39	259-4	Bugis x IR7858
19	267-9-1	Sriwijaya x IR148	I	IR20	Control variety
20	267-9-2	Sriwijaya x IR148	S	Salumpikit	Control variety
21	259-1	Bugis x IR7858			

Table 2. Traits identification of selected F6 lines based on criteria description of SES developed by IRRI (2002)

Score	Criteria	Description		
		Leaf rolling	Leaf drying	Recovery ability
0	Highly Tolerant	Leaves are healthy	No symptoms	100 % plant recovered
1	Tolerant	Leaves start to fold (shallow)	Slight tip drying	90-99% of plants recovered
3	Rather Tolerant	Leaves are folding (deep V-shape)	Tip drying extended up to ¼	70-89% of plants recovered
5	Moderate tolerant	Leaves are fully cupped (U-shape)	One-fourth to 1/2 of all leaves dried	40-69% of plants recovered
7	Moderate susceptible	Leaf margins are touching (0-shape)	More than 2/3 of all leaves fully dried	20-39% of plants recovered
9	Susceptible	Leaves are tightly rolled (V-shape)	All plants are dead. Length in most leaves thoroughly dried	0-19% of plants recovered

Field experiment and yield potential evaluation

A yield performance test of selected superior lines on previous experiments was carried out from March-July 2020 in Semarang Village, Bengkulu City. The materials used in this study were 16 selected superior lines in the F7 generation with the experiment carried out on a plot measuring 8 m x 6 m, a spacing of 20 x 20 cm, and by planting a seed. The first fertilization process was carried out at the age 14 days with a dose of 150 kg ha^{-1} of Urea, 100 kg ha^{-1} SP36, and 100 kg ha^{-1} KCl. The second fertilization was carried out at the age of 30 HST with a dose of 100 kg ha^{-1} urea, 100 kg ha^{-1} SP36, and 100 kg ha^{-1} KCl. Furthermore, intensive control was carried out against weeds, pests, and diseases, while observation of the agronomic characters of 10 plant per-plot samples was taken from each line number. The characters observed included plant height, number of panicles per-hill, panicle length, number of filled grains per-panicle, percentage of empty grain per-panicle, 1000 grain weight, grain weight per-hill, and yield per-plot.

RESULTS AND DISCUSSION

Identification of drought-tolerance level

Screening of F6 lines at the seedling stage was carried out to select the drought-tolerant rice. Table 2 shows the drought tolerance assessment carried out with the SES methods by comparing the treated lines with control varieties of Salumpikit and IR20. The symptoms, such as leaf curing, drying, and recovery ability, were identified after exposure to drought stress for 14 days, as shown in Figure 1. The criteria of 39 F6 lines were identified as highly to moderately tolerant to drought of 6, 5, 17, and 11 lines, respectively, as shown in Table 3. The scores of dry leaf of the 30 lines with high tolerance level were 0-1 with recovery ability of 90 to 100%, while the scores of the rest nine lines with moderate tolerance were 3-5, with recovery ability of 70 to 90% as shown in Table 4 and Figure 1.

Table 3. Screening of the 39 F6 lines for drought tolerance traits and identification of molecular markers of *DREB2A* and *BADH2* genes

Line number	Genotype	Crossing	The score of leaf rolling	The score of leaf drying	Score of recovery	Criteria	DREB2A gene	BADH2 gene
1	262.A1.4-1	Bugis x IR148	3	3	3	RT	+	+
2	260.A3.2	Bugis x IR7858	3	3	3	RT	+	+
3	260.A3.2	Bugis x IR7858	0	0	0	HT	+	+
4	262.A1.4-2	Bugis x IR148	3	3	3	RT	+	+
5	262.A1.4-3	Bugis x IR148	3	3	3	RT	+	+
6	260.A3.2	Bugis x IR7858	3	3	3	RT	+	+
7	262.A1.4-4	Bugis x IR148	3	3	3	RT	+	+
8	260.A3.2	Bugis x IR7858	5	5	5	MT	+	+
9	262.A1.4-5	Bugis x IR148	5	5	5	MT	+	+
10	262.A1.4-6	Bugis x IR148	3	3	3	RT	+	+
11	251-17	Bugis x IR148	3	3	3	RT	+	+
12	248-14-1	Bugis x IR7858	1	1	1	T	+	+
13	249-15-1	Bugis x IR7858	3	3	3	RT	+	+
14	250-16	Bugis x IR148	5	5	5	MT	+	+
15	247-13	Bugis x IR7858	3	3	3	RT	+	+
16	269-11	Sriwijaya x IR7858	1	1	1	T	+	+
17	248-14-2	Bugis x IR7858	0	0	0	HT	+	+
18	249-15-2	Bugis x IR7858	3	3	3	MT	+	+
19	267-9-1	Sriwijaya x IR148	0	0	0	HT	+	+
20	267-9-2	Sriwijaya x IR148	1	1	1	T	+	+
21	259-1	Bugis x IR7858	3	3	3	MT	+	+
22	259-6	Bugis x IR7858	1	1	1	T	+	+
23	259-9	Bugis x IR7858	5	5	5	MT	+	+
24	259-15	Bugis x IR7858	3	3	3	RT	+	+
25	260-21	Bugis x IR7858	3	3	3	RT	+	+
26	260-26	Bugis x IR7858	3	3	3	RT	+	+
27	262-43	Bugis x IR148	0	0	0	HT	+	+
28	262-48	Bugis x IR148	1	1	1	T	+	+
29	255-59	Sriwijaya x IR148	3	3	3	RT	+	+
30	253-2	Sriwijaya x IR148	5	5	5	MT	+	+
31	259-17	Bugis x IR7858	5	5	5	MT	+	+
32	259-3	Bugis x IR7858	3	3	3	RT	+	+
33	254-54	Sriwijaya x IR148	3	3	3	RT	+	+
34	258-60	Sriwijaya x IR7858	0	0	0	HT	+	+
35	255-56	Sriwijaya x IR148	0	0	0	HT	+	+
36	262-44	Bugis x IR148	5	5	5	MT	+	+
37	262-46	Bugis x IR148	5	5	5	MT	+	+
38	259-18	Bugis x IR7858	5	5	5	MT	+	+
39	259-4	Bugis x IR7858	3	3	3	RT	+	+
I	IR20	Control variaety	5	5	5	MT	-	-
S	Salumpikit	Control variety	1	1	1	T	+	+

Note: HT: Highly Tolerant (6 lines); T: Tolerant (5 lines); RT: Rather Tolerant (17 lines); MT: Moderate Tolerant (11 lines); + : gene was present

Table 4. The distribution of 39 F6 lines for identification of drought-tolerant lines

The number of lines	Drought response				The score of recovery ability				
	Highly- tolerant (score 0-1)	Rather tolerant (score 3)	Moderately tolerant (score 5)	Susceptible (Score 7-9)	1	3	5	7	9
11									
17									
11									
IR20									
Salumpikit									

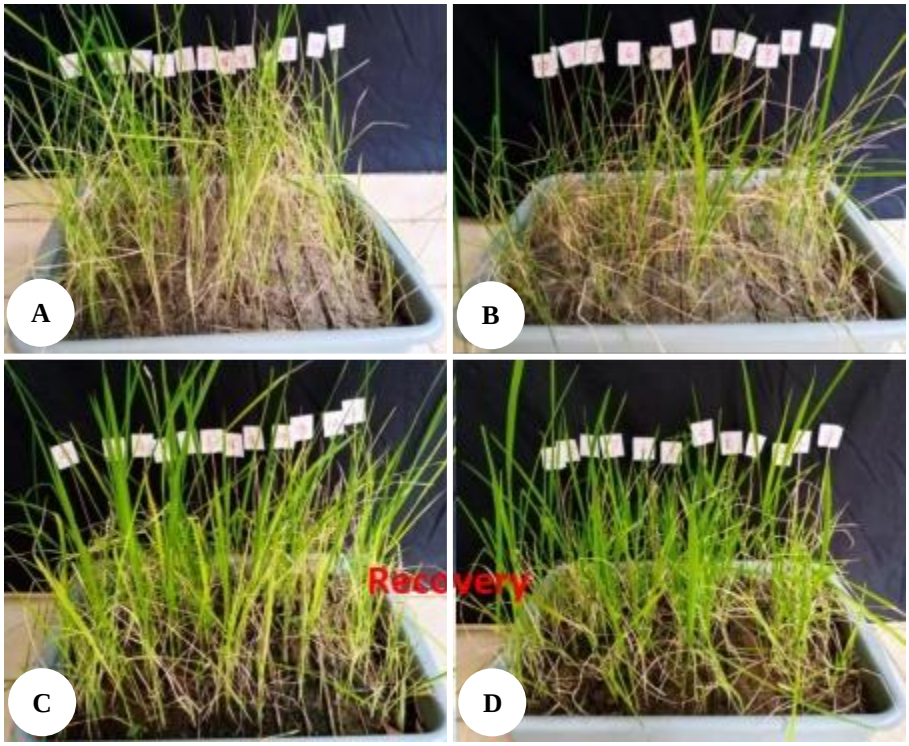

Figure 1. Responses of F6 progeny lines to drought observed on drying leaf (A-B) and recovery ability (C-D)

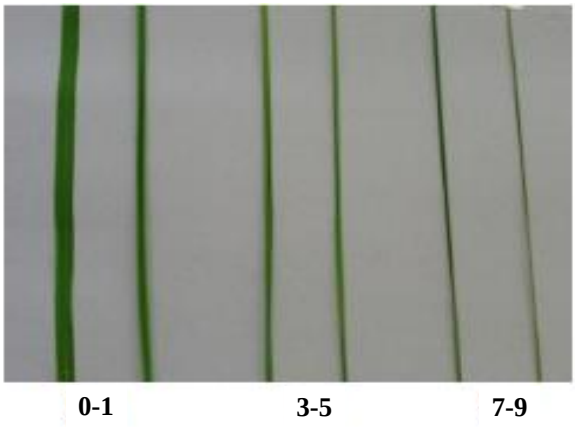

Figure 2. Description of rolling leaves based on SES Method; scores 0-1: start rolling to form V shape; 3-5: rolling to form V and U shapes inside leaves; 7-9: leaves are rolling totally

Molecular identification of drought tolerance genes

The molecular analysis using PCR products separated on agarose gel electrophoresis showed that the *DREB2A* gene was present in the 39 selected lines with the marker sizes approximately 250 bp as shown in Figure 3. This proves that the progeny of F6 lines originated from the parents IR148 + carrying QTL 12.1 and IR7858-1 are drought-tolerant. This evidence proved that the DREB2A genes control drought tolerance in rice plants. The visualization of the *BADH2* gene 39 selected lines showed a marker with a size of approximately 1300 bp as shown in Figure 4.

Performance of agronomic characters, yield and yield potential of superior lines

The performance of agronomic characters and yield potential of the 16 tested superior lines are shown in Table 5. Almost all tested lines uniformly performed as shown by plant height range of 101.1 and 140.5 cm with a standard deviation of 1.52-4.37. This shows that all tested lines were homozygous in the 8th generation (F7). Furthermore, the highest and lowest average number of panicles per-hill were 14.7 and 6.5, with the panicle length range of 24.61 - 27.6 cm. Furthermore, the number of filled grains per-panicles ranged from 99.5 - 150.07, while the percentage of unfilled grains was from 9.87%-26.66%, which are categorized as low based on the SES IRRI (2012). This led to variations in grain weight per-hill were 19-35.5 g per-hill.

The grain yield per-plot varied from the lowest at 458 g to the highest, at 1210 g. The agronomic characters that support the observed high grain yield were the high number of panicles, the low percentage of unfilled grain, and the high 1000 grains weight. The length of the panicles did not show any significant variation, i.e., range from 24.61-27.6 cm, as shown in Table 5.

Discussion

Seedlings' responses to drought stress tolerance were identified after 14 days after the stress treatment. Afterwhich, the tolerant lines continued to grow normally and their leaves remained fully open, whereas the moderately tolerant lines experienced the drying of leaf tips as shown in Figure 1. Kumar et al. (2014) stated that leaf rolling was delayed in drought-tolerant rice genotypes and

induced by loss of turgor as well as low osmotic regulation. Delayed leaf rolling in the tolerant genotype indicated that the turgor remained normal, and the plants were protected from dehydration, as shown in Figure 2. According to Bunnag and Pongthai (2013) and Swain et al. (2017), leaf rolling is one of the mechanisms used by plants to adjust the water potential and absorb groundwater in drought stress conditions. Swain et al. (2017) reported that the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments during the drought conditions. Furthermore, 30 and 48 lines were scored by 1, and 3, respectively. Out of these 78 lines assessed, 13 lines and the tolerant (CR 143-2-2) variety produced more than 1 and 2.7 t grain ha^{-1}, respectively, while the sensitive control variety (IR20) produced no grain at all. The IR20 variety is often used as a check for drought sensitivity, but our results show that IR20 was categorized as moderate in the drought stress treatment at the seedling phase. It is necessary to review the sensitivity and adaptability in the seedling phase.

Leaf rolling can reduce the surface area exposed to sunlight, thereby reducing transpiration rate in plants. This condition helps plants survive in a certain period when the availability of water in the environment decreases. The genes in rice plants that play a role in this process are the Roc5 (Rice outermost cell-specific genes) genes that encode the leucine zipper class IV transcriptional factor homeodomain. According to Zou et al. (2011), overexpression and suppression of these genes lead to leaf curling on the adaxial and abaxial sides of the plants. Delay in leaf rolling indicates a plant effort to maintain turgor and avoid dehydration. Water shortages tend to have lower rates of leaf rolling, which appears in plants with tolerant criteria score of 1, as shown in Table 3. It allowed the plant to survive drought at the low leaf tissue water potential (Sevanto 2018). Furthermore, the plants recovered after passing through a period of drought, thereby indicating the ability of plants to enhance their metabolic system (Bian et al. 2017; Wang et al. 2019).

Drought-tolerant rice varieties can be generated through crossbreeding by combining the tolerant character of the ancestors with other varieties that have high productivity. The use of molecular marker technology can help fasten the selection process, thereby making it accurate and faster. One of the markers related to drought tolerance is the QTL (quantitative trait locus) 12.1, which was produced by crossing the Vandana varieties of Indian rice and Indonesian Way Rarem (Mulyaningsih et al. 2010). One of the lines is IR148+, which was used as a parent in this study due to its ability to maintain rice yield in severe drought stress at the reproductive phase before flowering. The molecular marker of QTL 12.1 had an insignificant effect on some of the parameters observed under normal conditions (Bernier et al. 2007).

DREBs (Dehydration Responsive Element Bindings) are essential transcription factor proteins (TFs) used in regulating the expression of drought-responsive genes (Lata and Prasad 2011; Fujita et al. 2013). The *DREB2A* gene is essential as a regulator of drought-responsive genes, making it a marker of drought stress-tolerant.

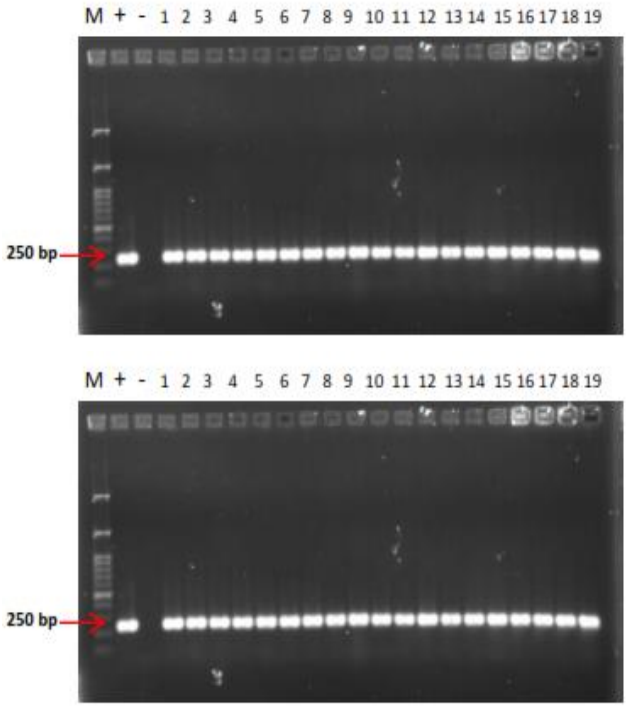

Figure 3. PCR amplification of *DREB2A* (250 bp) on 39 selected lines with Salumpikit and IR20 as positive and negative control respectively (M= DNA ladder of 100 kb)

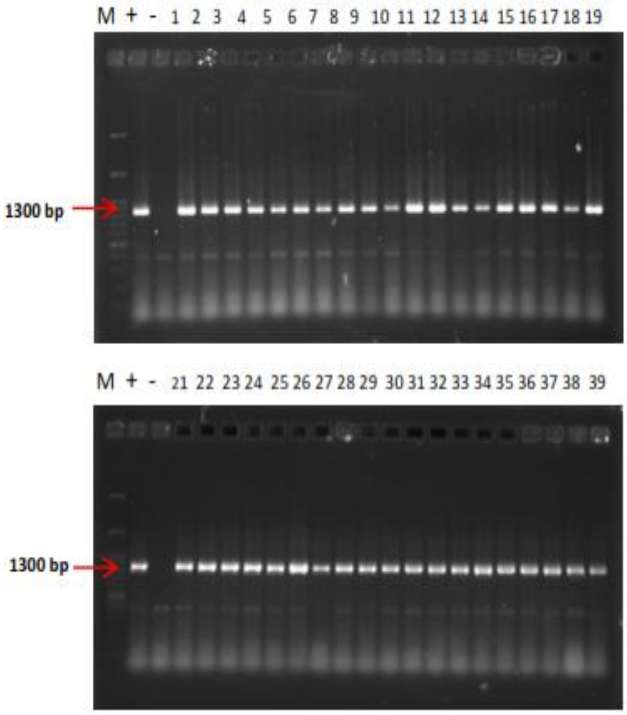

Figure 4. PCR amplification of *BADH2* (1300 bp) on 39 selected lines with Salumpikit and IR20 as positive and negative control respectively (M= DNA ladder of 100 kb)

Table 5. The performance of agronomic characters, yields and yield potential of superior lines in the field experiment

Accession	Plant height	Number of Panicle per- hill	Panicle length (cm)	Number of fill grains	% of unfilled gran per-panicle	1000 grains weight (g)	grains weight per- hill (g)	Yield per -plot (1x1 m^2) (g)	Yield potential (t ha^{-1})
	X ± SD (Mean ± standard deviation)								
BKL3-R51-1-253-18	113.1 ± 1.91	7.6 ± 1.35	26.08 ± 2.13	115.71 ± 26.70	11.06 ± 7.81	28.4 ± 1.26	19.0 ± 3.71	512	5.12
BKL3-R51-3-255-20	130.7 ± 2.87	9.9 ± 3.14	26.02 ± 1.94	150.07 ± 40.63	13.19 ± 5.81	27.4 ± 2.98	32.5 ± 15.89	519	5.19
BKL4-R51-1-256-21	105.4 ± 1.64	10.88 ± 2.15	24.77 ± 2.99	112.5 ± 30.22	17.96 ± 10.97	28.6 ± 1.89	29.2 ± 11.29	478	4.78
BKL4-R51-2-257-22	107.3 ± 2.58	8.5 ± 1.65	25.58 ± 1.99	111.28 ± 29.26	17.95 ± 8.19	27.9 ± 2.13	21.6 ± 8.43	431	4.31
BKL4-R51-3-258-23	101.1 ± 1.79	7.9 ± 1.72	25.05 ± 2.62	111.86 ± 40.49	12.29 ± 8.76	28.5 ± 2.27	18.7 ± 5.59	520	5.2
BKL1 B-1-259-1	111.6 ± 2.27	11.6 ± 1.95	24.61 ± 1.63	120.89 ± 30.07	12.71 ± 6.88	27.6 ± 1.84	31.8 ± 9.54	1005	10.05
BKL1 B-2-260-2	115.8 ± 3.67	10.8 ± 2.25	25.11 ± 2.17	112.93 ± 28.54	11.14 ± 6.15	26.6 ± 1.65	26.2 ± 5.61	712	7.12
BKL1 B-3-261-3	117.3 ± 2.45	14.7 ± 3.53	25.45 ± 2.23	110.04 ±29.74	9.87 ± 6.34	27.6 ± 1.26	33.6 ± 12.87	1038	10.38
BKL2 B-1-262-4	140.5 ± 3.24	12.8 ± 3.43	27.05 ± 2.25	105.92 ± 26.76	26.66 ± 9.4	29.00 ± 1.94	28.1 ± 7.25	719	7.19
BKL2 B-2-263-5	123.7 ± 2.31	13.4 ± 3.81	26.07 ± 2.47	103.57 ± 29.42	24.87 ± 10.84	28.8 ± 1.68	35.5 ± 22.14	750	7.5
BKL2 B-2-264-6	119.2 ± 1.55	12.0 ± 4.89	24.92 ± 1.57	110.5 ± 34.75	14.06 ± 8.33	29.2 ± 3.01	28.7 ± 13.71	1210	12.1
BKL3 B-1-265-7	108.0 ± 2.00	14.3 ± 2.58	25.63 ± 1.68	99.5 ± 19.76	16.93 ± 7.26	28.4 ± 1.84	28.2 ± 6.23	653	6.53
BKL3 B-2-266-8	107.1 ± 1.52	11.1 ± 1.79	27.6 ± 1.93	138.46 ± 34.52	21.94 ± 6.96	28.8 ± 1.39	36.7 ± 9.26	667	6.67
BKL3 B-3-267-9	112.3 ± 3.37	12.20 ± 3.29	25.68 ± 2.67	110.10 ± 39.48	22.56 ± 14.07	26.80 ± 1.87	19.1 ± 8.57	458	4.58
BKL4 B-1-268-10	127.3 ± 4.37	12.1 ± 3.38	25.55 ± 2.37	124.71 ± 36.6	21.72 ± 11.07	27.2 ± 1.93	31.9 ± 11.2	1206	12.06
BKL4 B-3-270-12	112.3 ± 4.03	6.5 ± 1.35	26.46 ± 2.63	108.32 ± 27.01	19.08 ± 11.18	28.4 ± 1.50	16.6 ± 5.62	640	6.4

The transcription factors in DREB2A are involved in the regulatory mechanisms that exist in some plants in response to drought, salinity, and heat stress (Lata and Prasad 2011; Mizoi et al. 2012). There are five *DREB2* genes in the rice genome, including *OsDREB2A, OsDREB2B, OsDREB2C, OsDREB2E*, and *OsABI4* (Matsukura et al. 2010; Srivastav et al. 2010). Expression of *OsDREB2A* in rice is caused by water deficit and exogenous ABA application, which leads to increased drought stress (Cui et al. 2011). The *OsDREB2B* transcript has a functional and non-functional form marked during drought conditions. Consequently, it can increase drought tolerance through alternative splicing induced by its pre-mRNA (Matsukura et al. 2010). All of these results indicate that *OsDREB2s* also play an essential role in the regulation of drought tolerance.

Huang et al. (2018) identified a new transcription factor gene such as *DREB2*, namely *OsDRAP1* (Responsive Drought Genes *AP2/EREBP*), located in the introgressed segment on chromosome 8 of DK151 and whose expression is highly regulated by drought at *DK151*, thereby showing its role in drought tolerance rice. Osmotic adjustment in cells is the primary response of plants to drought (Zivcak et al. 2016; Blum 2017). Previous studies indicated that osmoprotectant substances, namely glycine betaine, play an essential role in cell stabilization by balancing the protein quaternary and membrane structure against the adverse effects of salinity (Sakamoto and Murata 2000; Saxena et al. 2019). In addition, it facilitates osmotic adjustment by reducing the internal osmotic potential that contributes to water stress tolerance in plant cells. It also stabilizes the PSII and RuBisCO complexes during photosynthesis under stress conditions (Holmström et al. 2000; Huang et al. 2020). These are some of the positive effects of the application of exogenous betaine glycine in plants that grow under the pressure of salinity or drought stress. Plant cells can be protected from adverse effects of salinity-induced oxidative stress by exogenous application of glycine betaine (Demiral and Türkan 2004; Saxena et al. 2019).

The successful use of molecular markers that control complex traits for obtaining drought-tolerant superior rice varieties has been reported by Lanceras et al. (2004). Some of the studied traits included yield, root length, thickness, leaf curl, stomata sensitivity (Price et al. 2002; Courtois et al. 2003; Swain et al. 2017), and osmotic adjustment (Zivcak et al. 2016; Blum 2017; Kahraman et al. 2019). Betaine aldehyde dehydrogenase (BADH) is a key enzyme for the biosynthesis of glycine betaine. This is because several studies have reported the accumulation of glycine betaine and *BADH1* gene expression for tolerance to salinity, drought, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). DNA marker linked to *BADH2* gene was presented on the 39 selected lines with the marker size of approximately 1300bp (Shrestha 2011; Hasthanasombut et al. 2011) as shown in Figure 4.

The PCR assay of 39 selected lines for drought tolerance using *DREB2A* and *BADH2* primers is shown in Table 3. All tested lines showed positive results, they contained both genes and had the criteria of varying degrees of drought level in the seedling stage evaluation. However, the molecular analysis showed positive results as a drought-tolerant marker gene in the seedling stage, then evaluation at the productive stage needs to be carried out to obtain more accurate data, due to many genes contributing to regulation drought-responsive gene expression. Drought-tolerant plants can adapt to drought conditions, which are shown by high grain. The use of superior varieties is the most efficient technology to increase rice yield with low-cost production in the dry land. Therefore, developing a superior variety by crossbreeding is needed to produce superior potential lines. Furthermore, before releasing a new superior variety, potential selected lines need to be tested in various locations (multi-location trials/MLT).

The agronomic performance and yield of 16 superior lines showed that all lines reached homozygous in the 8th generation (F_7), where the plant height had a relatively low standard deviation. The number of panicles ranged from 14.7 had a high yield potential, with the value of a filled grain of 150.07 (Table 5). The new paradigm of rice breeding is the number of productive tillers between 8-12 tillers per-hill with the grains number per-panicles ranging from 150-200 (Peng and Khush 2003). Peng et al. (2008) stated that in the new type of rice variety breeding programs avoid extreme traits, such as 200-250 grain per-panicle which can produce panicles with low seed filling. Therefore, the increase in the second generation of new types of rice has been modified by IRRI to 150 grains per-panicle. Several lines have a potential yield of more than 10 t ha^{-1}, such as those with the accession number BKL1 B-1-259-1 and BKL1 B-3-261-3 to yield potential of 10.05 t ha^{-1} and 10.08 t ha^{-1}, respectively. Meanwhile, the lines with the BKL2 B-2-264-6 and BKL4 B-1-268-10 numbers had a potential yield of more than 12 t ha^{-1}, namely 12.1 and 12.06 t ha^{-1}, as shown in Table 5. These lines can be developed on dry land or as rice on rainfed land because the lines tested were identified as drought tolerant, as shown in Table 3.

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