Identification of drought tolerant and molecular analysis of DREB2A and BADH2 genes and yield potensial of lines from single crossing Bengkulu local rice varieties

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Abstract. Screening in the seedling stage of 39 progeny of F6 lines to drought stress was carried out in the greenhouse. Drought tolerant and sensitive varieties of IR 20 and Salumpikit, respectively, were used as control plants. The methods for traits identification of leaf curled, dried, and recovery ability after exposure to severe drought for two weeks was following the Standard Evaluation System (SES) developed by IRRI. Molecular analysis to detect the presence of the DREB2A gene was carried out by PCR amplification of genomic DNA using forward- and reverse- oligonucleotide primers of CCTCATTGGGTCAGGAAGAA and GGATCTCAGCCACCCACTTA, respectively, while for BADH2 gene using forward- and reverse- oligonucleotide primers of GGCCAAGTACCTCAAGGCGA and TGTCCCCAGCTGCTTCATCC, respectively. Molecular markers of DREB2A and BADH2 genes were also identified in 39 tested lines with approximately 250 and 2300 bp length, respectively. This study concluded that the progeny of F6 lines generating from the crossing of local varieties of IR7858 and IR148 is the potential to become a drought-tolerant variety upland rice. Line numbers BKL2 B-2-264-6 and BKL4 B-1-268-10 have a potential yield of more than 12 tonnes/ha. These line has the potential to be developed on rainfed lowland rice or dry land because it has drought resistance.

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

Running title: *Identification of DREB2A and BADH2 genes for drought tolerant*

INTRODUCTION

The development of upland rice variety is an alternative to increase national rice production in Indonesia because the extensification of lowland rice is increasingly difficult. This strategy is carried out through optimizing the use of uncultivated lands, where most of them have the potential for upland rice cultivation (Center for Research and Development 2006). The use of superior varieties, which has higher yields and tolerance to various obstacles so that it adapt well to climate change, is urgently needed to support efforts to increase rice yields in the dry land. Anticipating the impact of climate change on sustainable agricultural systems is carried out to produce technological innovations that are able to overcome and suppress the impacts caused. These technological innovations include superior varieties of drought-tolerant rice. Gene improvement to obtain superior varieties that are adaptive to the conditions of drought stress is an essential priority in rice breeding programs.

Assembling drought-tolerant rice varieties can be done through crossbreeding, which combines the resistant traits of the parents with other crops that have a high yield. Molecular marker technology can help selection more accurately than conventional. One of the markers related to drought tolerance is the QTL marker (quantitative trait locus) 12.1. The International Rice Research Institute (IRRI) had crossed the Vandana variety of Indian rice with Way Rarem from Indonesia. One of the filial is a crossing number of IR148+, which is derived from IR crossing 79971-B-369-B-B (Mulyaningsih et al. 2010). The crossing population has been showed to contain QTL 12.1 markers. The location of markers is on chromosome 12, between SSR markers RM28048 and RM 511 (Mc Couch et al. 2002). The presence of these markers can maintain yields in conditions of severe drought stress during the reproductive stage before flowering. In normal conditions, the marker QTL 12.1 did not have a significant effect on some of the parameters observed (Bernier et al. 2007).

The DREB2 gene controls drought stress in plants (Matsukura et al. 2010; Srivastav et al. 2010; Akhtar et al. 2012; Huang et al. 2018). DREBs (Dehydration Responsive Element Bindings) are transcription factor proteins (TFs) that are very important in regulating the expression of drought-responsive genes (Lata and Prasad 2011; Fujita et al. 2013). The homology of the DREB2 gene in rice is DREB2A (Sakuma et al. 2002). Some of the DREB2A target genes are MT2A, At1g69870, At3g53990, At1g22985, RD29A, LEA14, At2g23120 [9], RD29B, At1g52690, RD17 (Sakuma et al. 2006;

Qin et al. 2011), AtHsfA3, HSP18.2, and Hsp70 (Qin et al. 2011). The importance of the DREB2A gene is because it can be used as a regulator of various types of drought-responsive genes, making it a marker of drought stress-resistant genes.

Osmotic adjustment in cells is the primary response of plants to drought (Zivcak et al. 2016; Blum 2017). The reports of previous studies indicate that osmoprotectant substances, namely glycine betaine, plays an essential role in cell stabilization by balancing the structure of the protein quaternary and membrane structure against the adverse effects of salinity (Sakamoto and Murata 2000; Saxena et al. 2019). 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 of exogenous application of glycine in plants that grow on salinity stress have been shown to protect plant cells (Demiral and Türkan 2004; Saxena et al. 2019).

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

MATERIALS AND METHODS

The experiments were conducted at the University of Bengkulu. Screening study was done in the greenhouse of Agricultural Faculty from Februari to April 2020, while molecular analysis was done in the laboratory of the Department of Biology from May to July 2020. Plant materials were using the progeny of 39 lines that selected from F6 generations resulted from the 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). Var. Salumpikit and IR 20 as drought-tolerant and sensitive control varieties (Table 1).

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			

Screening of drought-tolerant rice of 39 F6 lines was carried out following the standard Evaluation System (SES) developed by IRRI (2002). The drought-susceptible variety (IR20) and local drought-tolerant variety (Salumpikit) were used as control. The test was carried out following the method of Kumar et al. (2015); Swain et al. (2017); Herawati et al. (2017). Plastic tubs sizes of 40 cm x 25 cm x 20 cm was filled with soil. Then, each tub was planted ten family lines and two control varieties. Each line was sown for 20 seeds in a row. Seedlings were watered intensively in 2 weeks after planting. After this stage, watering was stopped until the sensitive plants dried. Drought tolerance assessment was carried out based on the SES methods, as described in Table 2. Trait responses of the seedlings were recorded, then seedlings

were watered intensively for the next ten days. Recovery ability was recorded following the methods of SES, as described in Table 2.

Genomic DNA was isolated from fresh leaves at 14 days after treatment (DAT). Fragments of 0.1 g of rice leaf were ground in the mortar by adding liquid nitrogen. Isolation of total DNA was carried out by modifying the protocols of Wizard's Genomic DNA Purification Kit. The ground leaf was put into a 2 ml tube, then 600 µl of Nuclei Lysis Solution was added, followed shaking by vortex for 3 seconds. Then, the solution was heated in a water bath at 65ºC for 15 minutes. RNase of 3 µl was added followed incubation at 37 ° C for 15 minutes. Then, 200 µl Precipitation Solution was added, and the microtubes were centrifuged for 3 minutes at 13,000 rpm. The supernatants were removed to a 1.5 ml tube, and 600 µl of isopropanol was added. The microtubes were further centrifuged for 1 minute at room temperature. The solution was discarded, and DNA remained 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 for one night. The total isolated DNA is used as a template DNA for PCR amplification of DREB2A and BADH2 genes.

PCR amplification of the DREB2A gene using forward- and reverse- oligonucleotide primers of CCTCATTGGGTCAGGAAGAA and GGATCTCAGCCACCCACTTA, respectively (Jadhao et al. 2014; Kumar 2016; Lathif et al. 2018). While the amplification of the BADH2 gene was using forward- and reverse- oligonucleotide primers of GGCCAAGTACCTCAAGGCG and TGTCCCCAGCTGCTTCATCC, respectively (Robin et al. 2003). The PCR mixtures, including the DNA template, forward and reverse primers, buffer, dNTP (nucleotide mix) and Taq polymerase, were developed in the thermocycling. The program was started with 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 agarose gel 1% of 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.

In the season in 2020, a yield test of selected superior lines was carried out in March-July 2020 in Semarang Village, Bengkulu City. The materials used in this study were 16 selected superior lines in the F7 generation. The experiment was carried out on plot measuring 8 m x 6 m with a spacing of 20 x 20 cm, and 1 seed was planted. Fertilize twice, the first fertilization at the age of 14 days after planting (HST) with a dose of 150 kg/ha of Urea, 100 kg/ha SP36 and 100 kg/ha KCl. The second fertilization at the age of 30 HST with a dose of 100 kg/ha Urea, 100 kg/ha SP36 and 100 kg/ha KCl. Intensive control was carried out against weeds, pests and diseases. Observation of the agronomic characters of 10 plant/plot samples taken from each line number. The characters observed included plant height, number of panicles/hill, panicle length, number of filled grains/panicle, percentage of empty grain/panicle, 1000 grain weight, grain weight per hill, and yield per plot.

RESULTS AND DISCUSSION

Identification of drought tolerant traits

Screening of F6 lines at the seedling stage was carried out to select the drought-tolerant rice (Kumar et al. 2015; Swain et al. 2017; Herawati et al. 2017). Drought tolerant assessment following the methods of SES was done by comparing the treated plants with control varieties of Salumpikit and IR20 (Table 2). The symptoms were identified after exposed to drought stress for 14 days, including leaf curling, leaf drying, and ability to recover (Figure 1). The criteria of 39 F6 lines were identified as highly to rather tolerant, tolerant, and moderately tolerant to drought for a total number of 11, 19, and 9 lines, respectively (Table 3). The scores of dry leaf of the 30 lines with highly to rather a tolerance and tolerance were 0-1 which recovery ability was 90 to 100%, while the scores of the rest nine lines with moderate tolerance were 3-5 which recovery ability was 70 to 90% (Table 4, Figure 1).

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

Table 3. Screening of the 39 F6 lines for resistant traits and molecular identification of gene markers of DREB2A AND BADH2

Line number	Genotype	Crossing	The score of rolling leaf	The score of drought leaf	Score of recovery	Criteria	DREB2A genes	BADH2 genes
1	262.A1.4-1	Bugis x IR148	3	3	3	T	+	+
2	260.A3.2	Bugis x IR7858	3	3	3	T	+	+
3	260.A3.2	Bugis x IR7858	0	0	0	HT	+	+
4	262.A1.4-2	Bugis x IR148	3	3	3	T	+	+
5	262.A1.4-3	Bugis x IR148	3	3	3	T	+	+
6	260.A3.2	Bugis x IR7858	3	3	3	T	+	+
7	262.A1.4-4	Bugis x IR148	3	3	3	T	+	+
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	T	+	+
11	251-17	Bugis x IR148	3	3	3	T	+	+
12	248-14-1	Bugis x IR7858	1	1	1	RT	+	+
13	249-15-1	Bugis x IR7858	3	3	3	T	+	+
14	250-16	Bugis x IR148	5	5	5	MT	+	+
15	247-13	Bugis x IR7858	3	3	3	T	+	+
16	269-11	Sriwijaya x IR7858	1	1	1	RT	+	+
17	248-14-2	Bugis x IR7858	0	0	0	HT	+	+
18	249-15-2	Bugis x IR7858	3	3	3	T	+	+
19	267-9-1	Sriwijaya x IR148	0	0	0	HT	+	+
20	267-9-2	Sriwijaya x IR148	1	1	1	RT	+	+
21	259-1	Bugis x IR7858	3	3	3	T	+	+
22	259-6	Bugis x IR7858	1	1	1	RT	+	+
23	259-9	Bugis x IR7858	5	5	5	MT	+	+
24	259-15	Bugis x IR7858	3	3	3	T	+	+
25	260-21	Bugis x IR7858	3	3	3	T	+	+
26	260-26	Bugis x IR7858	3	3	3	T	+	+
27	262-43	Bugis x IR148	0	0	0	HT	+	+
28	262-48	Bugis x IR148	1	1	1	RT	+	+
29	255-59	Sriwijaya x IR148	3	3	3	T	+	+
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	T	+	+
33	254-54	Sriwijaya x IR148	3	3	3	T	+	+
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	T	+	+
I	IR20	Control variaety	5	5	5	MT	+	+
S	Salumpikit	Control variety	1	1	1	RT		

HT=High Tolerant (6 lines); RT=Rather Tolerant (5 lines); T= Tolerant (19 lines); MT= Moderate Tolerant (9 lines); + = gene was identified

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

The number of Lines	Drought Response				The score of Recovery Ability				
	Highly-Rather Tolerant (score 0-1)	Tolerant (score 3)	Moderate Tolerant (score 5)	Susceptible (Score 7-9)	1	3	5	7	9
11									
19									
9									
IR20									
Salumpikit									

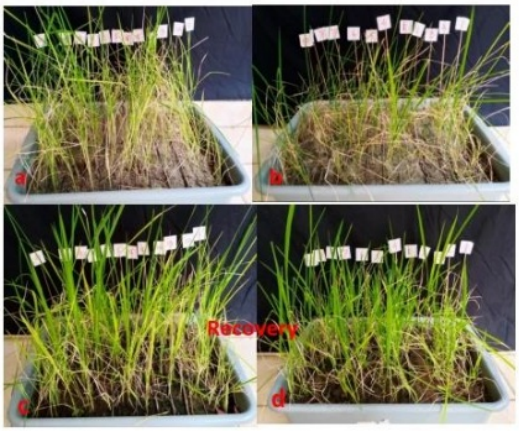

Figure 1. Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c dan d)

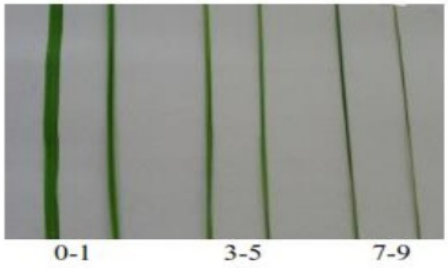

Figure 2. Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V; 3-5: rolling to form V and U inside leaves; 7-9: rolling leaves totally

Molecular identification of drought tolerant genes

Molecular analysis using PCR products showed that the DREB2A gene was visualized in 39 selected lines with the marker sizes approximately 250 bp (Tawfik et al. 2016; Lathif et al. 2018) (Figure 3). It proves that the progeny of F6 lines originated from the parents IR148 + carrying QTL and IR7858-1, which are drought-tolerant generated F6 lines that are the potential to be drought tolerant. This evidence proved that drought tolerance in rice plants is controlled by the DREB2A genes (Akhtar et al. 2012; Huang et al. 2018).

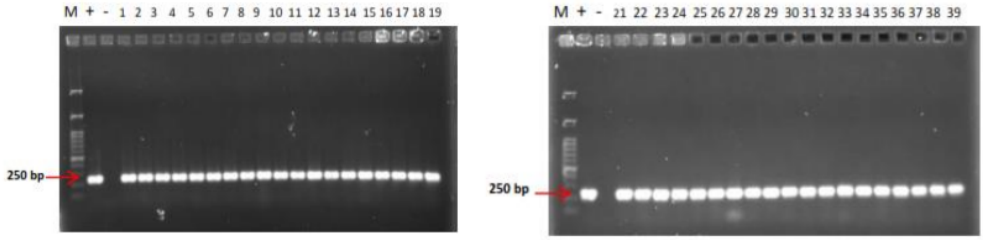

Figure 3. PCR amplification of DREB2A (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

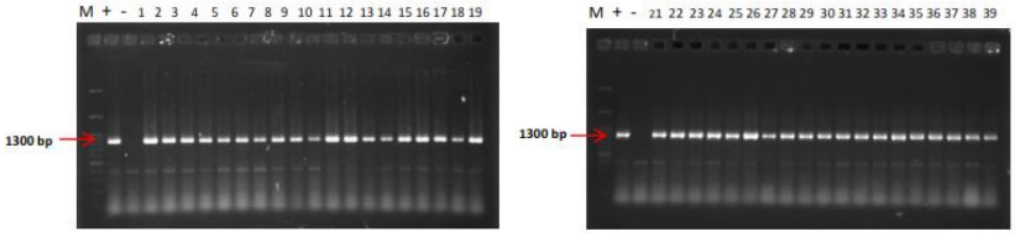

Figure 4. PCR amplification of BADH2 (1300 bp) on 39 selected lines generated from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

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 traits that have been studied include the yield, root length, root 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 known as a key enzyme for the biosynthesis of glycine betaine. Many researchers have reported the accumulation of glycine betaine and BADH1 gene expression for tolerance to salinity, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). Visualization of the BADH2 gene 39 selected lines showed a marker with a size approximately 1300 bp (Shrestha 2011; Hasthanasombut et al. 2011) (Figure 4).

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

The appearance of agronomic characters, yields and yield potential of the 16 superior lines tested are presented in Table 5. Almost all tested lines have shown uniformity as shown by the lowest average plant height appearance, namely 101.1 and the highest is 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). The highest average number of panicles/hill was 14.7 and the lowest was 6.5. however, the panicle length ranges from 24.61 - 27.6 cm. The number of filled grains/panicles ranged from 99.5 - 150.07, while the percentage of empty grains rice was categorized as low based on the SES IRRI (2012), which ranged from 9.87% - 26.66%. This led to variations in grain weight per hill, which was around 19-35.5 grams/hill .

Table 5. The performance of agronomic characters, yields and yield potential of superior lines

Assesion	Plant height	Number of Panicle/hill	Panicle length (cm)	Number of fill grains	% of empty/panicle	1000 grains weight (gram)	grains weight/hill (gram)	Yield/plot(1x1 m²) (gram)	Yield potential (ton/ha)
	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 yield of grain per plot varies from the lowest was 458 grams, and the highest, which was 1210 grams. If seen from the appearan[10]f agronomic characters, the high grain yield was supported by the characters of the large number of panicles, the low percentage of empty grain, and the weight of 1000 grains. The length of the panicles did not show any significant variation, which the range between 24.61-27.6 cm (Table 5).

Discussion

Seedlings' responses to drought stress were identified after 14 days without water. The tolerant lines continued to grow well, vigorous, and leaves remained fully open, whereas the moderate tolerant lines were dried on leaf tips (Figure 1). Kum[4] et al. (2014) reported that leaf rolling was delayed i[4]drought-tolerant rice genotypes. Leaf rolling in rice plants was induced by loss of turgor and low osmotic regulation. Delayed leaf rolling in the tolerant genotype showed that the turgor remained normal, and the plants were protected from dehydration (Figure 2). Leaf rolling is one of the mechanisms of plants to adjust the water poten[9]l, which enables the plants to absorb groundwater in drought stress conditions (Bunnag and Pongthai 2013; Swain et al. 2017).

Swain et al. (2017) reported that during the drought conditions and the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments were identified that 30 lines were scored [4]f 1, and 48 lines were scored of 3. Of these 78 assessments, 13 lines produced more than 1-ton grain/ha, tolerant lines (CR 143-2-2) produced more than 2.7 tons grain/ha, while sensitive plants as a control (IR20) did not produce any.

Leaf rolling can reduce leaf surface area exposed to sunlight, thereby reducing the rate of transpiration in plants. This condition will help plants to survive in a certain period when th[47]ailability of water in the environment decreases. The [43]es 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. Overexpression of these genes results in leaf curling on the adaxial side, whereas suppression of this gene causes leaf rolling on the abaxial side (Zou et al. 2011). Delaying leaf rolling indicates that a plant effort to maintain turgor and avoid dehydration. Water shortages tend to have lower rates of leaf rolling, which appeared in the plants that have tolerant criteria with a Score of 1 (Table 3). It allowed the plant to survive to drought at the low water potential of leaf tissue (Sevanto 2018). Plants wer[21]covery after passing through a period of drought indicated the ability of plants to improve 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 marking technology can help selection more accurate and faster. One of the markers related to drought tolerance is the QTL (quantitative trait locus) 12.1, which has been produced through the crossing of Vandana varieties of Indian rice and Way Rarem variety from Indonesia (Mulyaningsih et al. 2010). One of the lines is IR148+, which was used as a parent in this study. This marker can main[26]rice yield in severe drought stress at the reproductive phase before flowering. The molecular marker of QTL 12.[15]d not have a significant effect on some of the parameters observed under normal conditions (Bernier et al. 2007).

[11]DREBs (Dehydration Responsive Element Bindings) are transcription factor proteins (TFs) that are very important 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. [2]

Transcription factors in DREB2A are involved in the regulatory mechanisms that exist in some plants in response to drought, salinity, a[46]heat stress (Lata and Prasad 2011; Mizoi et al. 2012). There [2]e 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 can result in increased drought stress (Cui et al. 2011). The OsDREB2B transcript has a functional and non-functional form. It is ma[2]d during drought conditions, and consecutively 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 [35]rought tolerant.

Hu[2]g 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, 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, plays an essential role in cell stabilization by balancing the structure of the protein quaternary and membrane against the adverse effects of salinity (Sakamoto and Murata 2000; Saxena et al. 2019). Besides, it facilitates osmotic adjustment by reducing the potential of internal osmotic that contributes to the ability of plant cells tolerant to water stress. It also stabilizes the PSII and RuBisCO complexes during photosynthesis under stress conditions (Holmström et al. 2000; Huang et al. 2020). These are the positive effects of exogenous betaine glycine application 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 results of the PCR amplification of 39 selected lines for drought tolerance using DREB2A and BADH2 primers are presented in Table 3. All tested lines showed positive, containing both genes and had criteria with varying degrees from the results of the drought test in the seedling stage. Although the results of the molecular study showed positive results as a drought-tolerant marker gene in the seedling stage, evaluation at the productive stage needs to be done to obtain more accurate data. Drought-tolerant plants can adapt to drought conditions, which are shown on 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. Before releasing a new superior variety, potential selected lines need to be tested in various locations.

The agronomic performance and yield of 16 superior lines showed that all lines had reached homozygous in the 8th generation (F_7), where the plant height showed a relatively low standard deviation in all lines. The number of panicles ranging from 14.7 had a high yield potential, while the number of filled grains was 150.07 (Table 5). The new paradigm of new rice breeding is the number of productive tillers between 8-12 tillers/hill with the number of grains/panicles ranging from 150-200 grains (Peng and Khush 2003). Peng et al. (2008) stated that in the new type of rice breeding avoid extreme traits such as 200-250 grain/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/panicle. Several lines have a potential yield of more than 10 tonnes/ha, namely lines with the assesion number BKL1 B-1-259-1 and BKL1 B-3-261-3 have yield potential of 10.05 tonnes/ha and 10.08 tonnes/ha, 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 ton /ha, namely 12.1 and 12.06 tons/ha, respectively (Table 5). These lines have the opportunity to be developed on dry land or as rice on rainfed land because the lines tested were identified as drought resistance (Table 3).

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