Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] Submission Acknowledgement

1 pesan

Ahmad Dwi Setyawan <smujo.id@gmail.com> 3 Oktober 2020 20.30
Balas Ke: Ahmad Dwi Setyawan <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

Reny Herawati:

Thank you for submitting the manuscript, "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties" to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/6884
Username: renywati

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

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Biodiversitas Journal of Biological Diversity

Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] Editor Decision

4 pesan

Smujo Editors <smujo.id@gmail.com> 4 November 2020 11.32
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

Reny Herawati:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Our decision is to: Decline Submission
Note: We have invited c. 20 experts but no one wants to review. So, please make your "own-review" by sending your paper to at least two reviewers, and one professional language editor; then sending us your final revised paper along with comments from the two reviewers (incl. name & email address) and language editing certificate.

Smujo Editors
editors@smujo.id

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Biodiversitas Journal of Biological Diversity

T-Identification of drought tolerant and molecular analysis of DREB2A and BADH2 genes and yield potensial of lines from singl.doc
700K

Reny Herawati <reny.herawati@unib.ac.id> 5 November 2020 12.02
Kepada: Smujo Editors <editors@smujo.id>

Dear editor team,

Thank you for the quick response, we will comply as suggested by the editor. We will send the results as soon as possible

Best regards,

Dr. Reny Herawati et al

[Kutipan teks disembunyikan]

Reny Herawati <reny.herawati@unib.ac.id> 25 November 2020 13.02
Kepada: Smujo Editors <editors@smujo.id>

Dear editorial team,
as previously suggested, we attached the submit the final manuscript and supporting documents previously requested (proofreading, reviewer, certificate, and turnitin check).
We include 2 reviewers, namely:
1. Ir. Suprayogi, M.Sc., Ph.D (Rice Breeding from University of Jenderal Soedirman) email:suprayogi@unsoed.ac.id
2. Dr. Ir. Heni Safitri, M.Si (Rice Breeding, Research from Balai Besar Penelitian Tanaman Padi, Sikamandi) email:henisafitri2@gmail.com
We hope that our manuscript can be processed in the biodiversity journal. We look forward hearing from you soon

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

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 of 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 can 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. Genetic improvement to obtain superior varieties that are adaptive to the conditions of drought stress is an essential priority in rice breeding programs.

~~Assembling~~ Breeding drought-tolerant rice varieties can be done through crossbreeding, which combines the ~~resistant~~ tolerant 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;

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Comment [H1]: Really??? Any prove?

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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 lines 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

Comment [H5]: Salinity or drought? This manuscript talk about drought, not salinity.

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

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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

Comment [H8]: In the first sentence in this paragraph tell the reader that the test according to SES.

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 a 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)

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Score	Criteria	Description		
		Leaf Rolling	Leaf Drying	Recovery Ability
0	Highly Tolerant	Leaves 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 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									

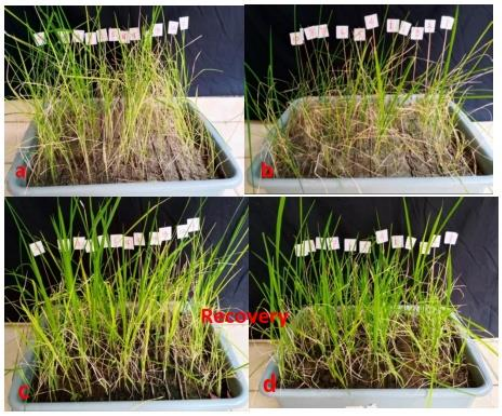

Figure 1. Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c dan d)

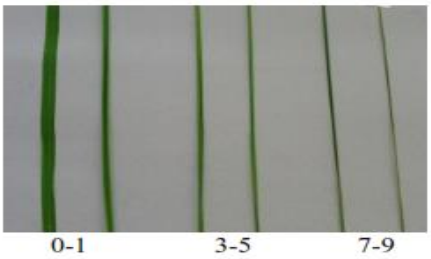

Figure 2. Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V; 3-5: rolling to form V and U inside leaves; 7-9: rolling leaves totally

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 12.1 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).

Comment [H10]: Cluster analysis is needed fo grouping the lines accroding agronomic and droug tolerance traits.

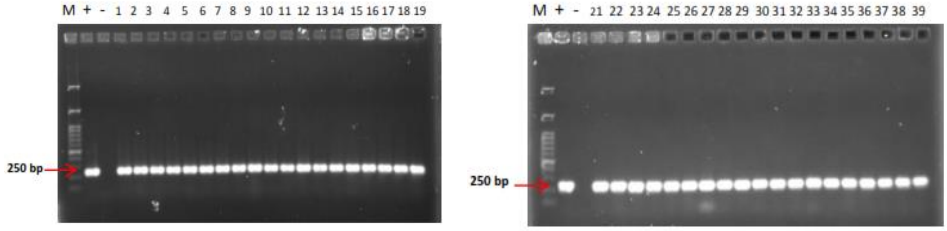

Figure 3. PCR amplification of DREB2A (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

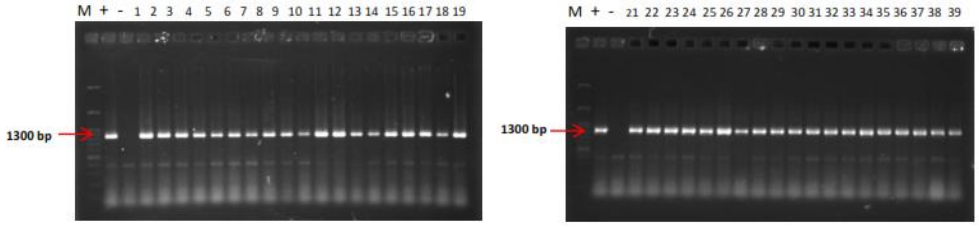

Figure 4. PCR amplification of BADH2 (1300 bp) on 39 selected lines generated from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

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 .

Comment [H11]: Which lines among the 39 li used??? The lines name were different.

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 m^2) (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 appearance of 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). Kumar et al. (2014) reported that leaf rolling was delayed in 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 potential, 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 of 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 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. 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 were recovery 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 maintain rice yield in severe drought stress at the reproductive phase before flowering. The molecular marker of QTL 12.1 did not have a significant effect on some of the parameters observed under normal conditions (Bernier et al. 2007).

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.

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 can result in increased drought stress (Cui et al. 2011). The OsDREB2B transcript has a functional and non-functional form. It is marked 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 of drought tolerant.

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, 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 8^{th} 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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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 of 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 can 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. Genetic 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;

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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

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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 a 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 % plant 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

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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	■								

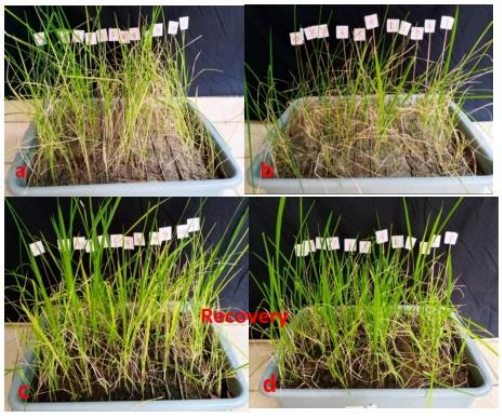

Figure 1. Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c dan d)

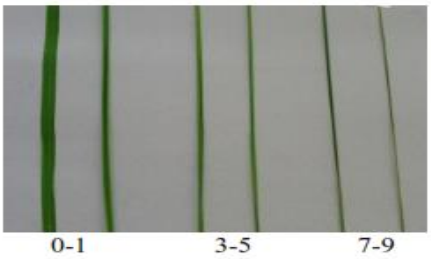

Figure 2. Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V; 3-5: rolling to form V and U inside leaves; 7-9: rolling leaves totally

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 12.1 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).

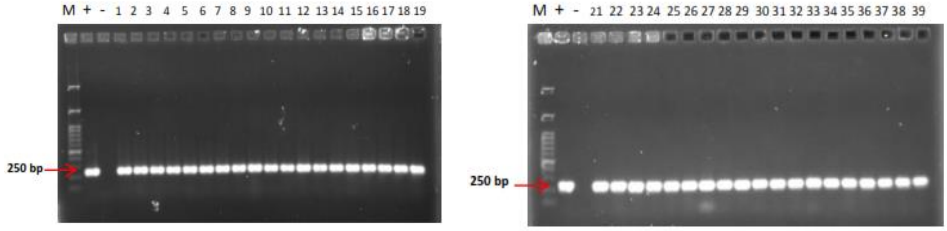

Figure 3. PCR amplification of DREB2A (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

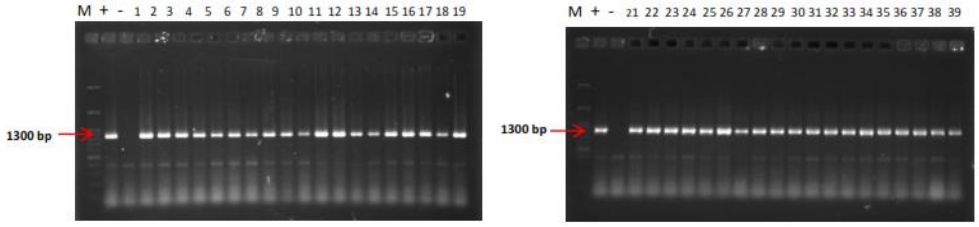

Figure 4. PCR amplification of BADH2 (1300 bp) on 39 selected lines generated from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

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 .

Comment [LC52]: hapus

Comment [LC53]: langsung menggunakan istilah F7 generation akan lebih pas

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 m^2) (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 appearance of 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).

Comment [LC54]: Seen from the agronom characters, ..

Comment [LC55]: hapus

Comment [LC56]: hapus

Comment [LC57]: hapus

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). Kumar et al. (2014) reported that leaf rolling was delayed in 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 potential, 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 of 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.

Comment [LC58]: hapus

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 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. 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 were recovery 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).

Comment [LC59]: hapus

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 maintain rice yield in severe drought stress at the reproductive phase before flowering. The molecular marker of QTL 12.1 did not have a significant effect on some of the parameters observed under normal conditions (Bernier et al. 2007).

Comment [LC60]: parents (?)

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.

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 can result in increased drought stress (Cui et al. 2011). The OsDREB2B transcript has a functional and non-functional form. It is marked 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 of drought tolerant.

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, 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).

Comment [LC61]: hapus

Comment [LC62]: they

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Molecular analysis of DREB2A and BADH2 genes and yield potential derived from single-cross of Bengkulu local rice varieties

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Abstract. This study aims to identify drought-tolerant and molecular analysis of DREB2A and BADH2 genes and yield potential from single-crossing varieties of rice in Bengkulu. The sensitive varieties of IR 20 and Salumpikit were used as control plants in the screening and seedling stages of the 39 F6 progeny lines, which were carried out in the greenhouse. In addition, the Standard Evaluation System (SES) developed by IRRI was used to the recovery ability of the curled and dried leaves after two weeks. The molecular analysis used to detect the presence of the DREB2A gene was carried out by PCR amplification and 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 also identified in 39 tested lines with approximately 250 and 2300 bp length. This result showed that the progeny of F6 lines generated from crossing local varieties of IR7858 and IR148 is potential to becoming a drought-tolerant variety of 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 and can be developed on rain field, low or dry land due to its drought resistance.

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

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

INTRODUCTION

Upland rice cultivation is an alternative means rice production used to increase the yearly number of rice in Indonesia, which has significantly decreased due to the rising difficulty associated with the extensification of lowlands. According to the Center for Research and Development (2006), this technique is carried out by optimizing the use of uncultivated lands, where the majorities have the potential for upland rice cultivation. The use of superior varieties with higher yields and tolerance to various obstacles is urgently needed to support efforts to increase rice yields in the dry land. Furthermore, it is important to anticipate the impact of climate change on sustainable agricultural systems to produce technological innovations that can overcome and suppress the impacts caused, such as the superior varieties of drought-tolerant rice. The genetic improvement to obtain superior varieties that are adaptive to the conditions of drought stress 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 crops. Molecular marker technology helps in selecting more accurate than conventional areas, 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, such as the filial with crossing number of 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 (Mc Couch 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) the DREB2 gene controls drought stress 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 that the homology of the DREB2 gene in rice is 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). 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). Previous studies carried out by Sakamoto & Murata (2000) and Saxena et al. (2019) indicated that osmoprotectant substances, such as 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. 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 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 a key enzyme for biosynthesis of glycine betaine. Several studies have reported the accumulation ability of glycine betaine and BADH1gene expression in tolerating salinity, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). The objective of this study is to identify drought-tolerant traits and molecular analysis of DREB2A and BADH2 genes using the progeny of F6 lines from the crossing of local varieties (Sriwijaya and Bugis) with drought-tolerant varieties of IR7858 and IR148+ positive QTL on the chromosome 12.1.

MATERIALS AND METHODS

This research was carried out at the University of Bengkulu, with drought evaluation 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 are the 39 lines selected from F_6 populations leading to the single cross 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 were used as drought-tolerant and sensitive control varieties respectively (Table 1).

Table 1: The selected F6 lines for traits and molecular identification of drought-tolerant genes of DREB2A and BADH2

Lines number	Genotype	Crossing	Line number	Genotype	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			

The standard Evaluation System (SES) developed by IRRI (2002) was used to evaluate the drought-tolerant of 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 tubs sizes of 40 cm x 25 cm x 20 cm 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 its 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.

Genomic DNA was isolated from fresh leaves 14 days after treatment (DAT) with cuttings of 0.1 g of rice leaf ground in the mortar by adding liquid nitrogen. 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 eppendorf tube, before adding and shaking 600 µl of Nuclei Lysis Solution for 3 seconds. In addition, the solution was heated in a water bath at 65ºC for 15 minutes, followed by the addition and incubation of 3 µl RNase at 37 ° C for 15 minutes. This was followed by the addition of 200 µl Precipitation Solution, with the microtubes centrifuged for 3 minutes at 13,000 rpm. The supernatants were collected into a 1.5 ml tube, before the addition of 600 µl of isopropanol. Furthermore, the microtubes were further centrifuged for 1 minute at room temperature with the solution 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 for one night. The total isolated DNA is used as a template for PCR amplification of DREB2A and BADH2 genes.

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 of 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 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 59^{o}C for 2 minutes, and extension at 72^{o}C for 2 minutes, and the final extension at 72^{o}C 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.

A yield test of selected superior lines 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 F_7 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. Fertilizer application was done twice, the first fertilizer was carried out 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, and the second fertilizer was carried out at the age of 30 HST with a dose of 100 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl. Furthermore, intensive control was carried out against weeds, pests and diseases, while observation of the agronomic characters of 10 plant/plot samples was taken from each line number. The observed characters 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). Furthermore, Table 2 shows the drought-tolerant assessment carried out with the SES methods by comparing the treated plants 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 drought for a total number of 11, 19, and 9 lines, respectively, as shown in Table 3. The scores of dry leaf of the 30 lines with high tolerant level were 0-1 with recovery ability of 90 to 100%, while the scores of the rest nine lines with moderate tolerant were 3-5, with recovery ability of 70 to 90% as shown in Table 4 and 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 % plant 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 were dead. Length in most leaves thoroughly 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	Tolerant	Moderate Tolerant	Susceptible	1	3	5	7	9
	(score 0-1)	(score 3)	(score 5)	(Score 7-9)					
11									
19									
9									

IR20

Salumpikit

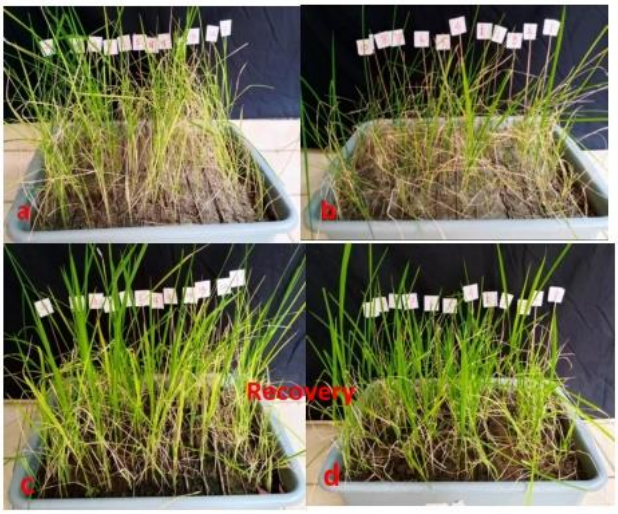

Figure 1: Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c and d)

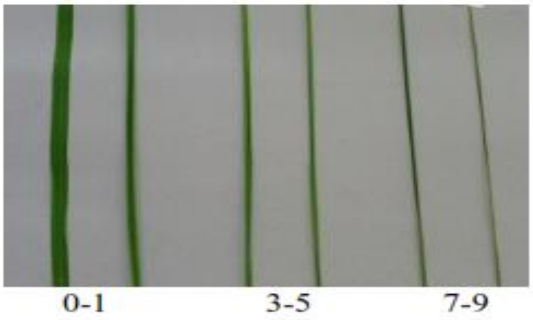

Figure 2: Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V; 3-5: rolling to form V and U inside leaves; 7-9: rolling leaves totally

Molecular identification of drought-tolerant genes

The molecular analysis using PCR products showed that the DREB2A gene was visualized in 39 selected lines with the marker sizes approximately 250 bp as shown in Figure 3 (Tawfik et al. 2016; Lathif et al. 2018). This proves that the progeny of F6 lines originated from the parents IR148 + carrying QTL 12.1 and IR7858-1, which are drought-tolerant generated from F6 lines. This evidence proved that drought tolerance in rice plants is controlled by the DREB2A genes (Akhtar et al. 2012; Huang et al. 2018).

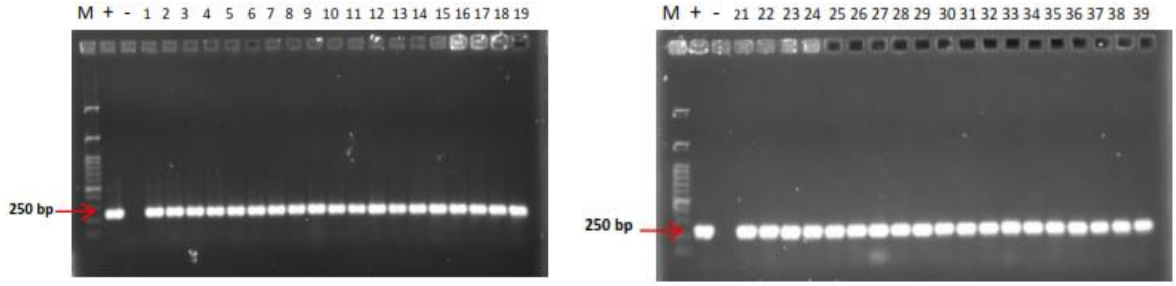

Figure 3: PCR amplification of DREB2A (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

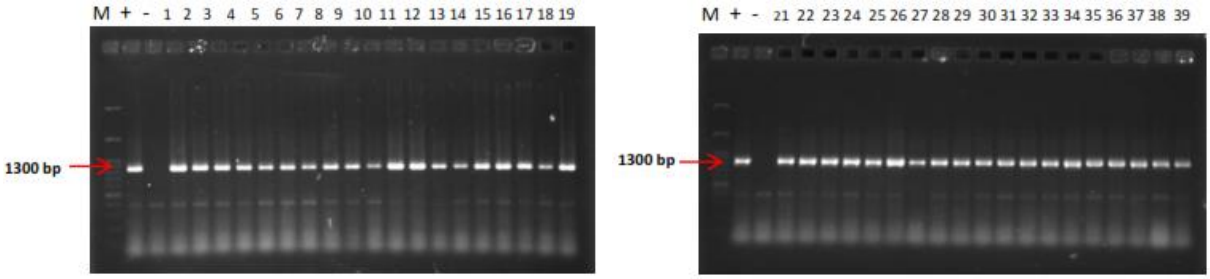

Figure 4: PCR amplification of BADH2 (1300 bp) on 39 selected lines generated from a single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

The successful use of molecular markers that control complex traits for obtaining varieties of drought-tolerant superior rice have been reported by Lanceras et al. (2004). Some of the studied traits include the 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, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). Furthermore, the visualization of the BADH2 gene 39 selected lines showed a marker with a size of approximately 1300 bp (Shrestha 2011; Hasthanasombut et al. 2011) as shown in Figure 4.

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

The appearance of agronomic characters and yield potential of the 16 tested superior lines are shown in Table 5. Almost all tested lines have indicated uniformity as shown by the lowest and highest average plant height, 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 F_7 generation. Furthermore, the highest and lowest average number of panicles/hill were 14.7 and 6.5, with the length ranging from 24.61 - 27.6 cm. Furthermore, 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 approximately 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 m^2) (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 at 458 grams to the highest, at 1210 grams. When determined from the appearance of agronomic characters, the high grain yield was supported by the characters of a 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 and range from 24.61-27.6 cm, as shown in Table 5.

Discussion

Seedlings' responses to drought stress were identified after 14 days without water. Furthermore, the tolerant lines continued to grow adequately and leave remained fully open, whereas the moderate tolerant lines were dried on 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 & Pongthai (2013) and Swain et al., (2017), leaf rolling is one of the mechanisms used by plants to adjust the water potential, and enable them to absorb groundwater in drought stress conditions. Swain et al. (2017) reported that during drought conditions, the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments. Furthermore, 30 and 48 lines were scored by 1, and 3, respectively. Out of these 78 assessments, 13 and tolerant (CR 143-2-2) lines produced more than 1-ton grain/ha, and 2.7 tons grain/ha, while sensitive plants as a control (IR20) did not produce any.

Leaf rolling can reduce the surface area exposed to sunlight, thereby reducing the rate of transpiration in plants. This condition help plants to 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 to drought at the low water potential of leaf tissue (Sevanto, 2018). Furthermore, plants recovery 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 parents with other varieties that have high productivity. The use of molecular marking technology can help hasten 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. 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 tolerant.

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. According to 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). In addition, it facilitates osmotic adjustment by reducing the internal occurrence possibility that contributes to the ability of plant cells to be 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 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 PCR amplification of 39 selected lines for drought tolerance using DREB2A and BADH2 primers are shown 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. However, 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 carried out 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. Furthermore, 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 reached homozygous in the F_7 generation, where the plant height had a relatively low standard deviation. The number of panicles ranging from 14.7 had a high yield potential, with the value of a filled grain of 150.07, as shown in Table 5. The new paradigm of rice breeding is the number of productive tillers between 8-12 tillers/hill with the grains/panicles ranging from 150-200 (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, such as those with the assesion number BKL1 B-1-259-1 and BKL1 B-3-261-3 to 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, as shown in Table 5. These lines have the opportunity to be developed on dry land or on rainfed land because they were identified as drought resistance, as shown inTable 3.

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Best regards,

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Reny Herawati <reny.herawati@unib.ac.id> 12 Desember 2020 15.46
Kepada: Smujo Editors <editors@smujo.id>

Dear editor,

We have submitted revision by sistem since 25 Nov 2020, but we don't have any notif until now...
[Kutipan teks disembunyikan]

Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] New notification from Biodiversitas Journal of Biological Diversity

1 pesan

Smujo Editors <smujo.id@gmail.com> 8 Januari 2021 16.31
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Supporting documents previously requested (proofreading, reviewer, certificate, and turnitin check)" regarding the submission "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Link: https://smujo.id/biodiv/authorDashboard/submission/6884

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] Editor Decision

1 pesan

Smujo Editors <smujo.id@gmail.com> 13 Januari 2021 22.53
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

Reny Herawati:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer A:

Dear Athors

After reviewing the manuscript, i reached the decision that:

1. The manuscript fulfill the science contribution and significances, therefore it feasible for publishing in Biodiversitas Journal.

2. There are still many comments that should be clariffied (see the reviewed version of the manuscript), and and the revision should be resubmitted.

3. Pay attention in the results parts concerning the references!

4. Thank you

Recommendation: Resubmit for Review

Reviewer C:

This is an extensive work with highly sufficient data for publication as a full research article. The methods are appropriate, sophisticated and robust. The results are well presented and discussion is comprehensive. The novelty of the research lies on the drought tolerance genes found to be present on the advanced rice lines tested in this study, but there are some issues that need to be explained regarding the association between tolerance markers and the phenotypic drought tolerance level observed in the seedling stage, as commented in the manuscript. In addition, many grammatical and editorial corrections are suggested. These issues need to be solved before further process of publication

Recommendation: Revisions Required

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

Abstract. This study aims to identify drought-tolerance~~ce~~ and molecular analysis of *DREB2A* and *BADH2* genes, as well as ~~and~~ yield potential from single-crossing varieties of rice in Bengkulu. The sensitive varieties of IR 20 and Salumpikit were used as control plants in the screening at~~and~~ seedling stages of the 39 F6 progeny lines, which were carried out in the greenhouse. ~~In addition, T~~the Standard Evaluation System (SES) developed by IRRI was used to evaluate the recovery ability of the curled and dried leaves after two weeks. The molecular analysis used to detect the presence of the *DREB2A* gene was carried out by PCR amplification of the~~and~~ 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 ~~also~~ identified in 39 tested lines with approximately 250 and 2300 bp length. This result showed that the progeny of F6 lines generated from the crossing of the local varieties of IR7858 and IR148 is potential to becom~~e~~ing a drought-tolerant variety of upland rice. Line numbers BKL2 B-2-264-6 and BKL4 B-1-268-10 have a potential yield of more than 12 t ~~onnes~~ha^{-1} and can be developed on rain field, low or dry land due to its drought resistance.

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

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

INTRODUCTION

Upland rice cultivation is an alternative means ~~rice production used~~ to increase the yearly rice production ~~number of rice~~ in Indonesia, which has significantly decreased due to the rising difficulty associated with the extensification of lowlands. According to the Center for Research and Development (2006), this ~~technique~~ is carried out by optimizing the use of uncultivated lands, where the majorities have the potential for upland rice cultivation. The use of superior varieties with higher yield~~s~~ and toleran~~tce~~ to various obstacles is urgently needed to support efforts to increase rice yield~~s~~ in the dry land~~s~~. Furthermore, it is important to anticipate the impact of climate change on sustainable agricultural systems by~~to~~ produc~~inge~~ 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 obtain superior varieties that are adaptive to the conditions of drought stress 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 crops. Molecular marker technology helps in selecting more accurate than conventional areas, 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, such as the filial with crossing number of 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 (Mc Couch 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) ~~the~~ *DREB2* gene controls drought stress 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 that the homolog~~y~~ of the *DREB2* gene in rice is *DREB2A*. 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

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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). Previous studies carried out by Sakamoto & Murata (2000) and Saxena et al. (2019) indicated that osmoprotectant substances, such as 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. 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 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 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 is to identify drought-tolerant traits and molecular analysis of *DREB2A* and *BADH2* genes in~~using~~ 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, with screening 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 are the progeny of 39 lines selected from F6 generations leading to 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). Table 1 shows the var. Salumpikit and IR 20 as drought-tolerant and sensitive controls.

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			

Driught tolerance screening

The standard Evaluation System (SES) developed by IRRI (2002) was used to screen the drought-tolerant rice of 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 tubs sizes of 40 cm x 25 cm x 20 cm 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

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watered for 2 weeks with the plants allowed to dry to determine its 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 fragments of 0.1 g of rice leaf ground in the mortar by adding liquid nitrogen. 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, before adding and shaking 600 µl of Nuclei Lysis Solution for 3 seconds. In addition, the solution was heated in a water bath at 65ºC for 15 minutes, followed by the addition and incubation of 3 µl RNase at 37 ° C for 15 minutes. This was followed by the addition of 200 µl Precipitation Solution, with the microtubes centrifuged for 3 minutes at 13,000 rpm. The supernatants were collected into a 1.5 ml tube, before the addition of 600 µl of isopropanol. Furthermore, the microtubes were further centrifuged for 1 minute at room temperature with the solution 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 for one night. The total isolated DNA is 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 of 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 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 59^{o}C for 2 minutes, and extension at 72^{o}C for 2 minutes, and the final extension at 72^{o}C 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.

Field experiment and yield potential evaluation

A yield test of selected superior lines 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 twice after planting (HST) for 14 days with a dose of 150 kg ~~/~~ha^{-1} of Urea, 100 kg/ha SP36 and 100 kg/ha KCl. The second fertilization was carried out at the age of 30 HST with a dose of 100 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha 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/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). Furthermore, Table 2 shows the drought-tolerant assessment carried out with the SES methods by comparing the treated plants 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 drought for a total number of 11, 19, and 9 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 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 % plant 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

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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 were dead. Length in most leaves thoroughly 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 ~~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	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	~~R~~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	~~R~~T	+	+
17	248-14-2	Bugis x IR7858	0	0	0	HT	+	+
18	249-15-2	Bugis x IR7858	3	3	3	RT	+	+
19	267-9-1	Sriwijaya x IR148	0	0	0	HT	+	+
20	267-9-2	Sriwijaya x IR148	1	1	1	~~R~~T	+	+
21	259-1	Bugis x IR7858	3	3	3	RT	+	+
22	259-6	Bugis x IR7858	1	1	1	~~R~~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	~~R~~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	~~R~~T		

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

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Comment [L13]: The controls showed a positi (IR 20) band, and according the SES, why the con (Salumkit) is Tolerant? The interpretation should b in added in the results section.

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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	■								

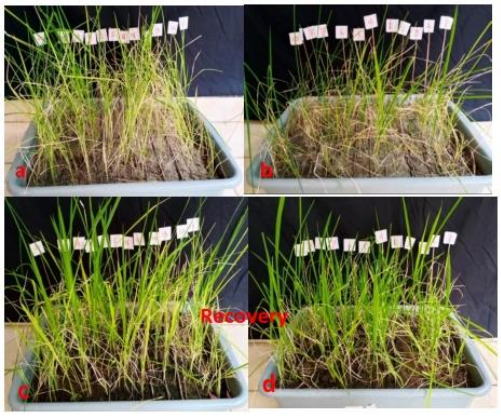

Figure 1: Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c and d)

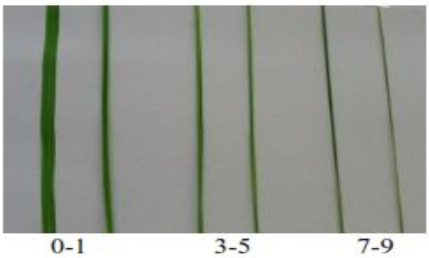

Figure 2: Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V; 3-5: rolling to form V and U inside leaves; 7-9: rolling leaves totally

Molecular identification of drought-tolerant genes

The molecular analysis using PCR products showed that the *DREB2A* gene was visualized in 39 selected lines with the marker sizes approximately 250 bp as shown in Figure 3 (Tawfik et al. 2016; Lathif et al. 2018). This proves that the progeny of F6 lines originated from the parents IR148 + carrying QTL 12.1 and IR7858-1, which are drought-tolerant generated from F6 lines. This evidence proved that drought tolerance in rice plants is controlled by the *DREB2A* genes (Akhtar et al. 2012; Huang et al. 2018).

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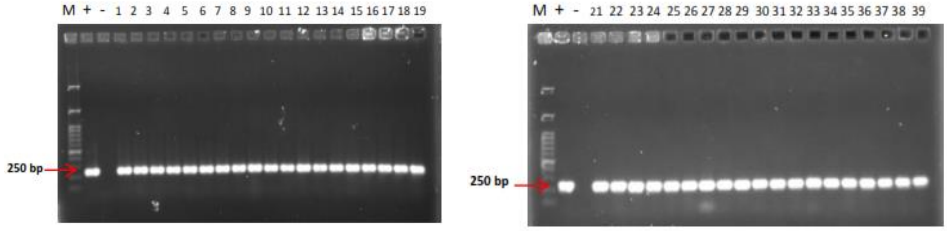

Figure 3: PCR amplification of *DREB2A* (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

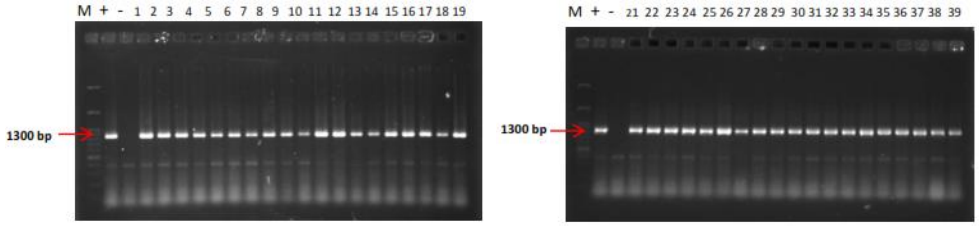

Figure 4: PCR amplification of *BADH2* (1300 bp) on 39 selected lines generated from a single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

The successful use of molecular markers that control complex traits for obtaining varieties of drought-tolerant superior rice have been reported by Lanceras et al. (2004). Some of the studied traits include the 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, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). Furthermore, the visualization of the *BADH2* gene 39 selected lines showed a marker with a size of approximately 1300 bp (Shrestha 2011; Hasthanasombut et al. 2011) as shown in Figure 4.

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

The appearance of agronomic characters and yield potential of the 16 tested superior lines are shown in Table 5. Almost all tested lines have indicated uniformity as shown by the lowest and highest average plant height appearance, 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 length ranging from 24.61 - 27.6 cm. Furthermore, 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 approximately 19-35.5 grams per hill.

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

Assesion	Plant height	Number of Panicle per hill	Panicle length (cm)	Number of fill grains	% of empty/panicle	1000 grains weight (gram)	grains weight per hill (gram)	Yield/plot(1x1 m^2) (gram)	Yield potential (ton/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

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Comment [L17]: This should be put on the discussion section. Results should present the data interpretation.. for the example, how is the band profile? Good or not? Whch line is positive? Why control has also positive band? What is the indication?

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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 at 458 g~~rams~~ to the highest, at 1210 g~~rams~~. When determined from the appearance of agronomic characters, the high grain yield was supported by the characters of a 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 and range from 24.61-27.6 cm, as shown in Table 5.

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Seedlings' responses to drought stress were identified after 14 days without water. Furthermore, the tolerant lines continued to grow adequately and leave remained fully open, whereas the moderate tolerant lines were dried on 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 & Pongthai (2013) and Swain et al.~~,~~ –(2017), leaf rolling is one of the mechanisms used by plants to adjust the water potential, and enable them to absorb groundwater in drought stress conditions. Swain et al. (2017) reported that during the drought conditions, the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments. Furthermore, 30 and 48 lines were scored by 1, and 3, respectively. Out of these 78 assessments, 13 and tolerant (CR 143-2-2) lines produced more than 1-ton grain/ha, and 2.7 tons grain/ha, while sensitive plants as a control (IR20) did not produce any.

Leaf rolling can reduce the surface area exposed to sunlight, thereby reducing the rate of transpiration in plants. This condition help plants to 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 to drought at the low water potential of leaf tissue (Sevanto, 2018). Furthermore, plants were recovery 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 marking technology can help hasten 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. 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 tolerant.

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. According to 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). In addition, it facilitates osmotic adjustment by reducing the internal occurrence possibility that contributes to the ability of plant cells to be 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 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 PCR amplification of 39 selected lines for drought tolerance using *DREB2A* and *BADH2* primers are shown 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. However, 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 carried out 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. Furthermore, 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 reached homozygous in the 8th generation (F_7), where the plant height had a relatively low standard deviation. The number of panicles ranging from 14.7 had a high yield potential, with the value of a filled grain of 150.07, as shown in Table 5. The new paradigm of rice breeding is the number of productive tillers between 8-12 tillers per hill with the grains/panicles ranging from 150-200 (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, such as those with the assesion number BKL1 B-1-259-1 and BKL1 B-3-261-3 to yield potential of 10.05 tonnes/ha and 10.08 t~~onnes~~/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~~on~~/ha^{-1}, namely 12.1 and 12.06 t~~ons~~/ha^{-1}, as shown in 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, as shown in Table 3.

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Molecular analysis of DREB2A and BADH2 genes and evaluation of yield potential of single-crossing varieties of rice in Bengkulu, Indonesia

Abstract. This study aimed to identify drought-~~tolerant~~ tolerance and molecular ~~analysis~~ characteristics of DREB2A and BADH2 genes and yield potential of single-cross varieties of rice in Bengkulu. The drought sensitive varieties of IR 20 and Salumpikit ~~were used as~~ (as the control plants) and 39 F6 progeny lines were used in the screening at seedling stages ~~of the 39 F6 progeny lines, which were carried out~~ in the greenhouse. In addition, the Standard Evaluation System (SES) developed by IRRI was used to assess the recovery ability of ~~the curled and dried leaves after two weeks~~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 also identified in 39 tested lines with approximately 250 and 2300 bp length. This result showed that the progeny of F6 lines generated from crossing local varieties of IR7858 and IR148 are potential ~~to becoming~~as drought-tolerant upland rice varieties~~of 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 and can be developed on rain-fed, lowland or dryland due to its drought ~~resistance~~tolerance.

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

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

INTRODUCTION

Upland rice cultivation is an alternative strategy for rice production to increase the annual ~~yearly number of~~ rice production in Indonesia, which has been significantly decreasing during the last decade due to the rising difficulty associated with the extensification of lowlands. According to the Center for Research and Development (2006), this technique is carried out by optimizing the use of uncultivated lands, ~~where the majorities have~~which are ~~the~~ potential for upland rice cultivation. The use of high yielding superior varieties with ~~higher yields and~~ tolerance to various obstacles is urgently needed to support efforts to increase rice yields in the dryland. Furthermore, it is important to anticipate the impact of climate change on sustainable agricultural systems to produce technological innovations that can overcome and suppress the impacts caused, such as the use of superior~~, varieties~~ drought-tolerant rice varieties. The genetic improvement to ~~obtain~~ produce superior varieties that are adaptive to the drought stress condition ~~of drought stress~~ 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 ~~crops~~ing varieties. Molecular marker technology can be used to more accurately selecting the desirable traits, ~~helps in selecting more accurate than conventional areas~~through masker 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, ~~such as~~which generated the filial with crossing number ~~of~~ 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 (Mc Couch 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) the 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 that in rice, ~~the homology of~~ the DREB2 gene ~~in rice~~ is homolog to DREB2A. 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

Comment [A1]: This is an extensive work with highly sufficient data for publication as a full research article. The methods are appropriate, sophisticated and robust. The results are well presented and discussion is comprehensive. The novelty of the research lies on the drought tolerance genes found to be present on the advanced rice lines tested in this study, but there are some issues that need to be explained regarding the association between tolerance markers and the phenotypic drought tolerance level observed in the seedling stage, as commented in the manuscript. In addition, many grammatical and editorial corrections are suggested. These issues need to be solved before further process of publication.

Comment [A2]: The title need to be made representing the content of the article. This on see not to.

Comment [A3]: This confusing in meaning, please revise

Comment [A4]: What is this?

al. 2011). The ~~importance of the~~ 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-~~resistant~~ 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 & Murata (2000) and Saxena et al. (2019) indicated that osmoprotectant substances, such as 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. 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 ~~that~~ grown ~~on~~ under salinity stress condition 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 BADH1gene expression in tolerating salinity, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). The objectives of this study ~~is~~ were ~~to~~ identify~~y~~ication of drought-~~tolerant~~ tolerance traits and molecular analysis of DREB2A and BADH2 genes using the progeny of F6 lines derived from the crossings of local varieties (Sriwijaya and Bugis) with drought-tolerant varieties of IR7858 and IR148+ positive QTL on the chromosome.

MATERIALS AND METHODS

This research was carried out at the University of Bengkulu~~, with~~. The screening of the rice lines was conducted at the ~~greenhouse~~ Greenhouse of Agricultural Faculty, Universitas Bengkulu, 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 ~~are~~ were the ~~progeny~~ progenies of 39 lines selected from F6 generations leading to 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). Table 1 shows the var. Salumpikit and IR 20 as drought-tolerant and sensitive controls.

Comment [A5]: This in confusing, please revis

Comment [A6]: This Table is not containing o the two varieties but also the 36 F6 lines tested. Please revise appropriately.

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			

The standard Evaluation System (SES) developed by IRRI (2002) was used to screen the drought-tolerant rice ~~of~~ 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 ~~tubs~~ trays ~~sizes~~ 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 its 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.

Genomic DNA was isolated from fresh leaves 14 days after treatment (DAT) with ~~fragments~~ a weight of 0.1 g of rice leaf was added with liquid nitrogen and then ground using a mortar ~~by adding liquid nitrogen~~. 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, ~~before adding and shaking~~added with 600 µl of Nuclei Lysis Solution and then shaken for 3 seconds. In addition, the solution was heated in a water bath at 65ºC for 15 minutes, ~~and followed by~~ then ~~addition~~ added ~~and incubation of~~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, ~~before~~ and then ~~addition~~ added with 600 µl isopropanol. Furthermore, the microtubes were further centrifuged for 1 minute at room temperature, then the ~~solution~~ 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 65 ºC for 1 hour or at 4ºC for overnight. The total isolated DNA was used as a template for PCR amplification of DREB2A and BADH2 genes.

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 of 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 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 59^{o}C for 2 minutes, and extension at 72^{o}C for 2 minutes, and the final extension at 72^{o}C 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.

A yield performance test of selected superior lines 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 twice after planting (HST) for 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 plot^{-1} samples was taken from each line number. The characters observed included plant height, number of panicles hill^{-1}, panicle length, number of filled grains panicle^{-1}, percentage of empty grain panicle^{-1}, 1000 grain weight, grain weight per hill, and yield per plot.

Comment [A7]: 100 mg?

Comment [A8]: From where were superior lin selected? From the previous screening? The time overlap with the time of screening, and before the molecular analysis, so how you selected the lines? Please clarify.

Comment [A9]: Only one seed was planted? Please clarify, and the reasons?

Comment [A10]: When and when????

RESULTS AND DISCUSSION

~~Identification~~ Assessment of drought ~~tolerant~~ tolerance ~~traits~~ level

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). ~~Furthermore,~~ Table 2 shows the drought-~~tolerant~~ tolerance assessment carried out with the SES methods by comparing the treated ~~plants~~ 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 ~~drought~~ to drought, ~~for a total number~~ of 11, 19, and 9 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 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	Moderately tolerant	Leaves are fully cupped (U-shape)	One-fourth to 1/2 of all leaves dried	40-69% of plants recovered
7	Moderately susceptible	Leaf margins are touching (0-shape)	More than 2/3 of all leaves are fully dried	20-39% of plants recovered

9	Susceptible	Leaves are tightly rolled (V-shape)	All plants ~~were~~ are dead. Length in most leaves thoroughly dried	0-19% of plants recovered

Comment [A11]: What length?

Table 3: Screening of the 39 F6 lines for ~~resistant~~ drought tolerance traits and molecular identification of ~~gene~~ molecular markers of DREB2A ~~AND~~ and BADH2 genes

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Line number	Genotype	Crossing	The score of leaf rolling ~~leaf~~	The score of ~~drought~~ leaf drying	Score of recovery	Criteria	DREB2A gene~~s~~	BADH2 gene~~s~~
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~~ly~~ Tolerant (6 lines); RT=Rather Tolerant (5 lines); T= Tolerant (19 lines); MT= Moderate Tolerant (9 lines); + = gene was ~~identified~~present

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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-Rather Tolerant (score 0-1)	Tolerant (score 3)	Moderately Tolerant (score 5)	Susceptible (Score 7-9)	1	3	5	7	9
11									
19									
9									
IR20									
Salumpikit									

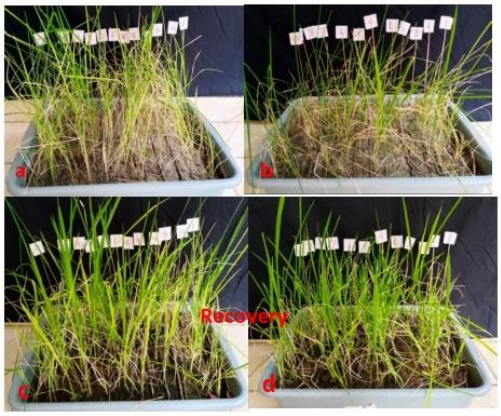

Figure 1: Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c and d)

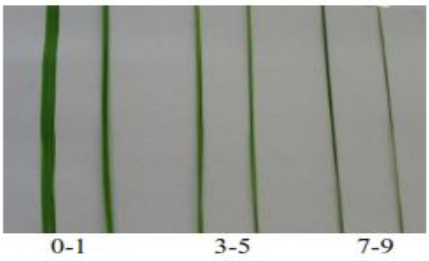

Figure 2: Description of rolling leaves based on SES Method; scores 0-1: started rolling to form V shape; 3-5: rolling to form V and U shapes inside leaves; 7-9: rolling leaves are rolling totally

Molecular identification of drought-tolerant tolerance genes

The molecular analysis using PCR products separated on agarose gel electrophoresis showed that the DREB2A gene was visualized present in the 39 selected lines with the marker sizes approximately 250 bp as shown in Figure 3 (Tawfik et al. 2016; Lathif et al. 2018). This proves that the progeny of F6 lines originated from the parents IR148 + carrying QTL 12.1 and IR7858-1, which are drought-tolerant generated from F6 lines. This evidence proved that drought tolerance in rice plants is controlled by the DREB2A genes (Akhtar et al. 2012; Huang et al. 2018).

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Comment [A12]: IR was used as the drought susceptible control variety but it was observed; Moderately Susceptible'. Please explain the reaso

Comment [A13]: Please indicate a, b, c, and d

Comment [A14]: Are these figure generated in the results of the preent study?? Or of the these authors? Please be clear.

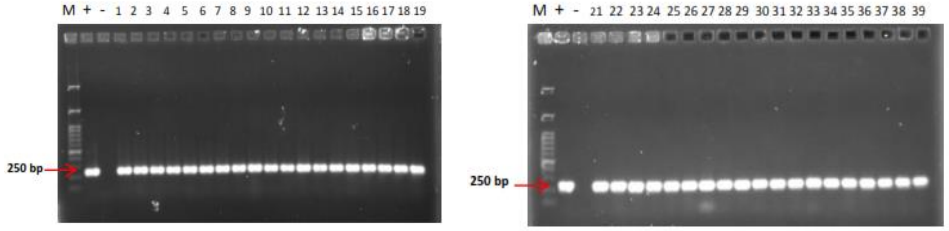

Figure 3: PCR amplification of DREB2A (250 bp) on 39 selected lines from single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - =negative control

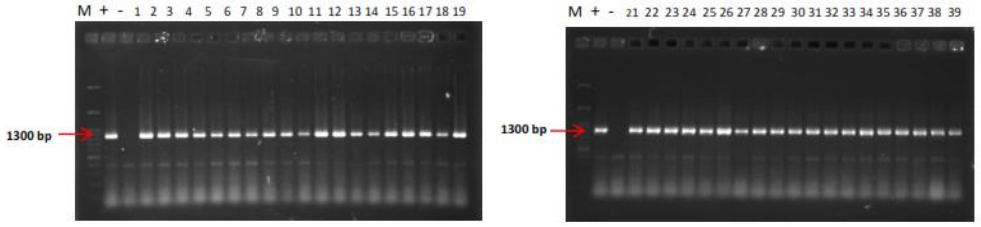

Figure 4: PCR amplification of BADH2 (1300 bp) on 39 selected lines generated from a single crossing of local variety with IR 20 and Salumpikit varieties (M= DNA ladder of 100 kb, + = positive control, - = negative control

The successful use of molecular markers that control complex traits for obtaining ~~varieties of~~ drought-tolerant superior rice varieties have been reported by Lanceras et al. (2004). Some of the studied traits include~~d~~ 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, dryness, and low temperatures (Lapuz et al. 2019; Kahraman et al. 2019). Furthermore, ~~the visualization of~~ the DNA marker linked to BADH2 gene was presente on the 39 selected lines with the marker size of approximately 1300 bp (Shrestha 2011; Hasthanasombut et al. 2011) as shown in Figure 4.

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

The ~~appearance~~ performance of agronomic characters and yield potential of the 16 tested superior lines are shown in Table 5. Almost all tested lines ~~have indicated~~ uniformly performed as shown by ~~the lowest and highest average~~ plant height ~~appearance~~ 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 hill^{-1} were 14.7 and 6.5, with the panicle???length ~~ranging~~ range ~~from~~ of 24.61 - 27.6 cm. Furthermore, the number of filled grains panicles^{-1} ranged from 99.5 - 150.07, while the percentage of unfilled grains ~~rice~~ was from 9.87% - 26.66%, which are 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 approximately~~ 19-35.5 grams hill^{-1}).

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

~~Assesion~~Accession	Plant height	Number of Panicle hill^{-1}	Panicle length (cm)	Number of fill grains	% of unfilled grain panicle^{-1}	1000-grain weight (gram)	grains weight hill^{-1} (gram)	Yield/plot(1x1 m^2) (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

Comment [A15]: Mention the name of the loc variety

Comment [A16]: Mention the names of check varieties

Comment [A17]: Mention the names of local variety and check/control varieties.

Comment [A18]: All tested lines contained bo genes, while their tolerance level were varied: Moderately tolerant, tolerant, rather tolerant,and highly tolerant. WHY would you expec that the tw markers bands are related to drought tolerance genes? Or there woud be other factors that may contribute to the tolerance, a=in addition to the tw gene? Please explain.

Comment [A19]: Drought???

Comment [A20]: Is this ranged considered homogen?

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 grain yield ~~of grain~~ per plot varied from the lowest at 458 grams to the highest at 1210 grams. ~~When determined from the appearance of~~The agronomic characters that supports the observed high grain yield were the high ~~supported by the characters of a large~~ number of panicles, the low percentage of unfilled grain, and the high ~~weight of~~ 1000-grain weight. The length of the panicles did not show any significant variation and ie ranged from 24.61-27.6 cm as shown in Table 5.

Discussion

Seedlings' responses to drought stress tolerance were identified after 14 days ~~without water~~after the stress treatment. ~~Furthermore~~Afterwhich, the tolerant lines continued to grow ~~adequately~~ normally and their leaves remained fully open, whereas the moderately tolerant lines experienced the drying~~dried~~ of the 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 & Pongthai (2013) and Swain et al. (2017), leaf rolling is one of the mechanisms used by plants to adjust the water potential, and enable them to absorb groundwater in drought stress conditions. Swain et al. (2017) reported that during the drought conditions, the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments. Furthermore, 30 and 48 lines were scored by 1, and 3, respectively. Out of these 78 lines ~~assessments~~assessed, 13 lines and the tolerant (CR 143-2-2) variety produced more than 1 ~~ton grain/ha,~~ and 2.7 tons grain/ha, respectively, while the sensitive ~~plants as a~~ control variety (IR20) ~~did not produce any~~produced no grain at all.

Leaf rolling can reduce the surface area exposed to sunlight, thereby reducing the rate of transpiration in plants. This condition help plants to 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 lef tissue water potential ~~of leaf tissue~~ (Sevanto 2018). Furthermore, the plants ~~were recovery~~ 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 ~~marking~~ marker technology can help ~~hasten~~ 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~~tolerance. 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 ~~tolerant~~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 in rice. ~~According to o~~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). In addition, it facilitates osmotic adjustment by reducing the internal occurrence possibility that contributes to the ability of plant cells to be 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 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 PCR ~~amplification~~ assay of 39 selected lines for drought tolerance using DREB2A and BADH2 primers are shown in Table 3. All tested lines showed positive results, they ~~containing~~ contained both genes and had the criteria of varying degrees ~~from the results of the~~of drought tolerance level in the seedling stage evaluation. However, the ~~results of 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. 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 of 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 ~~ranging~~ ranged from 14.7 had a high yield potential, with the value of a filled grain of 150.07~~, as shown in~~(-Table 5). The new paradigm of rice breeding is the number of productive tillers between 8-12 tillers /hill^{-1} with the grains number /panicles-1 ranging from 150-200 (Peng and Khush 2003). Peng et al. (2008) stated that in the new type ~~of~~ rice variety, breeding programmes avoid extreme traits~~,~~ such as 200-250 grain /panicle^{-1} 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^{-1}. Several lines have a potential yield of more than 10 tonnes/ha, such as those with the ~~assesion~~ accession number sBKL1 B-1-259-1 and BKL1 B-3-261-3 to 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, as shown in 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~~tolerant~~,~~ as shown inTable 3.

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Comment [A21]: This is not clear in meaning, please revise

Comment [A22]: Yes, in Figure 4, aAll tested lines contained both genes, while their tolerance l were varied: Moderately tolerant, tolerant, rather tolerant,and highly tolerant. WHY would you exp that the two markers bands are related to drought tolerance genes? Or there woud be other factors th may contribute to the tolerance, a=in addition to th two gene? Please explain.

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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. 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 length. This result showed that the progeny of F6 lines generated from the crossing of the local varieties of IR7858 and IR148 are potential as 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

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

INTRODUCTION

Upland rice cultivation is an alternative strategy to increase the annual rice production in Indonesia, which has has been significantly decreasing during the last decade due to the increasing in the conversion of lowland. 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 with 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 more accurately selecting the desirable traits, through masker 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 (Mc Couch 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, 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-tolerancet 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 & Murata (2000) and Saxena et al. (2019) indicated that osmoprotectant substances, such as 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. 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 condition 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 were 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. The screening of the rice lines 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.

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			

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 its 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.

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

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 then shaken for 3 seconds. In addition, the solution was heated in a water bath at 65ºC for 15 minutes, and 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 for 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 of 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 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 59^{o}C for 2 minutes, and extension at 72^{o}C for 2 minutes, and the final extension at 72^{o}C 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.

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	-	-

Line number	Genotype	Crossing	The score of leaf rolling	The score of leaf drying	Score of recovery	Criteria	DREB2A gene	BADH2 gene
S	Salumpikit	Control variety	1	1	1	T	+	+

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									

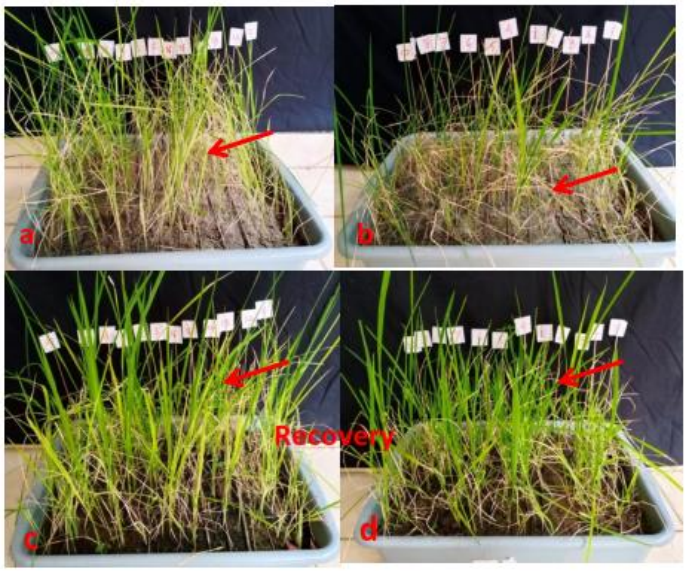

Figure 1: Responses of F6 progeny lines to drought observed on drying leaf (a and b) and recovery ability (c and d)

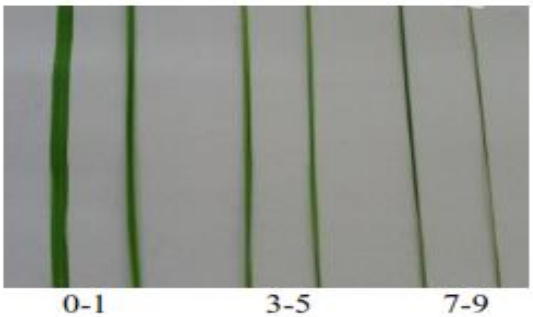

Figure 2: Description of rolling leaves based on SES Method; scores 0-1: start rolling to form V shape; 3-5: rolling to form V and U shapes inside leaves; 7-9: leaves are rolling totally

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 drought tolerance in rice plants is controlled by the *DREB2A* genes. The visualization of the *BADH2* gene 39 selected lines showed a marker with a size of approximately 1300 bp as shown in Figure 4.

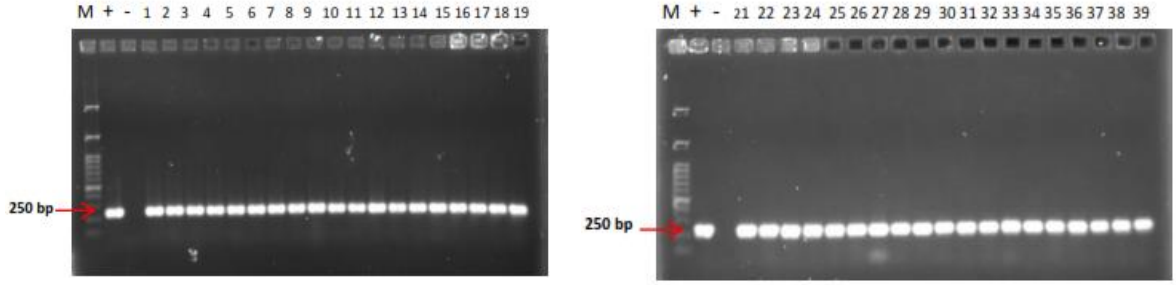

Figure 3: PCR amplification of *DREB2A* (250 bp) on 39 selected lines with Salumpikit and IR20 as positif and negative control respectively (M= DNA ladder of 100 kb)

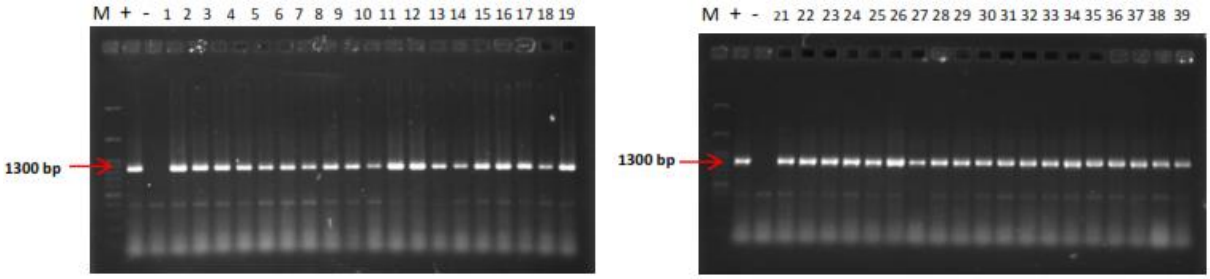

Figure 4: PCR amplification of *BADH2* (1300 bp) on 39 selected lines with Salumpikit and IR20 as positif and negative control respectively (M= DNA ladder of 100 kb)

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.

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 grain yield per-plot varied from the lowest at 458 g to the highest, at 1210 g. The agronomic characters that supports 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 and 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 & Pongthai (2013) and Swain et al. (2017), leaf rolling is one of the mechanisms used by plants to adjust the water potential, and enable them to absorb groundwater in drought stress conditions. Swain et al. (2017) reported that during the drought conditions, the level of groundwater was below 30 cm depth, of the 78 lines of drought-tolerant assessments. Furthermore, 30 and 48 lines were scored by 1, and 3, respectively. Out of these 78 lines assessed, 13 line 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 the rate of transpiration in plants. This condition help plants to 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 to 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. 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, 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). 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 have 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 presente on the 39 selected lines with the marker size of approximately 1300 bp (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 are 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 contribute to regulate 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 of 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 8^{th} 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 rice variety breeding programmes 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 have the opportunity to 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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Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] New notification from Biodiversitas Journal of Biological Diversity

3 pesan

DEWI NUR PRATIWI <smujo.id@gmail.com> 16 Januari 2021 17.00
Balas Ke: DEWI NUR PRATIWI <biodiv07@gmail.com>, Ahmad Dwi Setyawan <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Uncorrected Proof" regarding the submission "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Link: https://smujo.id/biodiv/authorDashboard/submission/6884

Ahmad Dwi Setyawan

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DEWI NUR PRATIWI <smujo.id@gmail.com> 16 Januari 2021 17.16
Balas Ke: DEWI NUR PRATIWI <biodiv07@gmail.com>, Ahmad Dwi Setyawan <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

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[Kutipan teks disembunyikan]

Yosep S. Mau <smujo.id@gmail.com> 16 Januari 2021 19.09
Balas Ke: "Yosep S. Mau" <yosepmau@yahoo.com>, Ahmad Dwi Setyawan <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Uncorrected Proof" regarding the submission "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".
[Kutipan teks disembunyikan]

Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] New notification from Biodiversitas Journal of Biological Diversity

1 pesan

NURHASANAH NURHASANAH <smujo.id@gmail.com> 20 Januari 2021 13.41
Balas Ke: NURHASANAH NURHASANAH <nurhasanah_2710@yahoo.com>, Ahmad Dwi Setyawan <editors@smujo.id>
Kepada: Reny Herawati <reny.herawati@unib.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Uncorrected Proof" regarding the submission "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Link: https://smujo.id/biodiv/authorDashboard/submission/6884

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

Reny Herawati <reny.herawati@unib.ac.id>

[biodiv] Editor Decision

3 pesan

Smujo Editors <smujo.id@gmail.com> 19 Januari 2021 14.42
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: RENY HERAWATI <reny.herawati@unib.ac.id>, ALNOPRI <author@smujo.id>

RENY HERAWATI, ALNOPRI, MASDAR, MARULAK SIMARMATA, SIPRIYADI, MIMI SUTRAWATI:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties".

Our decision is to: Accept Submission

Smujo Editors
editors@smujo.id

Biodiversitas Journal of Biological Diversity

Smujo Editors <smujo.id@gmail.com> 19 Januari 2021 14.43
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: RENY HERAWATI <reny.herawati@unib.ac.id>, ALNOPRI <author@smujo.id>

RENY HERAWATI, ALNOPRI, MASDAR, MARULAK SIMARMATA, SIPRIYADI, MIMI SUTRAWATI:

The editing of your submission, "Identification of Drought Tolerant and Molecular Analysis of DREB2A and BADH2 Genes and Yield Potensial of Lines from Single Crossing Bengkulu Local Rice Varieties," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/6884

Smujo Editors
editors@smujo.id

[Kutipan teks disembunyikan]

Smujo Editors <smujo.id@gmail.com> 19 Januari 2021 14.44
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: RENY HERAWATI <reny.herawati@unib.ac.id>, ALNOPRI <author@smujo.id>

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