

Polymorphism analysis of drought tolerance gene *OsDREB2A* in Indonesian local rice from Lampung, Indonesia

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Abstract. *Chrisnawati L, Ernawati E, Yulianty, Hariri MR. 2022. Polymorphism analysis of drought tolerance gene OsDREB2A in Indonesian local rice from Lampung, Indonesia. Biodiversitas 23: 6352-6357.* Drought stress is a significant threat to rice cultivation and the selection of drought-tolerant rice plants is needed to find superior plants. The *OsDREB2A* gene plays a role in the regulation of drought tolerance and is widely used as a marker. Therefore, this study aims to analyze the *OsDREB2A* gene polymorphism in the local rice variety of Lampung Lumbang Sewu Cantik, compared to the Inpago 8 drought-tolerant and the IR64 sensitive variety. DNA extraction was performed on fresh leaves using the GENE AID Genomic DNA Mini Kit, amplification by PCR was carried out with the *OsDREB2A* primer pair, while sequence analysis was conducted with MEGA X and confirmed using the BLAST program. The sequencing results showed that the three rice varieties had the same sequence length of 250 bp. The Lumbang Sewu Cantik variety has the same sequence as Inpago 8, while IR64 has an insertion that causes a frameshift mutation. Furthermore, phylogenetic analysis showed that the Lumbang Sewu Cantik variety was in a similar group with tolerant varieties, while IR64 was in a different group. The similarity of the *OsDREB2A* Lumbang Sewu Cantik sequence is presumably associated with tolerance to drought.

Keywords: Drought stress, Lampung local rice, Lumbang Sewu Cantik, *OsDREB2A*

INTRODUCTION

A big challenge facing the world currently is climate change caused by global warming, which has several consequences, such as the lack of water leading to deficiency not only in the soil but also in the plant tissue. Most of the water in plants will be lost due to the increase in evaporation and temperature (Sultan et al. 2019). This phenomenon leads to drought stress against plants which is a problem for agriculture around the world. Global warming affects crop production (Sharma et al. 2022) and reduces rice supplies (Moonmoon and Islam 2017; Sarwendah et al. 2022). This is a challenge in the future, considering that rice is the most vulnerable crop to climate change (Mohanty et al. 2013)

Drought stress reduces rice production by inhibiting cell growth, cell proliferation, and differentiation, causing the accumulation of reactive oxygen species (Upadhyaya and Panda 2019; Liu et al. 2019). Rice is very sensitive to drought conditions, specifically in the germination and early seedling growth stages (Vibhuti et al. 2015). Drought stress causes a decrease in ATP production and respiration, which trigger seed germination failure. Furthermore, it leads to a decrease in plant height, leaf width, and biomass (Panda et al. 2021), while also affecting the morphology, anatomy, physiology, and biochemistry. Fresh biomass of plants contains about 80-95% water which plays a role in physiological processes. Hence, water deficit will affect the metabolic processes (Seleiman et al. 2021).

Drought triggers plant defenses involving various mechanisms at the molecular and cellular levels, which will activate antioxidant defense systems against oxidative damage (Laxa et al. 2019). Plants contain ABA, a phytohormone involved in tuning responses to drought stress (Alazem and Lin 2017). It induces root growth for water uptake, promotes stomatal closure to minimize transpiration, and plays a role in the expression of genes involved in osmoprotectant, osmoregulation, and control of stress damage as well as improvement in drought conditions to provide stress tolerance (Zhu 2016). Drought also triggers the activation of various genes involved in plant defense, one of which is the *DREB2A* gene. This gene encodes a protein, which acts as a transcription factor that regulates plant responses to abiotic stress by hydrating cells through ABA-independent signaling pathways (Hrmova and Hussain 2021). The transcription factors play an important role in the regulation of genes that control plant stress responses (Song et al. 2016). In rice, drought induces the expression of the *OsDREB2A* gene (Zhang et al. 2013). Currently, the *OsDREB2a* gene is widely used as a marker of plant resistance to abiotic stress because it can distinguish between drought-tolerant and sensitive plants (Rini 2019).

Several investigations have been conducted on the association of the *OsDREB2A* polymorphism with drought tolerance in rice. Al Azzawi et al. (2020) selected Iraqi rice to obtain drought-tolerant cultivars using *OsDREB2A* as a molecular marker. Another study was conducted by Jadhao et al. (2014) by associating allelic variations of *OsDREB2A*

with drought tolerance characters. Furthermore, mutations in the gene have been shown to reduce the ability to bind to cis-regulatory target genes such as *rd29A* in local Indonesian Java rice varieties (Lathif et al. 2018). Differences in the *OsDREB2A* gene sequence cause alterations in the protein structure (Niu et al. 2020; Gumi et al. 2018) which can change plant responses to drought stress (Liu et al. 2013).

The development of drought stress tolerance rice is an effective strategy to adapt to increasing global temperatures. This can be implemented by exploring the potential for local rice resistance to drought. Evaluation of Indonesian local rice against drought has been carried out, but species endemic to Lampung Province have not been studied. Lumbang Sewu Cantik is a local drought-resistant rice from Lampung that has long been cultivated by local farmers in the highlands. Previous studies revealed that during the germination phase, Lumbang Sewu Cantik adapted to osmotic stress through the induction of 20% PEG (Chrisnawati et al. 2022). Exploring the potential for resistance of local rice germplasm can be useful in conserving genetic resources that can be used in the development of new drought-resistant varieties. Therefore, this study aims to analyze the *OsDREB2A* gene polymorphism in local rice of Lumbang Sewu Cantik variety from Lampung, Indonesia, compared to Inpago 8 drought-stress resistant and IR64 sensitive varieties to evaluate its drought tolerance.

MATERIALS AND METHODS

Materials

The samples used were Lumbang Sewu Cantik as local rice varieties, as well as Inpago 8 and IR64 as resistant and sensitive controls, respectively. Lumbang Sewu Cantik was obtained from Pardasuka Pringsewu Village, Lampung, Indonesia.

Procedures

DNA extraction

DNA extraction was performed on fresh leaves using the GENE AID Genomic DNA Mini Kit. Initially, 100 mg leaves were cut and placed in liquid nitrogen. The leaves sample were ground to a powder and transferred to a 1.5 ml microtube, then 5 µl of RNase A and 400 µl of GP1 Buffer or GPX1 Buffer were added and mixed by the vortex. The mixture was incubated at 60°C for 10 minutes, and the tube was inverted every 5 minutes during the process. Subsequently, Elution Buffer (200 µl per sample) was pre-heated to 60°C and then 100 µl of GP2 Buffer was added, mixed by vortex and incubated on ice for 3 minutes. A Filter Column was placed in a 2 ml Tube, then the solution was transferred, centrifuged at 1,000 x g for 1 minute, and discarded. The supernatant was transferred to a new 1.5 ml microtube, while a 1.5 volume of GP3 Buffer was added and vortexed for 5 seconds. Afterward, 400 µl of W1 Buffer was added to the GD Column and centrifuged at 14-16,000 x g for 30 seconds. The flow-through was discharged then the GD Column was inserted into the

Collection Tube 2 ml and centrifuged for 3 minutes at 14-16,000 x g to dry the column matrix. The dried GD Column was transferred to a clean 1.5 ml microcentrifuge tube then 100 µl of TE was added to the middle of the column matrix. To ensure the TE was completely absorbed, it was left for five minutes and then centrifuged for 30 seconds at 14-16,000 x g to elute the purified DNA.

DNA amplification

DNA amplification (PCR) was performed using primer pair *DREB2A* (F = 5'-CCT CAT TGG GTC AGG AAG AA-3' and R = 5'-GGA TCT CAG CCC ACT TA-3') and PROMEGA GoTaq Flexi DNA Polymerase with a 25 µL mixture of reaction from the laboratory namely (5 L 5X buffer, 2 L MgCl₂ 25 mM, 0.5 L dNTPs 10 mM, 0.5 L forward primer 10 mM, 0.5 L reverse primer 10 mM, 0.14 L Taq Polymerase, 14.36 L free nuclease water, and 2 L DNA template). The reaction was carried out at temperature conditions for PCR, namely initial denaturation of 95°C-5 minutes and 35 cycles, denaturation 94°C-1 minute, annealing 59°C-2 min, elongation 72°C-2 min, final elongation 72°C-10 min, and maintained at 14°C. The PCR results were then visualized using 1% agarose on TBE and BIOTIUM Gel Red Nucleic Acid Gel Stain under UV light.

Data analysis

The DNA sequences were read and edited using MEGA X software, with the forward and reverse sequences of each sample combined to form a consensus DNA sample. *DREB2A* sequence confirmation was carried out using the BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to search for homologs in the GenBank database. Sequence alignment with comparison was performed using the ClustalW method, while the phylogenetic tree was inferred with the UPGMA method. Phylogenetic analyses were conducted by MEGA X, while the phylogenetic tree construction in which related taxa clustered together in a bootstrap test was performed with 1000 replicates.

RESULTS AND DISCUSSION

PCR amplification

DNA isolation from the samples of Lumbang Sewu Cantik, Inpago 8 (drought-resistant rice), and IR64 (sensitive rice) was successfully carried out, producing sufficient and good-quality DNA. The results of the *DREB2A* gene PCR amplification showed a single band with a size of 250 bp, as presented in Figure 1. The PCR products were then sequenced and the data showed that the three rice varieties had the same sequence length of 250 bp. Furthermore, sequencing was carried out to determine the *OsDREB2A* polymorphism from the three varieties.

Sequence analysis

BLAST confirmation in each sample *OsDREB2A* sequence showed 100% query cover compared to *Oryza sativa* Indica Group cultivar Pokkali dehydration responsive element binding protein 2a (*DREB2A*) mRNA, complete cds (JQ341059.1). The percentage of similarity

shown was 100% (Table 1), while Pokkali cultivar was selected as a comparison for drought resistance other than Inpago 8. Pokkali is known to have resistance to abiotic stress (de Silva et al. 2014).

Multiple alignments were performed on the sequences of Lumbung Sewu Cantik, Inpago 8, IR64, and the comparison of Pokkali sequences (access number JQ341059mutation.1). Furthermore, to facilitate analysis, identical symbols were used in the MEGA X software for DNA base similarities and differences in the four sequences and the results displayed highlights for samples with different sequences. Lumbung Sewu Cantik, Inpago 8, and Pokkali have identical bases compared to IR64, as demonstrated in Figure 2. The *OsDREB2A* gene in IR64 underwent a frameshift caused by the insertion, as observed in the two GA bases (1 and 2) early in the sequence (Figure 3). The frameshift mutation caused the IR64 sequence skeleton to shift and the sequence to be longer. When the two GA bases (1st and 2nd base) were removed at the beginning of the IR64 sequence and the 3-203rd base was observed on IR64, the same DNA sequence was obtained as the 1-201st base on Lumbung Sewu Cantik, Inpago 8, and Pokkali. The sequence skeleton was shifted back to two bases due to the insertion of GA. Meanwhile, the 202nd base (Guanine base) in Lumbung Sewu Cantik, Inpago 8, and Pokkali, was not the same as the 204th base (Adenine) at IR64 (Figure 3). This means that there was a transition mutation in the *OsDREB2A* IR64 gene.

Phylogenetic analysis based on the *OsDREB2A* gene sequence showed that two branches separated drought-resistant from sensitive rice (Figure 4). The Lumbung Sewu Cantik rice is thought to be drought-resistant along with Inpago 8 and Pokkali, while IR64 as control was separated from the group.

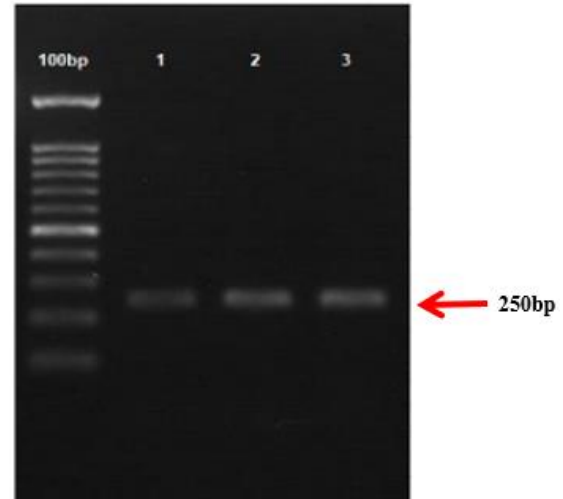


Figure 1. PCR amplification showed that the sample DNA had a size of 250bp. DNA sample in the picture; 1= Inpago 8 (resistant control), 2= IR64 (sensitive control), and 3= Lumbung Sewu Cantik (local rice)

Table 1. BLAST confirmation results of *OsDREB2A* rice samples against *Oryza sativa* Indica Group cultivar Pokkali dehydration responsive element binding protein 2a (DREB2A) mRNA, complete cds (JQ341059.1)

Sample	Max score	Total score	Query cover	E value	Per. ident	Acc. Len	Accession
Lumbung Sewu Cantik	375	375	100%	3.00E-102	100.00%	846	JQ341059.1
Inpago 8	379	379	100%	3.00E-103	100.00%	846	JQ341059.1
IR64	381	381	100%	7.00E-104	100.00%	846	JQ341059.1



Figure 2. Multiple alignments of each *OsDREB2A* sequence. The dot symbol shows the similarity of the nucleotide bases in the sequence. The highlight color indicates that the sequence is different

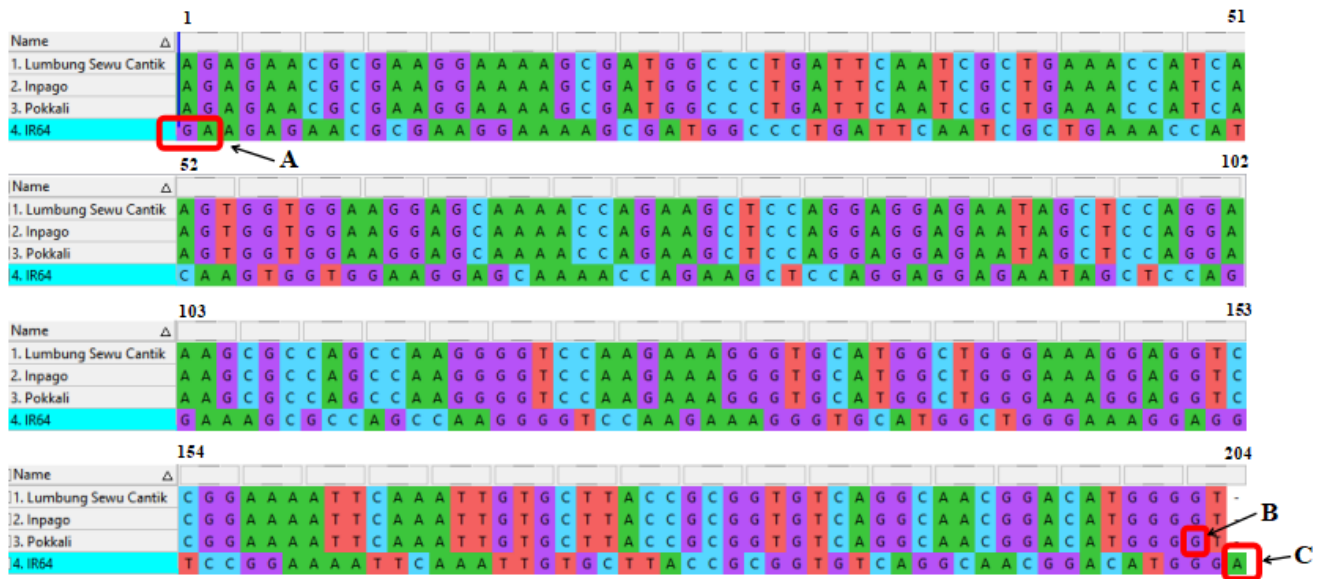


Figure 3. Multiple alignment analysis of each *OsDREB2A* sequence showing the nucleotide bases of each sequence. A= GA base insertion at the beginning of the sequence causes a -frameshift; B and C= a transition mutation between G and A base

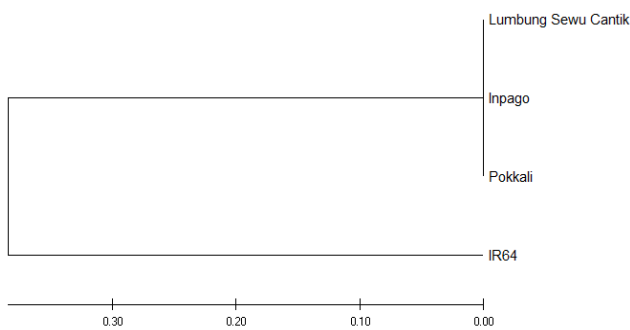


Figure 4. Phylogenetic tree using UPGMA method

Discussion

This study investigated the correlation between the *OsDREB2A* gene and drought resistance in Lampung local rice, the Lumbung Sewu Cantik variety, which is widely cultivated by local farmers in the highlands. The result showed that there is an *OsDREB2A* sequence polymorphism between Lumbung Sewu Cantik and the comparative variety. Polymorphism is caused by insertion that leads to a frameshift and a transition mutation. Lumbung Sewu Cantik has exactly the same *OsDREB2A* sequence as Inpago 8 used as a comparison of drought-resistant rice. Inpago 8 is a drought-tolerant variety developed for dry land with low rainfall (BB Padi 2022). In addition, a comparison of other resistant varieties from GenBank, namely Pokkali, was used. The *OsDREB2A* Pokkali gene was widely transformed for transgenic plants resistant to osmotic stress (Borah et al. 2017) and as a comparison variety for drought resistance (Zakiyah et al. 2021; Jayaweera et al. 2016). Sequence analysis showed that Lumbung Sewu Cantik also has the same sequence as Pokkali, while the different *OsDREB2A* sequence was only

shown by IR64, which was used as a comparison of sensitive variety and yielded an inferior response under drought stress (Miftahudin et al. 2020).

Furthermore, *OsDREB2A* is involved in the abiotic stress response and its expression is induced by drought. It is also a member of the *DREBP* subfamily of AP2/ERF transcription factors in rice (*Oryza sativa*) (Zhang et al. 2013). Transcription factors play an important role in upstream gene regulation of plant stress response pathways (Javed et al. 2020). In a previous study, it was found that the *OsDREB2A* mutation changed the structure of the B-sheet (Zakiyah et al. 2021), thereby causing alterations in protein function (Abrusan and Marsh 2016) which inhibited the binding to the promoter region (Lathif et al. 2018). It is known that the B-sheet structure is the *OsDREB2A* binding area on the promoter of the target gene which is commonly referred to as the DRE box (Chen et al. 2020) and changes in this region affect the expression of the target gene. Based on the results, the *OsDREB2A* sequence of Lumbung Sewu Cantik did not experience mutations in the analyzed sequences. The mutation was found only in IR64, hence, the phylogenetic tree shows two separate groups. Lumbung Sewu Cantik, Inpago 8, and Pokkali are in the same group, while IR64 is separated into different branches.

The sequence analysis showed that Lumbung Sewu Cantik did not have different sequences of *OsDREB2A* with drought-tolerant varieties Inpago 8 and Pokkali, but very different from IR64 sensitive plants, which experienced insertion mutations. The similarity of the *OsDREB2A* Lumbung Sewu Cantik sequence with tolerant plants is presumably associated with the level of tolerance to drought. A previous study showed that this variety could maintain the length and dry weight of the radicle when induced with drought through PEG 20% at the germination phase, with a heavier radicle weight than Inpago 8 and

IR64 (Chrisnawati et al. 2022). This shows that Lumbung Sewu Cantik has the potential to be drought-tolerant (Chrisnawati et al. 2021). Root length is a morphological character that is widely observed in drought tolerance testing and associated with plant resistance to drought stress (Polania et al. 2017). Plants with longer roots are expected to be able to extract a lot of water in dry soil (Cai et al. 2022). Long roots will make it easier for plants to reach water sources. Hence, rice with elongated roots has the potential to become drought-tolerant. In addition, plants with large root dry weight also have good adaptation to water deficit (Dien et al. 2017). The root biomass describes root density, which determines the response to drought stress (Guo et al. 2022). Root density is needed to deal with hydraulic conductivity in land with limited water (Zhang et al. 2020). During soil drying, roots with high hydraulic conductivity will be more responsive in reducing transpiration. This is beneficial for the plant to maintain growth (Cai et al. 2022).

Based on the results, the phylogenetic tree exhibited two distinct branches between tolerant and sensitive varieties with very minor modifications in the nucleotide sequence of the *OsDREB2A* gene. The conserved region in the consensus sequence *OsDREB2A* was found in Inpago 8, Pokkali, and Lumbung Sewu Cantik. In contrast, the insertion or deletion of nucleotides in the initial region of the sequence was found in IR64-sensitive plants. The insertion of two nucleotides G and A, at the beginning of the sequence caused a shift in the IR64 nucleotide frameshift, although the order did not change significantly. However, the shift in the sequence framework culminated in many different haplotypes compared to all *OsDREB2A*. The transition mutation that occurred at the end of the sequence, where G was replaced by A, adds to the haplotype differences. Differences in the *OsDREB2A* sequence in IR64 indicate poor adaptation to water-deprived environments. Therefore, it was concluded that Lumbung Sewu Cantik has potential drought tolerance properties based on the *OsDREB2A* gene, but an agronomic analysis is needed to confirm this result.

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