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Exploration of *Oryza sativa* drought-responsive element binding protein 2A (*OsDREB2A*) gene in several local Indonesian rice varieties

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Abstract. *Yuliza, Salamah A, Puspitaningrum H. 2024. Exploration of* Oryza sativa *drought-responsive element binding protein 2A (OsDREB2A) gene in several local Indonesian rice varieties. Biodiversitas 25: 574-582.* Drought is one of the abiotic stress factors that reduces rice productivity in Indonesia. *OsDREB2A* is a member of the DREBP subfamily of AP2/ERF transcription factors and participates in drought stress by directly binding to DRE elements to regulate downstream gene expression. However, further research is still needed to observe each *OsDREB2A* gene in local Indonesian rice varieties. This research aims to explore the *OsDREB2A* gene in several local Indonesian rice varieties, namely from Java (Ciherang, Situbagendid, Way Apo, Inpara 32 and Inpari 42), Kalimantan (Black Rice), Aceh (Sigupai) and West Sumba (Pare Lambem and Pare Bakato Kaka). DNA was isolated from the leaves of each variety, amplified using PCR, and then electrophoresed and sequenced. Sequencing data were analyzed using DNA Baser, BioEdit and then visualized using the SWISS-MODEL server, Rice Genome Annotation Project Database, and chromosome map tools. The results showed that nine samples had 100% query cover with the Pokkali *OsDREB2A* (KU159743.1) cultivar sequence, 99.86% similar percent identity compared to cultivar R180 and WAB540-1-B-P-6-1-1, 99.75% similar percent identity compared to cultivar FL-478 and 99.62% similar percent identity compared to cultivar Nona Bokra. The amino acid structure of each cultivar refers to chromosome 1 at the LOC_Os01g07120 locus.

Keywords: Drought, *OsDREB2A* gene, local rice varieties, sequencing

INTRODUCTION

Climate change significantly impacts agriculture and food supply; fluctuations in temperature and precipitation lead to decreased crop productivity and quality (Hasan and Hussain 2020). Rice is one of the most vulnerable crops to climate change (Schneider and Asch 2020; Saud et al. 2022). At the same time, a significant portion of the Indonesian population relies on rice as their main source of carbohydrates (Hafizah et al. 2020). Rice is a crucial food commodity in Indonesia, significantly ensuring national economic stability and contributing to community income (Sukmayanto and Listiana 2022). However, according to the data from the Indonesian Central Bureau of Statistics, Indonesian rice production in 2021 decreased by 0.42% compared to the previous year, and one of the factors contributing to this decline is weather-related conditions (BPS 2022).

Prolonged periods of drought resulting from extreme weather conditions can subject crops to stress, reducing food productivity (Lesk et al. 2016; Lamaoui et al. 2018). Under drought conditions, crops undergo physical, functional, and chemical changes. This stress impacts the crops' productivity, growth, reproduction, and overall development. Additionally, drought diminishes crucial chlorophyll and pigment levels necessary for photosynthesis. Extended stomata closure results in a lack of carbon dioxide (CO_2), negatively impacting photosynthesis. Plants experience oxidative stress, causing substantial harm. Plants activate their innate protective mechanisms to defend against Reactive Oxygen Species (ROS) and prevent severe damage. Responses like decreased transpiration and leaf curling serve as primary strategies to limit water loss and counter the harsh effects of drought. Resilient plants have adapted through deep root systems, leaf curling, hormonal signaling, and efficient transpiration (Batra et al. 2014; Shahzad et al. 2016; Zargar et al. 2017).

Selecting drought-tolerant rice varieties can be a potential approach to overcome the decreased rice harvest due to drought (Kumar et al. 2014). Molecular-based selection is necessary to confirm its genotype tolerance (Gupta and Shaw 2021). This approach involves identifying specific genes or markers associated with stress tolerance. The *Oryza sativa* Dehydration Response Element 2A (*OsDREB2A*) can be used as a molecular marker for drought in rice. The *OsDREB2A* belongs to the dehydration -responsive element-binding protein/C-repeat binding factors (DREB/CBF) Transcription Factor group, which is associated with *cis-acting dehydration-responsive element*/ C-repeat. The DREB gene plays an important role in encoding transcription activators for the expression of salinity, drought, and cold conditions (Zhang et al. 2013; Chrisnawati et al. 2022). Abscisic acid (ABA), a key regulator in plant drought response, induces the DREB protein. DREB interacts with other drought transcription

factors, including NAC (No Apical Meristem/NAM), ATAF (Arabidopsis Transcription Activation Factor), and CUC (Cup-shaped Cotyledon), MYB (Myeloblastosis), AREB (Abscisic Acid Responsive Element Binding Protein), ABRE (Abscisic Acid Responsive Element), and DRE/CRT (Dehydration Responsive Element/C-Repeat), through an ABA-independent pathway (Shaheen et al. 2018; Sidorenko and Chebotar 2022).

Several studies have been conducted on using plants' *OsDREB2A* gene related to drought tolerance. According to a study by Hassan 2021, the *OsDREB2A* gene was inserted into tomato genotypes using the Agrobacterium-mediated transformation method. The study found that the transgenic tomato lines showed enhanced drought tolerance compared to the wild type (Hassan et al. 2021). The interaction between OsSaIT, a Jacalin domain-containing protein, and *OsDREB2A* imparts drought stress tolerance in plants (Sahid et al. 2021). Another study has evaluated the use of *OsDREB2A* as a transcription factor for Rice Tungro Spherical Virus (RTSV) in drought responses (Encabo et al. 2020).

The inventory of local rice varieties is essential to obtain potential donor genes for developing stress-tolerant varieties, which is crucial for food security and sustainable agriculture programs (Kamruzzaman 2017). Molecular data is important to support the advantageous information of each local rice variety. Combining molecular and agronomic data can provide a more comprehensive understanding of varieties and develop strategies to improve their cultivation and utilization. Therefore, this study aims to explore the *OsDREB2A* gene in some local rice varieties in Indonesia.

MATERIALS AND METHODS

Location of research

The research was conducted in the Molecular Biology Preparation Laboratory and Integrated Instrumentation Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java, Indonesia.

Material

The samples used in this study were Java local rice varieties (Ciherang, Situbagendid, Way Apo, Inpara 32, and Inpari 42), Kalimantan local rice variety (Black Rice), Aceh local rice variety (Sigupai) and West Sumba local rice varieties (Pare Lambem and Pare Bakato Kaka).

Methods

DNA extraction

The DNA extraction was performed based on the protocol provided by the VIOGENE plant DNA isolation kit. Initially, The 100 mg leaf was ground using a mortar in liquid nitrogen. The sample was transferred into a 1,5 mL microcentrifuge tube, and 400 µL PX1 and 5 µL RNAse were added. Next, the sample was vortexed and incubated for 30 minutes at 65°C. During the incubation, the tube was inverted every 7 minutes. After the incubation, 130 µL of PX2 was added to the lysate and vortexed. Then, the mixture was incubated on ice for 10 minutes. Subsequently, the tube was placed into a shearing tube and centrifuged at 13,000 rpm for 2 minutes. The supernatant was transferred to a new tube, and PX3 200 µL and ethanol 400 µL were added. The mixture was inverted 5 times and transported to the mini-column tube, then centrifuged for 1 minute at 10,000 rpm. The liquid at the bottom of the tube was discarded, and the mini-column tube was centrifuged at 10,000 rpm for 1 minute. Afterward, WS buffer 700 µL was added to the mini-column tube twice and centrifuged at 13,000 rpm for 1 minute. The flow-through sample from the top of the mini-column tube was placed on a new tube, and 20 µL of TE was added to the middle of the column. It was left for 5 minutes and centrifuged at 13,000 for 2 minutes to elute the purified DNA. The top part of the mini-column tube was discarded. The DNA sample was stored in the 1.5 mL tube at a temperature of -20°C.

DNA amplification

PCR (polymerase chain reaction) is used to amplify the target gene of a pair of primers. The specific *OsDREB2A* designed primers have a forward primer base sequence of 5'-ATG CTG TTT CGA TTT GTG TCT TG-3' and a reverse primer base sequence of 5'-CTA ATA GGA GAA AAG GCT AAA C-3' (Zakiyah et al. 2021). The target gene fragment length is approximately 800 bp. The procedure involves a reaction volume of 20 µL. The reaction mixture comprises 10 µL of GoTaq Green Master Mix, 7 µL of nuclease-free water, 1 µL of 10 mM forward primer, 1 µL of 10 mM reverse primer, and 1 DNA template. The GoTaq Green Master Mix is a premixed ready-to-use solution containing dNTPs, Taq DNA polymerase, MgCl2, and reaction buffers for efficient amplification of DNA templates at optimal concentrations by PCR. The PCR program used for 35 cycles consists of a pre-denaturation step at 94°C for 2 minutes, next by denaturation at 94°C for 20 seconds, annealing at 46.4°C for 10 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 3 minutes. Agarose gel electrophoresis was performed to visualize the PCR amplification results. The first step was to prepare a 1% agarose gel using 1X TAE buffer. The agarose gel electrophoresis results were visualized using a gel documentation system or a UV light box.

Data analysis

The sequencing data of the fragment base of *OsDREB2A* was obtained in the *.abi file format from P.T Genetika Science. The data was analyzed using many software. DNA Baser generates a consensus nucleotide sequence from forward and reverse sequences. A BLAST (Basic Local Alignment Search Tool) search on the NCBI was used to identify homologous sequences in the GenBank database to confirm the obtained nucleotide sequence. BioEdit software performed sequence alignment to analyze the differences between the GenBank database's sample and reference sequences. Afterward, nucleotide sequences were translated into amino acid sequences by using BioEdit software. The SWISS-MODEL server was used to visualize the generated 3D protein structures from the amino acid sequence (https://swissmodel.expasy.org). The chromosome coordinates of protein sequences were

visualized using the Rice Genome Annotation Project Database (http://rice.uga.edu/) and chromosome map tool (http://viewer.shigen.info/ oryzavw/maptool/MapTool.do).

RESULTS AND DISCUSSION

PCR amplification

Amplification of the *OsDREB2A* gene from nine local rice varieties showed a single PCR product at approximately 800 bp (Figure 1).

Sequence analysis

Using BLAST on the NCBI website, each sample of several Indonesian rice varieties showed 100% query cover compared to Oryza sativa Indica Group cultivar Pokkali DREB2A transcription factor gene, complete cds (KU159743.1). The 775-808 bp sequences of nine Indonesian rice varieties were submitted to GenBank as accession no. PP171509-PP171513 and PP187271-PP187274, and the percent Identity shown was 100% (Table 1). The Pokkali cultivar is a standard of abiotic stress tolerance for drought and salt parameters (Nounjan et al. 2018). Each sample of several Indonesian rice varieties also showed 99.86% percent identity similarity with cultivars R180 and WAB540-1-B-P-6-1-1, 99.75% percent with cultivar FL-478, and 99.62% percent with cultivar Nona Bokra.

BioEdit performed the alignments on the sequences of Padi Hitam, Sigupai, Ciherang, Situ Bagendid, Way Apo, Inpari 32, Inpari 42, Pare Bakato Kaka, Pare Lambem, and the comparison to Pokkali, R180, WAB540-1-B-P-6-1-1, FL-478, Nona Bokra sequences. The results of 9 varieties showed no different sequences of each *OsDREB2A* gene (Figure 2). The alignment process of Padi Hitam, Ciherang, Situ Bagendid, and Way Apo started at the same sequence as Pokkali cultivar. Meanwhile, Inpari 32, Inpari 42, Pare Bakato Kaka, and Pare Lambem aligned at the 43rd base, and Sigupai variety started the alignment at the 19th base.

The nucleotide sequence was converted to an amino acid sequence using the bioEdit application. The alignment of amino acid sequences from 9 samples showed no differences with the Pokkali cultivar. Based on the data from NCBI, The *Oryza sativa* Indica Group cultivar Pokkali *DREB2A* transcription factor gene, complete cds (KU159743.1), has a DNA binding site located on amino acid residues 83 to 138. Nine test samples and comparisons did not experience mutations on the DNA binding site. The nucleotide alignment results show that R180 and WAB540-1-B-P-6-1-1 have many nucleotide differences from sequence 23rd to sequence 67th compared to the others. R180 and WAB540-1-B-P-6-1-1 have an insertion of the base Guanine at 61st base. R180 has a substitution at base 55 from Cytosine to Adenine. Nona Bokra has a substitution at base 772 from Adenine to Guanine. Furthermore, the cultivars Nona Bokra and FL-478 have substitutions at bases 790th and 791st of the bases Cytosine and Guanine to Adenine. R180 and WAB540-1-B-P-6-1-1 have deletions in bases 827th, 828th and 829th.

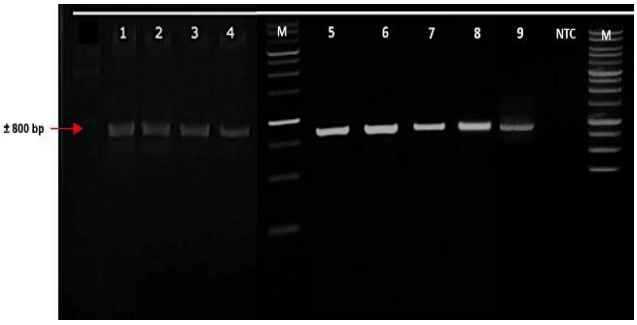

Figure 1. Amplification of *OsDREB2A* gene fragments from nine different local rice varieties. 1: Pare Lambem, 2: Pare Bakato Kaka, 3: Inpari 42, 4: Inpari 32, 5: Sigupai, 6: Situbagendid, 7: Ciherang, 8: Padi hitam and 9: Way Apo, M: 1kb DNA marker, NTC: Non-template control

Table 1. BLAST results of 9 rice samples against *Oryza sativa* Indica Group cultivars and *Oryza glaberrima* cultivars

Description	Max score	Total score	Query cover	E value	Acc. length	Percent identity	Accession
Padi Hitam	1493	1493	100%	0	808	100%	PP171510
Sigupai	1439	1439	100%	0	779	100%	PP171511
Ciherang	1474	1474	100%	0	794	100%	PP171509
Situ Bagendid	1461	1461	100%	0	791	100%	PP171512
Way Apo	1471	1471	100%	0	796	100%	PP171513
Inpari 32	1430	1430	100%	0	774	100%	PP187272
Inpari 42	1428	1428	100%	0	773	100%	PP187271
Pare Lambem	1404	1404	100%	0	760	100%	PP187274
Pare Bakato Kaka	1432	1433	100%	0	775	100%	PP187273
Oryza sativa Indica cultivar Pokalli *DREB2A*, TF *genes, complete cds*	1430	1430	100%	0	849	100%	KU159743.1
Oryza sativa Indica Group cultivar FL-478 *DREB2A* transcription factor gene, *complete cds*	1456	1456	100%	0	849	99.75%	KU159744.1
Oryza sativa Indica Group cultivar Nona Bokra *DREB2A* transcription factor gene, *complete cds*	1450	1450	100%	0	849	99.62 %	KU159746.1
Oryza sativa isolate R180 *DREB2A* gene, *complete cds*	1356	1356	92%	0	825	99.86%	MN436785.1
Oryza sativa isolate WAB540-1-B-P-6-1-1 *DREB2A* gene, *complete cds*	1356	1356	92%	0	825	99.86%	MN436788.1

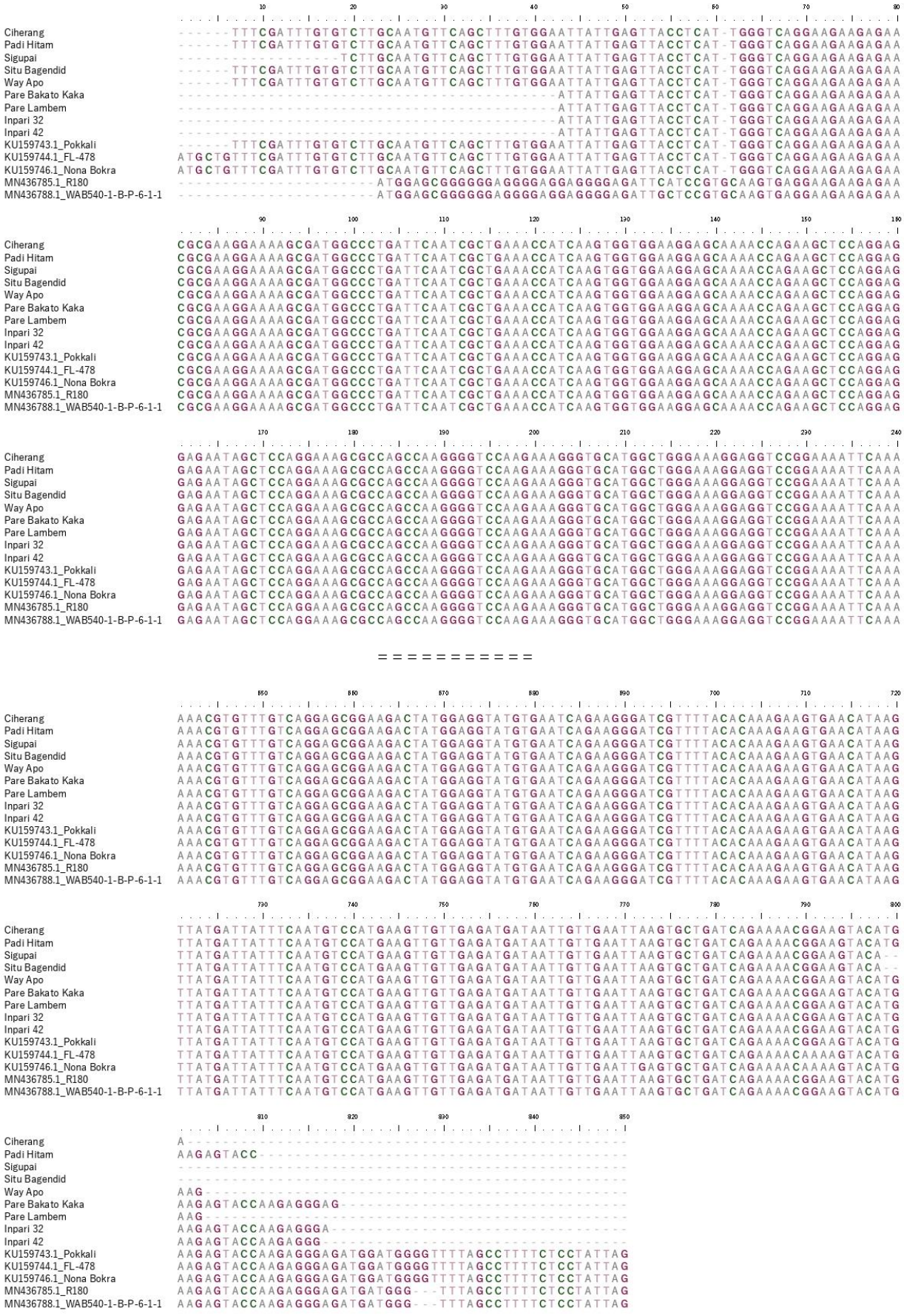

Figure 2. Multiple alignments of nine local varieties of Indonesian rice *OsDREB2A* gene. The red double-dash line (===) shows similar base of each variety and reference seq and depicts due to its excessive sequence length

The amino acid alignment results show that R180 and WAB540-1-B-P-6-1-1 have differences in amino acids from sequences 8 to 21 but do not cause a frame shift in the following amino acid sequence. FL- 478 and the Nona Bokra cultivar changed the 264th amino acid from Glutamic Acid to Lysine. Changes in R180 and WAB540-1-B-P-6-1-1 at bases 827th, 828th and 829th eliminate the Tryptophan protein at 275th (Figure 3).

The program SWISS-Model server was used to obtain the protein structure of nine test samples and the comparison cultivars, and the results showed similarities between them (Table 2). The protein structure of each variety contains α-helix, β-strand , and structure coil. The alterations of amino acids at R180, WAB540-1-B-P-6-1-1, FL- 478, and Nona Bokra do not occur in the alpha helix and beta regions; instead, they exclusively take place in the coil structure region (Figure 4).

According to the rice genome project, each of the nine protein sequences corresponds to chromosome 1 with the locus ID LOC_Os01g07120 (Table 3). Furthermore, according to the NCBI server, the LOC_Os01g07120 locus consists of two exons, the first exon spanning from base 1 to 463, and the second exon spanning from base 464 to 1472. The alignment results for the *OsDREB2A* primer used against the sequence at LOC_Os01g07120 are found at bases 518-539 and 1327-1349. This observation suggests that the *OsDREB2A* sequences obtained from the results of specific PCR primers in the nine varieties are contained within a single exon.

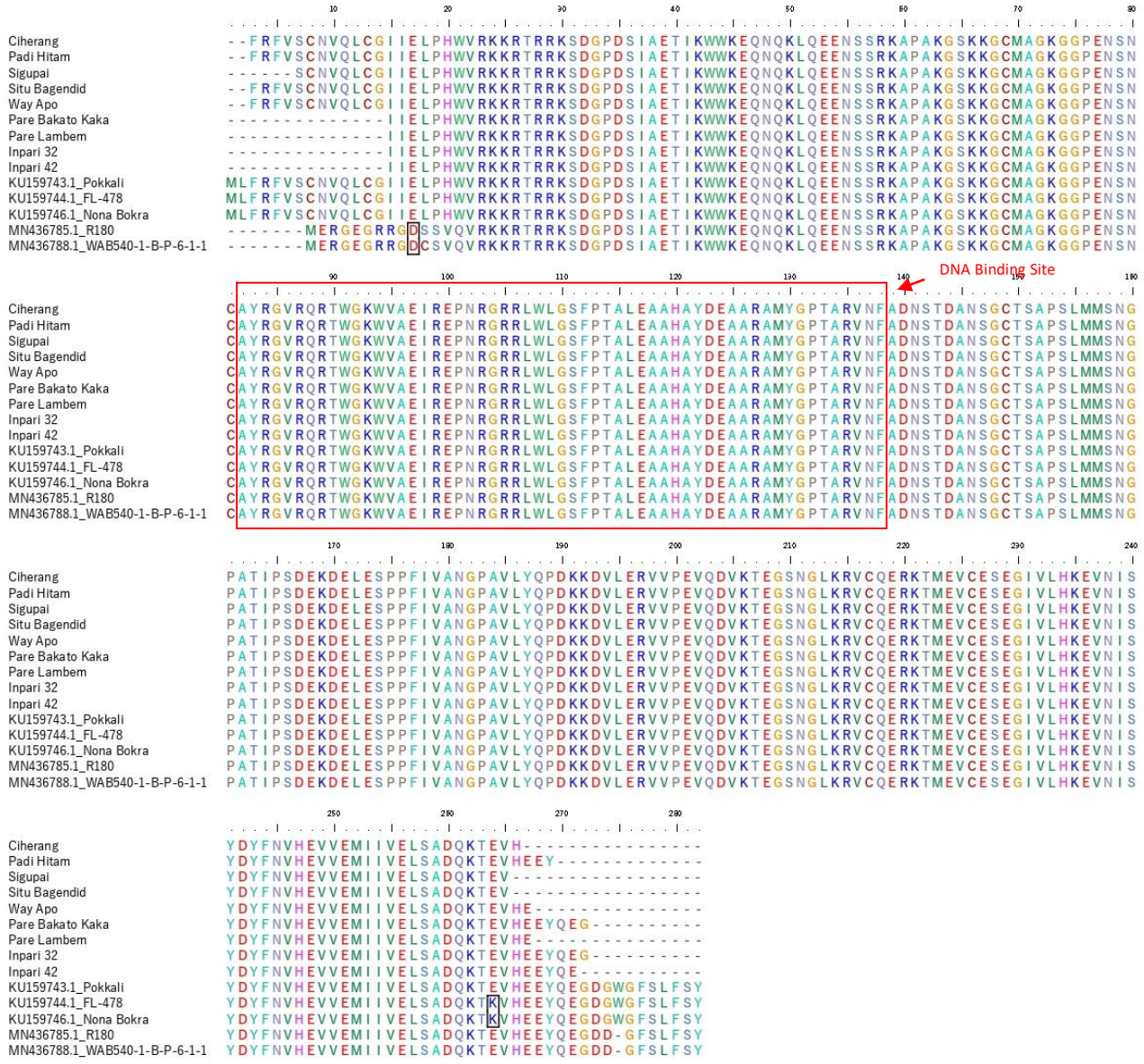

Figure 3. Multiple alignments of *OsDREB2A* amino acid sequences covering the DNA Binding Site from nine local varieties of Indonesian rice with eight other reference amino acid sequences

Table 2. *OsDREB2A* protein model generated from each nine local varieties of Indonesian rice sequences and comparisons. A: α-helix, B: β-strand, C: structure coil

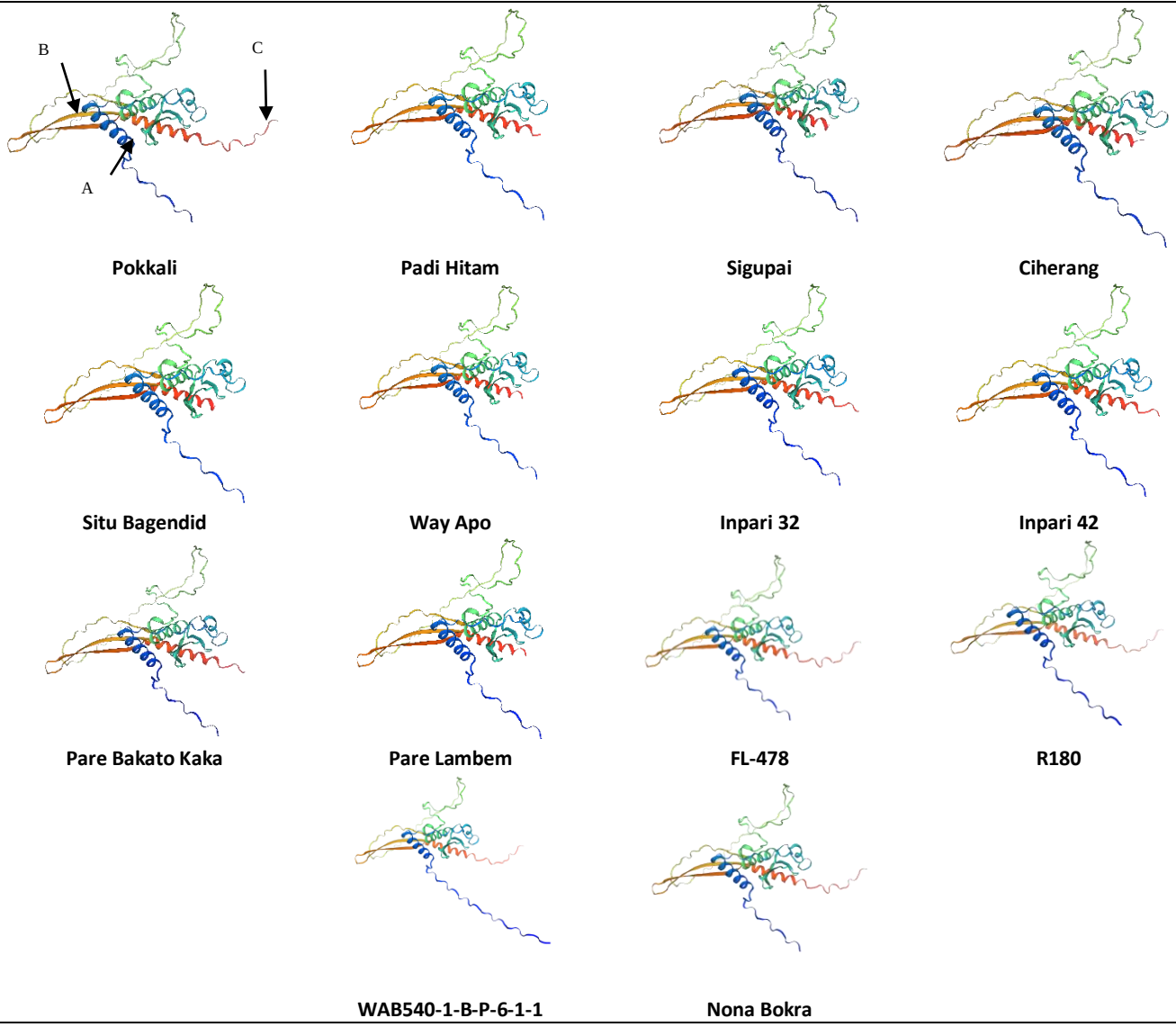

Table 3. Gene attributes of locus LOC_Os01g07120

Locus ID	Locus name	Alternative splice form	Chr.	CDS Coordinates (5'-3')	No. of amino acids	Mol. weight (kDa)
LOC_Os01g07120	LOC_Os01g07120.1	LOC_Os01g07120.2	1	3357287 - 3358443	282	31.59

Note: *Chr*: Chromosome

Discussion

In rice plants, there are five types of DREB2 genes, namely *OsDREB2A*, *OsDREB2E*, *OsDREB2B*, *OsABI4*, and *OsDREB2C*. However, only three of these genes exhibit upregulation under environmental conditions, namely *OsDREB2A*, *OsDREB2B*, and *OsDREB2C*. *OsDREB2B*, in particular, exhibits notably elevated expression levels in cold conditions. In contrast, *OsDREB2A* and *OsDREB2C* display peak expression levels during drought conditions, and their expression remains unaffected by cold environmental conditions (Herath 2016). The dehydration-responsive element binding proteins (DREBs) are crucial APETALA type (AP2/ERF) transcription factors that activate a group of genes related to abiotic stress. This process ultimately enhances the plants' ability to withstand and tolerate stressful conditions (Cao et al. 2020).

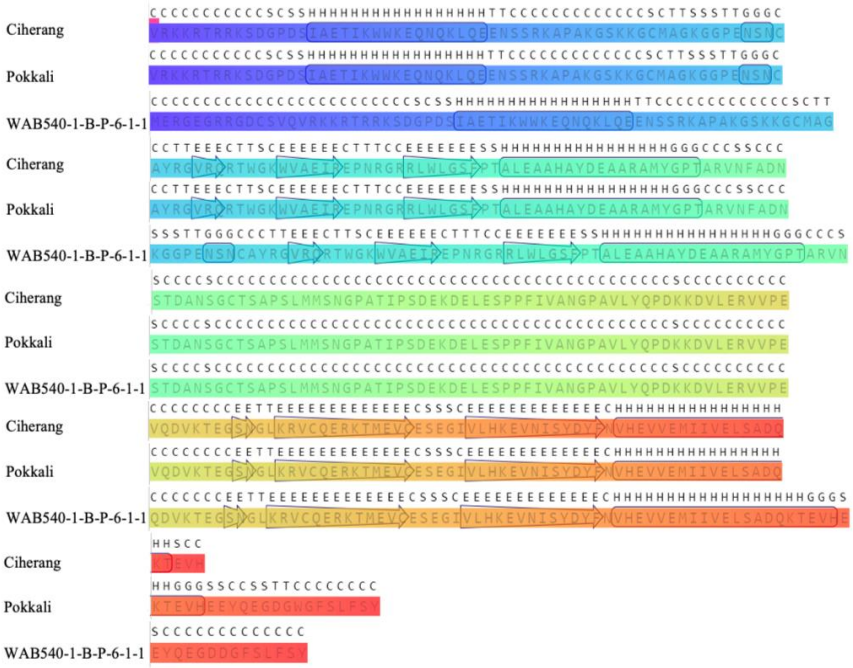

Figure 4. Location and number of α-helix, β-strand, and coil structures in the amino acid sequence of cultivar WAB540-1-B-P-6-1-1 compared to Sigupai and Pokkali cultivars. Amino acids taking part in corresponding secondary structures 'E' denotes extended strand, 'T' denotes β turn, 'C' denotes random coil, 'H' denotes α-helix and "S" denotes β-strand

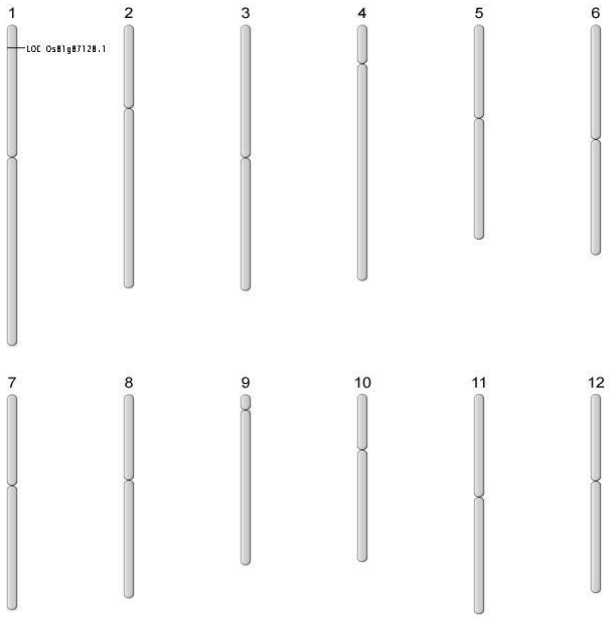

Figure 5. Chromosomal localization of the *OsDREB2A* gene by locus ID LOC_Os01g07120

The alignment of the nine test samples reveals nucleotide base similarity with the Pokkali cultivar (Figure 2), which indicates that the Java local rice varieties (Ciherang, Situbagendid, Way Apo, Inpara 32, and Inpari 42), the Kalimantan local rice variety (Padi Hitam), the Aceh local rice variety (Sigupai), and the West Sumba local rice varieties (Pare Lambem and Pare Bakato Kaka) can be classified as drought-tolerant. The other comparison Within plant stress responses, there are two key factors in responding to stress: the ABA-dependent pathway (involving AREB/ABFs) and the ABA-independent pathway (featuring DREB2A). *OsDREB2A* holds particular importance as the DREB2A transcription factor operates within the ABA-independent pathway, enabling adaptation to osmotic stress even without ABA involvement. Therefore, it increases the flexibility of plant adaptation to its environment involves transcription factors in osmotic stress response, particularly AREB/ABFs, which heavily rely on the presence and levels of ABA hormone (Yoshida et al. 2014).

Suppose there is a co-overexpression between the DREB2A and APX genes in rice plants. In that case, it can enhance drought tolerance by physiologically activating the antioxidative defense to alleviate reactive oxygen species (ROS) generated under drought stress. DREB2A functions as a transcription factor in drought responses, while APX acts as an antioxidant enzyme to mitigate reactive oxygen species. Their cooperation significantly aids plants in coping with environmental stressors more effectively (Sandhya et al. 2021).

Cross-communication mechanisms govern the coordination of the ABA-independent and ABA-dependent signaling pathways. The expression of the DREB2A gene is subject to regulation by ubiquitin E3 ligase enzymes, including DRIP1 and DRIP2. Interestingly, the expression of DREB2A can be suppressed by GRF7 (GROWTH-REGULATING FACTOR7), which interacts with a specific promoter region of DREB2A (Qin et al. 2008). When stress is absent, cells generate limited quantities of a

brief DREB2 mRNA, producing incomplete and non-operational DREB2a proteins. Additionally, GRFs inhibit the DREB2 gene's expression. When plants sense stress, miR396 reduces the levels of GRF transcripts, enabling the activation of DREB2 gene transcription. The activated DREB2 proteins play a pivotal role in triggering stress-induced expression of transcription factors, which further regulate secondary alterations in stress-related gene expression. These changes encompass downstream genes responsible for protective or reparative functions. Stress-related miRNAs are activated, orchestrating developmental adjustments in response to environmental conditions (Guerra et al. 2015).

The nucleotide similarity among the nine samples has been confirmed through chromatogram data. Processing of the raw DNA chromatogram data is necessary to assess the quality scores for each base. In sequence variations, further investigation will be conducted on the vertical axes, which indicate the intensity of the four color-coded base indicators (Oz et al. 2016). While the nine local rice varieties exhibit nucleotide similarity in their *OsDREB2A* sequences, it is important to note that the subcellular localization of the *OsDREB2A* gene may vary. As indicated by Herath (2016), *in silico* predictions suggest that *OsDREB2A* could be localized in both the nucleus and cytoplasm. Additionally, findings from Chen's (2020) study on the model plant Arabidopsis highlight that mutations in the *DREB2A* sequence do not significantly impact the protein's ability to bind target proteins.

The DNA binding sites in the nine varieties show no sequence distinctions compared to the references (Figure 2). The cultivars of FL-478, Nona Bokra, R180, and WAB540-1-B-P-6-1-1 have several different nucleotides but also do not have nucleotide differences in the DNA binding protein region (Figure 3). The DREB DNA binding domain comprises beta strands and alpha helices and binds to cis-elements on target gene promoters (Rehman and Mahmood 2015). The AP2 amino acid sequence of the DREB protein consists of 3 β-strands, which represent a folded structure within the amino acid chain, and a single α-helix containing valine and glutamic acid residues. This combination of structures characterizes the typical features of the AP2/EREBP domain. The AP2/ERF domain in rice contains a conserved YRG amino acid sequence at its N-terminal end. This segment possesses basic and hydrophilic properties crucial for the DNA binding capacity of the DREB transcription factor. These amino acid residues aid the DREB protein in interacting with and binding to DNA, facilitating gene regulation.

Additionally, within the AP2/ERF domain, the carboxylic end (C-terminal) also contains conserved residues. These residues form an amphipathic α-helix structure at the C-terminal, crucial for interactions with other proteins. The interaction between DREB and other proteins enables better coordination in response to the environment. Specific amino acid residues/segments also recognize the DNA sequences targeted for binding by the DREB protein (Jadhao et al. 2014).

Furthermore, variations are only noted in the length of protein tails of these nine samples (Table 2) due to incomplete sequences of the *OsDREB2A* (only 800 bp). The protein sequences of the *OsDREB2A* gene in all nine rice varieties correspond to chromosome 1 (Figure 5). This observation is consistent with Herath's (2016) in silico analysis, which indicated that DREB2s are distributed across chromosomes 1, 3, 5, and 8. More specifically, *OsDREB2A* is on chromosome 1, *OsDREB2E* on chromosome 3, *OsDREB2B* and *OsABI4* on chromosome 5, and *OsDREB2C* on chromosome 8. Following acquiring *OsDREB2A* sequence data from nine local rice varieties in Indonesia, further investigation is warranted using qPCR analysis to assess the gene's expression levels under abiotic drought stress conditions.

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REFERENCES

BPS [Badan Pusat Statistik]. 2022. Harvest Area and Rice Production in Indonesia 2021. Central Bureau of Statistics. Jakarta. [Indonesian]

Batra NG, Sharma V, Kumari N. 2014. Drought-induced changes in chlorophyll fluorescence, photosynthetic pigments, and thylakoid membrane proteins of Vigna radiata. J Plant Interact 9 (1): 712-721. DOI: 10.1080/17429145.2014.905801.

Cao S, Wang Y, Li X, Gao F, Feng J, Zhou Y. 2020. Characterization of the AP2/ERF transcription factor family and expression profiling of DREB subfamily under cold and osmotic stresses in *Ammopiptanthus nanus*. Plants 9 (4): 455. DOI: 10.3390/plants9040455.

Chen CY, Lin PH, Chen KH, Cheng YS. 2020. Structural insights into Arabidopsis ethylene response factor 96 with an extended N-terminal binding to GCC box. Plant Mol Biol 104: 483-498. DOI: 10.1007/s11103-020-01052-5.

Chrisnawati L, Ernawiati E, Yulianty Y, Hariri MR. 2022. Polymorphism analysis of drought tolerance gene *OsDREB2A* in Indonesian local rice from Lampung, Indonesia. Biodiversitas 23 (12): 6352-6357. DOI: 10.13057/biodiv/d231232.

Encabo JR, Macalalad-Cabral RJA, Matres JMK, Coronejo SCTP, Jonson GB, Kishima Y, Choi IR. 2020. Infection with an asymptomatic virus in rice results in a delayed drought response. Funct Plant Biol 47 (3): 239-249. DOI: 10.1071/FP19241.

Guerra D, Crosatti C, Khoshro HH, Mastrangelo AM, Mica E, Mazzucotelli E. 2015. Post-transcriptional and post-translational regulations of drought and heat response in plants: a spider's web of mechanisms. Front Plant Sci 6: 57. DOI: 10.3389/fpls.2015.00057.

Gupta A, Shaw BP. 2021. Field-and laboratory-based methods of screening salt tolerant genotypes in rice. Crop Pasture Sci 72 (2): 85-94. DOI: 10.1071/CP20393.

Hafizah D, Hakim DB, Harianto H, Nurmalina R. 2020. The role of rice's price in the household consumption in Indonesia. Agriekonomika 9 (1): 38-47. DOI: 10.21107/agriekonomika.v9i1.6962. [Indonesian]

Hasan A, Hussain Z. 2020. Climate change and its impact on food security. Res J Human Soc Sci 11 (1): 91-96. DOI: 10.5958/2321-5828.2020.00016.9.

Hassann, El-Shafeynm, Khodarysea, El-Shabrawih, Badra. 2021. Expression of *OsDREB2A* in Transgenic Tomato Improves Drought Tolerance. Rom Biotechnol Lett 26: 3145-3154. DOI: 10.25083/rbl/26.6/3145-3154.

Herath V. 2016. Small family, big impact: *In silico* analysis of DREB2 transcription factor family in rice. Comput Biol Chem 65: 128-139. DOI: 10.1016/j.compbiolchem.2016.10.012.

Jadhao KR, Samal KC, Pradhan SK, Rout GR. 2014. Studies on molecular characterization of DREB gene in indica rice (*Oryza sativa* L.). Hereditary Genet 3 (3): 1-12. DOI: 10.4172/2161-1041.1000133.

Kamruzzaman MD, Marjuk AL, Alam M. 2017. Local rice varieties in climate vulnerable areas of Bangladesh: prospects and barriers. In: Proceedings of 7th international symposium, SEUSL, 7th and 8th December 2017. [Bangladesh]

Kumar A, Dixit S, Ram T, Yadaw RB, Mishra KK, Mandal NP. 2014. Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. J Exp Bot 65 (21): 6265-6278. DOI: 10.1093/jxb/eru363.

Lamaoui M, Jemo M, Datla R, Bekkaoui F. 2018. Heat and drought stresses in crops and approaches for their mitigation. Front Chem 6 (26). DOI: 10.3389/fchem.2018.00026.

Lesk C, Rowhani P, Ramankutty N. 2016. Influence of extreme weather disasters on global crop production. Nature 529 (7584): 84-87. DOI: 10.1038/nature16467.

Nounjan N, Chansongkrow P, Charoensawan V, Siangliw JL, Toojinda T, Chadchawan S, Theerakulpisut P. 2018. High performance of photosynthesis and osmotic adjustment are associated with salt tolerance ability in rice carrying drought tolerance QTL: physiological and co-expression network analysis. Front Plant Sci 9: 1135. DOI: 10.3389/fpls.2018.01135.

Öz E, Kurt S, Asyalı MH, Kaya H, Yücel Y. 2016. Feature based quality assessment of DNA sequencing chromatograms. Appl Soft Comput 41: 420-427. DOI: 10.1016/j.asoc.2016.01.025.

Qin F, Sakuma Y, Tran LSP, Maruyama K, Kidokoro S, Fujita Y, Yamaguchi SK. 2008. Arabidopsis DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress–responsive gene expression. The Plant Cell 20 (6): 1693-1707. DOI: 10.1105/tpc.107.057380.

Rehman S, Mahmood T. 2015. Functional role of DREB and ERF transcription factors: Regulating stress-responsive network in plants. Acta Physiologiae Plantarum 37: 178. DOI: 10.1007/s11738-015-1929-1.

Sahid S, Roy C, Shee D, Datta R, Paul S. 2021. Jacalin domain-containing protein OsSalT interacts with *OsDREB2A* and OsNAC1 to impart drought stress tolerance in planta. Environ Exp Bot 183: 104362. DOI: 10.1016/j.envexpbot.2020.104362.

Sandhya J, Ashwini T, Manisha R, Vinodha M, Srinivas A. 2021. Drought tolerance enhancement with co-Overexpression of DREB2A and APX in indica rice (*Oryza sativa* L.). Amer J Plant Sci 12 (2): 234-258. DOI: 10.4236/ajps.2021.122014.

Saud S, Wang D, Fahad S, Alharby HF, Bamagoos AA, Mjrashi A, Hassan S. 2022. Comprehensive impacts of climate change on rice production and adaptive strategies in China. Front Microbiol 13: 926059. DOI: 10.3389/fmicb.2022.926059.

Schneider P, Asch F. 2020. Rice production and food security in Asian Mega deltas—A review on characteristics, vulnerabilities and agricultural adaptation options to cope with climate change. J Agron Crop Sci 206 (4): 491-503. DOI: 10.1111/jac.12415.

Shaheen T, Mahmood-ur-Rahman MFA, Zaib P, Mehboob-ur-Rahman I. 2018. Identification, characterization, homology modeling and protein-protein interactions of cotton (*Gossypium arboreum* L.) DREB gene. Intl J Agric Biol 20 (5): 1055-106. DOI: 10.17957/IJAB/15.0603.

Shahzad MA, Jan SU, Afzal F, Khalid M, Gul A, Sharma I, Ahmad P. 2016. Drought stress and morphophysiological responses in plants. In: Water Stress and Crop Plants: A Sustainable Approach. John Wiley & Sons, Chichester, UK. DOI: 10.1002/9781119054450.ch27.

Sidorenko MV, Chebotar SV. 2022. Genetic determination of drought resistance in common wheat (*Triticum aestivum* L.). Visnik ukrains' kogo. Tovaristva Genetikiv I Selekcioneriv 20 (1-2): 31-47. DOI: 10.7124/visnyk.utgis.20.1-2.1511.

Sukmayanto M, Hasanuddin T, Listiana I. 2022. Analysis of rice farming production and income in Central Lampung Regency. Jurnal Ekonomi Pertanian dan Agribisnis 6 (2): 625-634. DOI: 10.21776/ub.jepa.2022.006.02.26.

Yoshida T, Mogami J, Yamaguchi-Shinozaki K. 2014. ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Curr Opin Plant Biol 21: 133-139. DOI: 10.1016/j.pbi.2014.07.009.

Zakiyah AS, Khasna EN, Ardana IKKG, Fikriani CN, Anggraini NB, Novitasari D, Suharti S. 2021. *OsDREB2A* gene polymorphism does not affect salinity tolerance potency of local rice varieties from Banten. Hayati J Biosci 28 (2):123-123. DOI: 10.4308/hjb.28.2.123.

Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Salgotra, RK. 2017. Impact of drought on photosynthesis: Molecular perspective. Plant Gene 11: 154-159. DOI: 10.1016/j.plgene.2017.04.003.

Zhang XX, Tang YJ, Ma QB, Yang CY, Mu YH, Suo HC, Nian H. 2013. *OsDREB2A*, a rice transcription factor, significantly affects salt tolerance in transgenic soybean. PLoS One 8 (12): e83011. DOI: 10.1371/journal.pone.008301.