NUSANTARA BIOSCIENCE
Vol. 11, No. 1, pp. 35-43
May 2019

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n110107

Short Communication: Sequence variation of DREB2 gene as a potential molecular marker for identifying resistant plants toward drought stress

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Manuscript received: 5 July 2018. Revision accepted: 14 February 2019.

Abstract. *Rini DS. 2019. Short Communication: Sequence variation of DREB2 gene as a potential molecular marker for identifying resistant plants toward drought stress. Nusantara Bioscience 11: 35-43.* Drought stress is the most destructive environmental factor affecting agricultural productions worldwide. Drought stress affects around 60 million hectares of agricultural land in Indonesia. Therefore, the selection of crops that can grow well and survive under drought is necessary to overcome the problem of decreased crop yield during stress conditions. DREB2 (Dehydration Responsive Element Binding2) belongs to the Transcription Factor (TF) gene families as a master regulator of plant responses under stress. Due to increased expression by stress, this gene can be used as a molecular marker to identify resistant plants toward drought. However, study the sequence variation of DREB2 is required to understand the character of this gene related to its function at a molecular level. The sequence of DREB2 in several plants was obtained from NCBI and aligned to determine the similarity of the regions. Even though variations were found in N-terminal of DREB2 sequences studied, there was a high similarity of the sequences in the DNA binding domain. It has been predicted that this domain is part of a conserved region in the sequence of DREB2 gene. This domain is then proposed to be used for primer designing purposes to study DREB2 gene expression between plant species.

Keywords: DREB2, drought stress, sequence variation

INTRODUCTION

As environmental constraints, drought stress can limit crop productivity due to its effect in impairing normal plant growth. Therefore, high yield productivity of crop under normal as well as under drought conditions is crucial for food security of the growing population. Plant, however, adapt to drought stress through a variety of physiological, biochemical, and molecular mechanisms at the cellular and whole organism levels (Basu et al. 2016).

Molecular analysis of plant responses toward drought stress is one of the most interesting subjects in the modern biological sciences (Hirayama and Shinozaki 2010). A better understanding of this molecular mechanism is fundamentally required to engineer the transgenic drought-tolerant plants genetically. Through the availability of the complete genome sequence of some model plants, such as *Arabidopsis thaliana* and *Oryza sativa*, a number of genes have been described to respond to drought stress. These genes were identified by using genetic, reverse genetic, and molecular biology methods (Bartels and Sunkar 2005; Yamaguchi-Shinozaki and Shinozaki 2005).

Genes induced by drought stress can broadly be classified into two groups regarding its function. The first group contains the genes which the proteins encoded are designed as functional proteins. These proteins probably function as metabolic proteins for stress tolerance through protecting the cells from stress effects by the removal of toxic elements, restoration of cellular homeostasis, and eventual recovery of normal growth pattern. These proteins include water channel protein, proline, detoxification enzymes, antifreeze proteins, and late embryogenesis abundant (LEA) proteins (Shinozaki and Yamaguchi-Shinozaki 2007; Gao et al. 2008; Jan et al. 2012). The second group is the genes which proteins encoded function as regulatory proteins involved in further regulation of signal transduction and stress-responsive gene expression. The proteins belonging to this group are calmodulin-binding protein, protein kinases, protein phosphatases, and transcription factors (Shinozaki and Yamaguchi-Shinozaki 2007).

DREBs (Dehydration Responsive Element Bindings) are important transcription factors (TFs) regulating the expression of many stress-inducible genes (Lata and Prasad 2011). TFs function to play an essential role in multiple abiotic stress responses through binding to regulatory elements in the promoter region of downstream stress-responsive genes, thereby promoting or suppressing its function (Mitsuda and Ohme-Takagi 2009; Nuruzzaman et al. 2013; Mun et al. 2017). DREB family of transcription factors which consist of two subfamilies, namely CBF/DREB1 (C-Repeat Binding Factor/Dehydration Responsive Element Binding1) and DREB2 (Dehydration Responsive Element Binding2) (Liu et al. 1998), are involved in two separate low temperature and dehydration signal transduction pathways, respectively (Nakashima et al. 2000; Sakuma et al. 2006). In *Arabidopsis* plant, DREB transcription factors binds to DRE/CRT (Dehydration-

Responsive Element/C-Repeat) *cis*-elements which the conserved DNA-binding motif is *A/GCCGAC* (Yamaguchi-Shinozaki and Shinozaki 2005; Lucas et al. 2011) that function to modulate the expression of stress-responsive genes in an ABA-independent signaling pathway (Riechmann et al. 2000; Khan 2011) .

DREB2 was first identified in *Arabidopsis* plant. This gene was screened by using a yeast one-hybrid screen method and driven by using the *CAMV35S* promoter as the bait (Liu et al. 1998). Afterward, *DREB2* homologous genes have been successfully isolated from many economically important cereal crops such as wheat, pearl millet, foxtail millet, and maize (Egawa et al. 2006; Qin et al. 2007; Agarwal et al. 2010; Lata et al. 2011). Temporal gene expression studies of DREB2 gene in various crops have revealed that this gene is not only induced by drought but also by heat and salinity (Liu et al. 1998; Sakuma et al. 2006). Their overexpression can significantly improve the stress tolerance of the transgenic plant. Overexpression of GmDREB2 in tobacco plant driven by *CAMV35S* promoter showed an increased tolerance level of a plant towards drought (Chen et al. 2007). Moreover, transgenic canola plant containing *Arabidopsis* DREB2A confers tolerance to salinity stress (Shafeinie et al. 2014).

It has been widely known that variation of gene sequences presents within and among various plant populations (Gonzalez-Martinez et al. 2006; Arana et al. 2010; Constanzo and Jia 2010; Yi et al. 2018). Such variations, in turn, will also underlie genetic diversity between organisms. Many studies also indicate the presence of sequence variation in drought-responsive genes in a plant (Pomponio et al. 2013; Yu et al. 2013; Xia et al. 2017). In this study, however, we focused on analyzing the sequence variations of DREB2 transcription factor (TF) genes in plants related to their function as a master regulator to manage and organize the expression of down-stream drought-responsive genes located in the ABA-independent signaling pathway.

MATERIALS AND METHODS

Sequence materials

Nucleotides and amino acids of DREB2 sequences tested in this study were obtained from the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov) in FASTA format. Only the complete cds (coding sequence) of DREB2 sequences from 42 species of monocotyledon and dicotyledon plants (Table 1) was used for data analysis.

Data analysis

Phylogenetic trees of DREB2 sequences on various plants studied were constructed by MEGA 7 tool (http: //www.megasoftware.net/mega.html) (Tamura et al. 2007) based on the distance matrix with the neighbor-joining (NJ) method. The tree was drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and were in the units of the number of base differences per site. All positions containing gaps and missing data were eliminated. The neighbor-joining (NJ) tree was performed to present the expected phylogenetic relationship of DREB2 gene among the tested plant species.

The multiple alignments of the nucleotide sequence of DREB2 were conducted by using BioEdit Sequence Alignment Editor (Hall 1999). The protein multiple sequences alignments of DREB2 was performed by using CLC sequence viewer 8 (CLC Bio, Aarhus Denmark). The result obtained will identify the homology region between the sequences studied. Then, the consensus sequences of the alignment results were calculated from multiple sequence alignment taking into account the quality value for each base.

RESULTS AND DISCUSSION

Since it was identified first in *Arabidopsis* plant (Liu et al. 1998), DREB2 gene has been recognized to contain a conserved AP2/ERF (APETALA2/Ethylene Responsive Factor) domain (Sakuma et al. 2002) which function was to recognize DRE (Dehydration Responsive Element) sequence (Mitsuda and Ohme-Takagi 2009). The AP2 domain consists of a three-stranded β-sheet and one α-helix which is almost parallel to the β-sheet, and it contacts to the DNA sequence of gene target via Arg and Trp residues located in the β-sheet (Liu et al. 1998).

Sequence variation is one of necessity occurred during the adaptation process to various environmental conditions. Sequence variation is taken together with the environmental effect lead to the presence of diversity among organism. In this study, DREB2 sequences of plant species obtained from the NCBI database are varied both in length and in sequence. A systematic phylogenetic analysis was carried out on the basis of the nucleotide sequence of DREB2 gene in 42 plants obtained from the NCBI database. This phylogenetic tree was performed by MEGA 7 with the Neighbor-Joining (NJ) method. The result of phylogenetic analysis (Figure 1) shows that all the six sub-groups of DREB2 gene can be easily classified in the plants tested. Sub-group A and B all consist of monocotyledon plants in the family of Poaceae. Interestingly, *Triticum aestivum* and *Triticum turgidum* that also belong to a member of the Poaceae family, are placed separately in the sub-group D and E, respectively. Both plants are in the same group with dicots plants, such as *Glycine max* and *Coffea arabica*. However, *Agropyron mongolicum*, a member of Poaceae plants, is distantly related to DREB2 of *Triticum turgidum* and clustered in the same region, sub-group E, like those from dicotyledon plants.

Multiple alignment sequence of cds (coding sequence) DREB2 (Figure 2) was performed in Poaceae plants with all the members are included in the clade (sub-group) A (Figure 1). Poaceae plants used in this alignment analysis are *Saccharum officinarum* (JQ736812.1), *Sorghum bicolor* (DQ403725.1), *Setaria italica* (NM_001310053.1), *Hemarthria compressa* (KC203598.1), *Poa pratensis*

(AY553331.1), *Aegilops biuncialis* (FR719742.1), and *Buchloe dactyloides* (EF512460.1). It has been identified that the DREB gene contains the basic region and an acidic region in its N-terminal and C-terminal region, respectively. The basic region functions as a nuclear localization signal whereas an acidic region might play as an activation domain for transcription. Since the TFs only function in the nucleus, the regulation of their entry into the nucleus is mediated by NLS (Akhtar et al. 2012). However, its middle region functions as a negative regulatory domain in the regulation of DREB2 activity (Akhtar et al. 2012).

The result of multiple alignments performed by BioEdit Sequence Alignment Editor shows the sequence variation at the N-terminal domain (Figure 2.A). The homology region of DREB2 nucleotide is presented at the C-terminal domain of the sequence (Figure 2C). However, a high similarity nucleotide sequence is also identified in the region located in between N-terminal stretch which is rich in basic and hydrophilic residue and C-terminal region capable of forming an α-helix strand. The high similarity region of the nucleotide sequence indicates the conserved region of DREB2 gene (Figure 2B). Variation of a few nucleotides in this region is supposed to be critical for amino acid changes.

Table 1. List of plant species, NCBI accession number, and references for DREB2 analysis in this study

Plant species	NCBI accession number	References
Aegilops biuncialis	FR719742.1	Yang et al. (2010)
Aegilops speltoides	KF731665.1	Tavakol et al. (2014)
Aegilops tauschii	KF731663.1	Tavakol et al. (2014)
Agropyron mongolicum	MG385678.1	Yu and Han (2017)
Betula luminifera	KP245823.1	Zhang (2014)
Buchloe dactyloides	EF512460.1	Hu et al. (2007)
Caragana korshinskii	GU573848.1	Wang et al. (2010)
Chrysanthemum vestitum	EF633987.2	Liu et al. (2008)
Coffea arabica strain *laurina*	JQ687375.1	Joet (2012)
Cynodon dactylon	AY462118.1	Xie and Wang (2003)
Eucalyptus globulus	HM992944.1	Baltierra and Krauskopf (2010)
Glycine max	AY296651.1	Li et al. (2005)
Halimodendron halodendron	EU872018.1	Yin et al. (2008)
Helianthus annuus	AY508007.1	Diaz-Martin et al. (2005)
Hemarthria compressa	KC203598.1	Chen et al. (2016)
Hordeum brevisubulatum	AY728807.1	Wan et al. (2004)
Ipomoea batatas	KU578260.1	Yang et al. (2016)
Lilium longiflorum	MG811543.1	Wu et al. (2018)
Lycopersicon esculentum	AF500012.1	Cheng et al. (2002)
Malus prunifolia	JN204427.1	Zhao et al. (2011)
Malus sieversii	JQ790526.1	Zhao et al. (2013)
Manihot esculenta	JN615576.1	An and Zhang (2011)
Nicotiana tabacum	EU727156.1	Liu and Feng (2008)
Phyllostachys edulis	EU295483.1	Liu et al. (2011)
Poa pratensis	AY553331.1	Shen et al. (2004)
Populus euphratica	NM001304317.1	Chen et al. (2009)
Populus hopeiensis	GU207863.1	Zhang et al. (2009)
Populus trichocarpa	EF151454.1	Yin et al. (2006)
Saccharum arundinaceum	KJ670161.1	Augustine and Subramonian (2014)
Saccharum officinarum	JQ736812.1	Liu et al. (2012)
Schedonorus arundinaceus	AY436639.1	Lv et al. (2003)
Setaria italica	NM001310053.1	Lata et al. (2010)
Solanum lycopersicum	HQ698902.1	Hichri et al. (2010)
Solanum lycopersicum	NM001247830.1	Aoki et al. (2010); Hichri et al. (2016)
Solanum tuberosum	JN125858.1	Bouaziz et al. (2012)
Sophora moorcroftiana	KM527093.1	Yao et al. (2016)
Sorghum bicolor	DQ403725.1	Bihani et al. (2006)
Triticum aestivum	AY781345.1	Xu et al. (2004)
Triticum turgidum subsp. *durum*	GU785008.1	Morran et al. (2010)
Triticum urartu	KF731664.1	Tavakol et al. (2014)
Vigna unguiculata	JQ066264.2	Sadhukhan et al. (2014)
Zoysia japonica	KP676132.1	Wie et al. (2015)

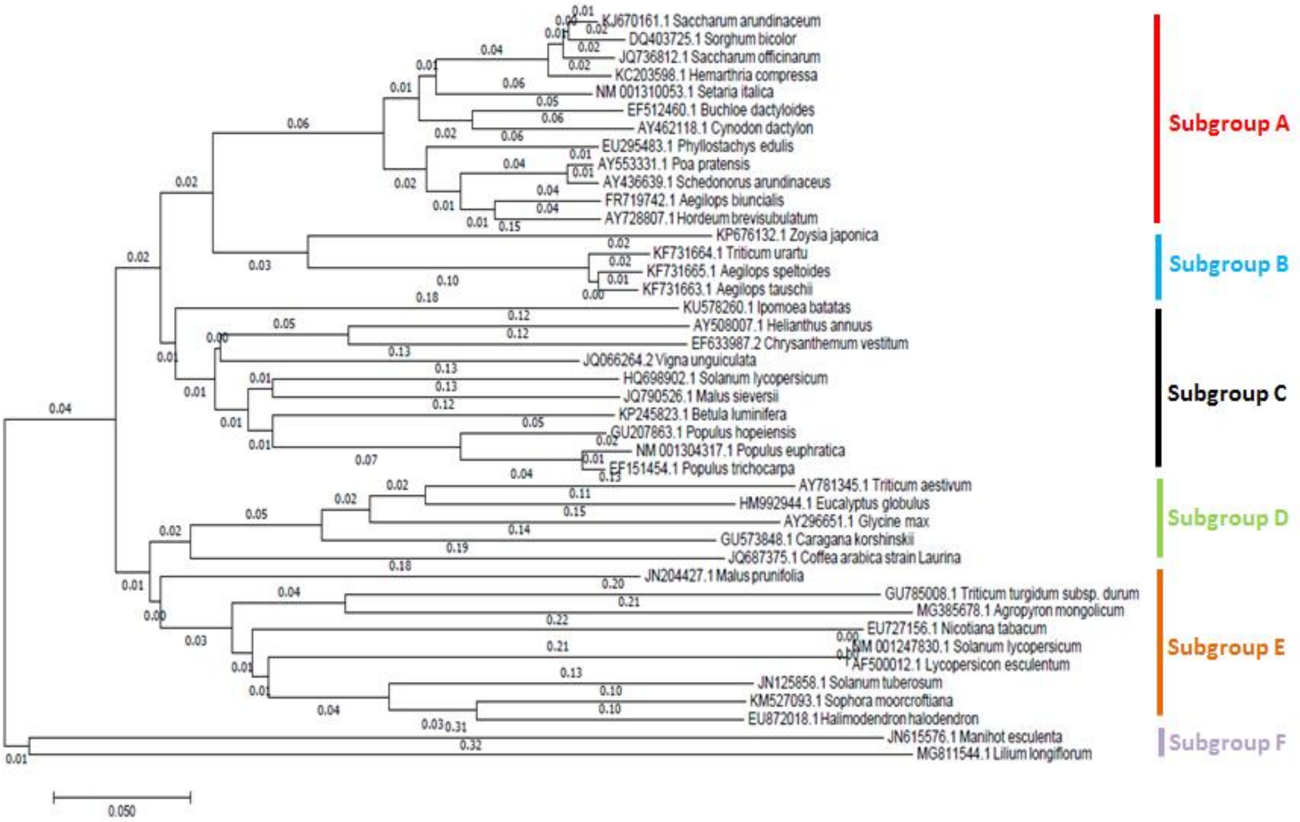
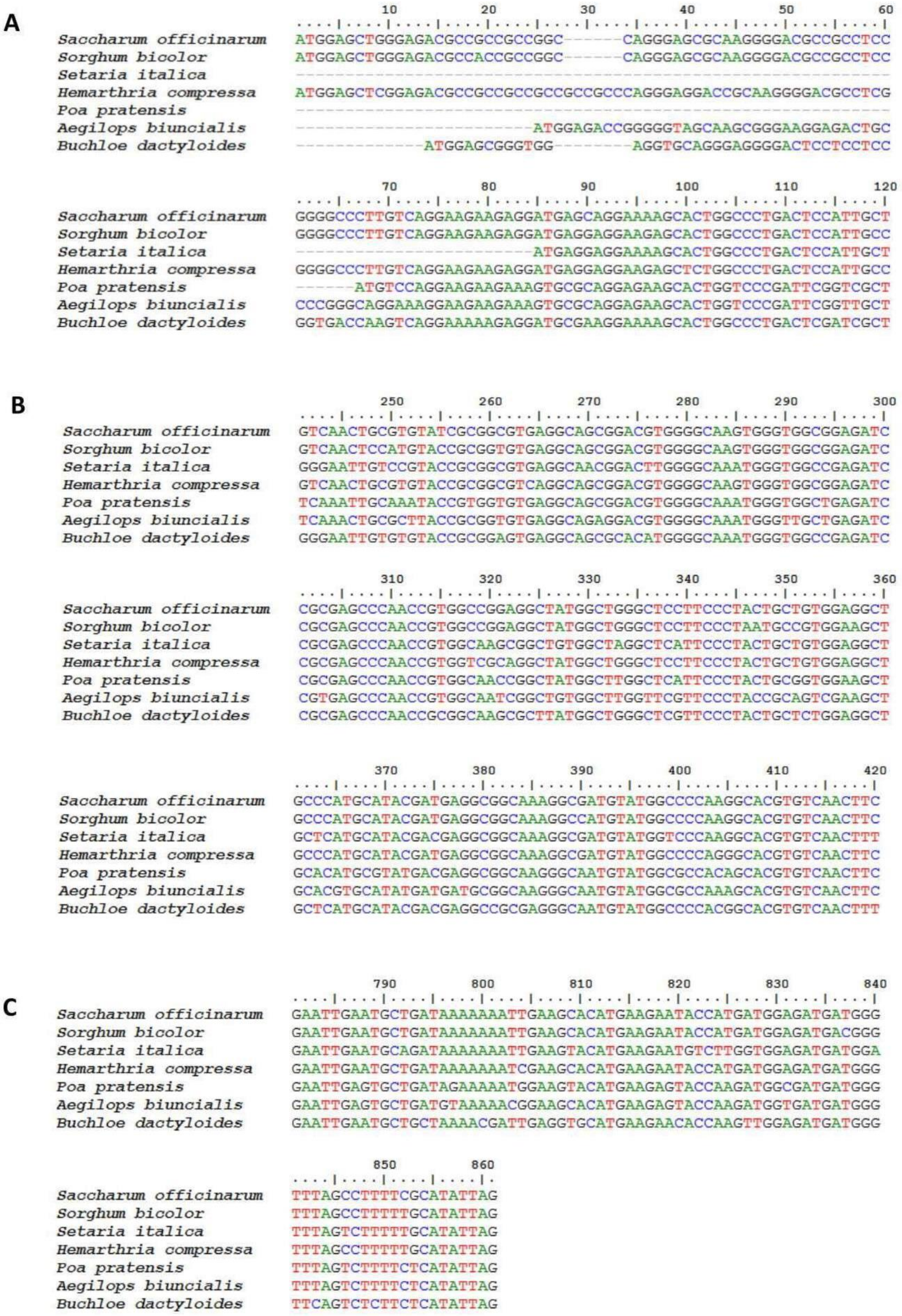


Figure 1. Phylogenetic tree of DREB2 gene in various plant species. The phylogenetic analysis was inferred using MEGA7 tool with the Neighbor-Joining method based on the DREB2 nucleotide sequences of 42 plant species. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

By using multiple sequence alignment, protein homology analysis of DREB2 was performed in a number of monocotyledons and dicotyledons species (Figure 3). A highly conserved amino acid region is identified in the AP2/ERF domain having the motif sequence of YRGVRQRTWGKWVAEIREPNR in between NLS (Nuclear Localization Signal) and acidic regions.

Therefore, it can be concluded that the AP2 domain has been evolutionarily conserved in the plant species. It is also predicted that the AP2 binding domain shares a similar activity in between plant species to recognize the DRE sequence. Induced strongly by drought, DREB2 is thought to be a major transcription factor functioning under drought stress (Sakuma et al. 2006).

Several amino acid sequences can be generated from a single gene through the alternative splicing process which leads to expansion of the gene products for any given gene. The common events of AS are exon skipping (ES), intron retention (IR), and also the alternative of 5' or 3' splicing events (Kannan et al. 2018). Posttranscriptional mechanisms based on alternative splicing and RNA processing involved in responses to abiotic stress (Ding et al. 2014). Therefore, it has been predicted that AS plays a regulatory role in plant stress responses (Duque 2011). Many studies have reported the AS events in the DREB2 gene in various plants, but the functional regulation of this splicing process primarily depends on the species (Egawa et al. 2006; Liu et al. 2017). By using data obtained from INSDC (International Nucleotide Sequence Database Collaboration) and RefSeq as NCBI Reference Sequence Database, a number of AS events have been recognized in crop plant (Table 2). The alternative splicing of DREB2s is important for plants to repress functional protein accumulation because the high level of DREB2s causes growth defects. Due to its function as a post-transcriptional mechanism, AS process contributes to both transcriptome and proteome diversities which play as an important role in gene regulation and tissue-specific expression (Ramani et al. 2011). The basic role of the splicing mechanism is the recognition of introns and exons by the splicing machinery. The key component of AS process is the spliceosome that functions to recognize the splice sites. AS is tightly regulated by cis-elements within exons and surrounding introns as well as trans-acting factors that bind to these cis-elements. The environmental stresses can induce AS process in pre-mRNAs of stress-responsive genes due to their sensitivity to the stress. In this case, the functions of their gene product are predicted by comparing the homology sequence with a known protein.



A

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                              10        20        30        40        50        60
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum ATGGAGCTGGGAGACGCCGCCGCCGGC------CAGGGAGCGCAAGGGGACGCCGCCTCC
Sorghum bicolor       ATGGAGCTGGGAGACGCCACCGCCGGC------CAGGGAGCGCAAGGGGACGCCGCCTCC
Setaria italica       ------------------------------------------------------------
Hemarthria compressa  ATGGAGCTCGGAGACGCCGCCGCCGCCGCCGCCCAGGGAGGACCGCAAGGGGACGCCTCG
Poa pratensis         ------------------------------------------------------------
Aegilops biuncialis   ------------------------ATGGAGACCGGGGGTAGCAAGCGGGAAGGAGACTGC
Buchloe dactyloides   -------------ATGGAGCGGGTGG--------AGGTGCAGGGAGGGGACTCCTCCTCC

                              70        80        90       100       110       120
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum GGGGCCCTTGTCAGGAAGAAGAGGATGAGCAGGAAAAGCACTGGCCCTGACTCCATTGCT
Sorghum bicolor       GGGGCCCTTGTCAGGAAGAAGAGGATGAGGAGGAAGAGCACTGGCCCTGACTCCATTGCC
Setaria italica       ------------------------ATGAGGAGGAAAAGCACTGGCCCTGACTCCATTGCT
Hemarthria compressa  GGGGCCCTTGTCAGGAAGAAGAGGATGAGGAGGAAGAGCTCTGGCCCTGACTCCATTGCC
Poa pratensis         ------ATGTCCAGGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCCGATTCGGTCGCT
Aegilops biuncialis   CCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCCGATTCGGTTGCT
Buchloe dactyloides   GGTGACCAAGTCAGGAAAAAGAGGATGCGAAGGAAAAGCACTGGCCCTGACTCGATCGCT
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B

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                             250       260       270       280       290       300
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum GTCAACTGCGTGTATCGCGGCGTGAGGCAGCGGACGTGGGGCAAGTGGGTGGCGGAGATC
Sorghum bicolor       GTCAACTCCATGTACCGCGGTGTGAGGCAGCGGACGTGGGGCAAGTGGGTGGCGGAGATC
Setaria italica       GGGAATTGTCCGTACCGCGGCGTGAGGCAACGGACTTGGGGCAAATGGGTGGCCGAGATC
Hemarthria compressa  GTCAACTGCGTGTACCGCGGCGTCAGGCAGCGGACGTGGGGCAAGTGGGTGGCGGAGATC
Poa pratensis         TCAAATTGCAAATACCGTGGTGTGAGGCAGCGGACGTGGGGCAAATGGGTGGCTGAGATC
Aegilops biuncialis   TCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGCAAATGGGTTGCTGAGATC
Buchloe dactyloides   GGGAATTGTGTGTACCGCGGAGTGAGGCAGCGCACATGGGGCAAATGGGTGGCCGAGATC

                             310       320       330       340       350       360
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum CGCGAGCCCAACCGTGGCCGGAGGCTATGGCTGGGCTCCTTCCCTACTGCTGTGGAGGCT
Sorghum bicolor       CGCGAGCCCAACCGTGGCCGGAGGCTATGGCTGGGCTCCTTCCCTAATGCCGTGGAAGCT
Setaria italica       CGCGAGCCCAACCGTGGCAAGCGGCTGTGGCTAGGCTCATTCCCTACTGCTGTGGAGGCT
Hemarthria compressa  CGCGAGCCCAACCGTGGTCGCAGGCTATGGCTGGGCTCCTTCCCTACTGCTGTGGAGGCT
Poa pratensis         CGCGAGCCCAACCGTGGCAACCGGCTATGGCTTGGCTCATTCCCTACTGCGGTGGAAGCT
Aegilops biuncialis   CGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCGTTCCCTACCGCAGTCGAAGCT
Buchloe dactyloides   CGCGAGCCCAACCGCGGCAAGCGCTTATGGCTGGGCTCGTTCCCTACTGCTCTGGAGGCT

                             370       380       390       400       410       420
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum GCCCATGCATACGATGAGGCGGCAAAGGCGATGTATGGCCCCAAGGCACGTGTCAACTTC
Sorghum bicolor       GCCCATGCATACGATGAGGCGGCAAAGGCCATGTATGGCCCCAAGGCACGTGTCAACTTC
Setaria italica       GCTCATGCATACGACGAGGCGGCAAAGGCGATGTATGGTCCCAAGGCACGTGTCAACTTT
Hemarthria compressa  GCCCATGCATACGATGAGGCGGCAAAGGCGATGTATGGCCCCAGGGCACGTGTCAACTTC
Poa pratensis         GCACATGCGTATGACGAGGCGGCAAGGGCAATGTATGGCGCCACAGCACGTGTCAACTTC
Aegilops biuncialis   GCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTC
Buchloe dactyloides   GCTCATGCATACGACGAGGCCGCGAGGGCAATGTATGGCCCCACGGCACGTGTCAACTTT
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C

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                             790       800       810       820       830       840
                      ....|....|....|....|....|....|....|....|....|....|....|....|
Saccharum officinarum GAATTGAATGCTGATAAAAAAATTGAAGCACATGAAGAATACCATGATGGAGATGATGGG
Sorghum bicolor       GAATTGAATGCTGATAAAAAAATTGAAGCACATGAAGAATACCATGATGGAGATGACGGG
Setaria italica       GAATTGAATGCAGATAAAAAAATTGAAGTACATGAAGAATGTCTTGGTGGAGATGATGGA
Hemarthria compressa  GAATTGAATGCTGATAAAAAAATCGAAGCACATGAAGAATACCATGATGGAGATGATGGG
Poa pratensis         GAATTGAGTGCTGATAGAAAAATGGAAGTACATGAAGAGTACCAAGATGGCGATGATGGG
Aegilops biuncialis   GAATTGAGTGCTGATGTAAAAACGGAAGCACATGAAGAGTACCAAGATGGTGATGATGGG
Buchloe dactyloides   GAATTGAATGCTGCTAAAACGATTGAGGTGCATGAAGAACACCAAGTTGGAGATGATGGG

                             850       860
                      ....|....|....|....|.
Saccharum officinarum TTTAGCCTTTTCGCATATTAG
Sorghum bicolor       TTTAGCCTTTTTGCATATTAG
Setaria italica       TTTAGTCTTTTTGCATATTAG
Hemarthria compressa  TTTAGCCTTTTTGCATATTAG
Poa pratensis         TTTAGTCTTTTCTCATATTAG
Aegilops biuncialis   TTTAGTCTTTTCTCATATTAG
Buchloe dactyloides   TTCAGTCTCTTCTCATATTAG
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Figure 2. Multiple alignment sequence (MSA) of cds DREB2 gene in Poaceae plants. The MSA method was performed by using BioEdit Sequence Alignment Editor. Poaceae plants used in this alignment analysis are *Saccharum officinarum* (JQ736812.1), *Sorghum bicolor* (DQ403725.1), *Setaria italica* (NM_001310053.1), *Hemarthria compressa* (KC203598.1), *Poa pratensis* (AY553331.1), *Aegilops biuncialis* (FR719742.1), and *Buchloe dactyloides* (EF512460.1). A. N-terminal domain of DREB2 gene, B. DNA binding domain of DREB2 gene, C. C-terminal region of DREB2 gene

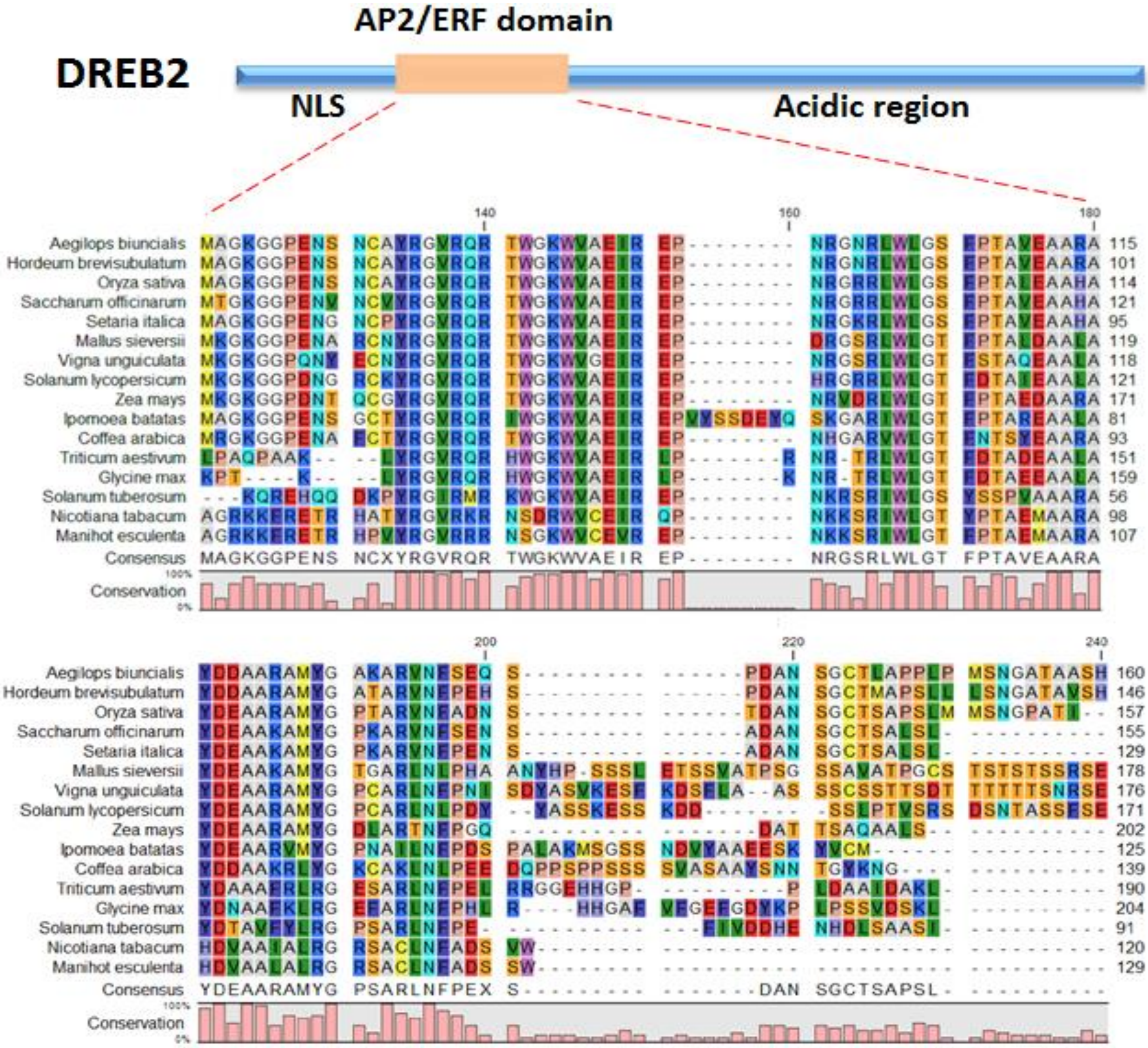

Figure 3. The highly conserved region of DREB2 protein. This conserved region belongs to AP2 domain that functions to bind to DRE of the down-stream targeted gene

The induced expression of a certain gene is a key event in helping the plant to set up an effective defensive state under stress conditions. Genes expressed under drought conditions are thought to function in the protecting cells from stress, not only by producing the metabolic proteins but also by regulating of down-stream genes for signal transduction cascades of the stress response (Lata and Prasad, 2011). Among the regulatory genes, TFs have acquired widespread attention due to their significant role in plant stress tolerant. Meanwhile, changes in the gene expression after given stress and the reprogramming of molecular machinery are also regulated by the transcription factor. In addition, a number of drought stress associated genes encoding transcription factors that in turn control other various genes, are also involved in diverse physiological and molecular reactions to drought stress.

Understanding molecular biology and biotechnical tools is a key factor underlying the modern efforts to develop transgenic stress tolerant plants. The DREB2 transcription factor is one of the most promising candidate genes for this purpose in conferring drought tolerance. Therefore, genetic modification of the expression of these regulatory genes can greatly influence plant stress tolerance due to their regulation in many downstream stress-responsive genes at a given time (Wang et al. 2016). As members of the AP2 family, DREB2 is known to control plant response to drought stress. Their overexpression can significantly improve stress tolerance in the transgenic plant (Sakuma et al. 2006). Transgenic *Arabidopsis* plants over-expressing ZmDREB2A showed improved drought stress tolerance (Qin et al. 2007). The transformation of GmDREB2 in tobacco plant under CAM35S promoter induced the drought stress tolerance plant having 4.5-fold higher proline content (Chen et al. 2007). Meanwhile, genetic transformation of tobacco plants overexpressing CpDREB2 exhibited an increased amount of proline and greater tolerance level to drought (Agarwal et al. 2010).

Table 2. Alternative splicing of DREB2 in *Zea mays* and *Sorghum bicolor* plants

Source	CDS Region in Nucleotide	Protein	Name	Organism	References
INSDC	JF915837.1 422-1168 (+)	AFI71290.1	Dehydration responsive element binding protein 2 isoform b	*Zea mays*	Liu and Peng (2011)
RefSeq	NC_024466.2 96825865-96826611 (+)	NP_001292874.1	Dehydration-responsive element binding protein 2A isoform 2	*Zea mays*	Ware (2017)
RefSeq	NC_024466.2 96825865-96826611 (+)	XP_020397925.1	Dehydration-responsive element binding protein 2A isoform X4	*Zea mays*	Liu and Peng (2011)
INSDC	CM000784.4 96825865-96826611 (+)	AQK93332.1	Dehydration-responsive element-binding protein 2A	*Zea mays*	Ware (2017)
RefSeq	NC_024466.2 96823667-96826611 (+)	NP_001292873.1	Dehydration-responsive element binding protein 2A isoform 1	*Zea mays*	Jiao et al. (2017)
NSDC	JF915834.1 156-3100 (+)	AFI71285.1	Dehydration responsive element binding protein 2 isoform c	Zea mays	Liu and Peng (2011)
RefSeq	NM_001112406.2 156-1115 (+)	NP_001105876.2	dehydration-responsive element binding protein 2A isoform 3	Zea mays	Gu et al. (2016)
INSDC	JF915841.1 137-1279 (+)	AFI71296.1	Dehydration responsive element binding protein 2 isoform c	*Sorghum bicolor*	Liu and Peng (2011)
INSDC	JF915838.1 137-2893 (+)	AFI71291.1	Dehydration responsive element binding protein 2 isoform c	*Sorghum bicolor*	Liu and Peng (2011)
INSDC	JF915840.1 400-1197 (+)	AFI71295.1	Dehydration responsive element binding protein 2 isoform b	*Sorghum bicolor*	Liu and Peng (2011)
INSDC	JF915838.1 2096-2893 (+)	AFI71293.1	Dehydration responsive element binding protein 2 isoform b	*Sorghum bicolor*	Liu and Peng (2011)
INSDC	JF915838.1 137-2893 (+)	AFI71292.1	Dehydration responsive element binding protein 2 isoform a	*Sorghum bicolor*	Liu and Peng (2011)

DREB2 is one of the important genes for crop improvement either through engineering stress tolerance or through crop breeding strategies since it is being the major TF that binds to the cis-acting elements of most of the osmotic stress-inducible genes responsible for providing tolerance traits to the plants under stress conditions. Temporal gene expression studies of DREB2 gene in various crops have revealed that DREB2 is induced particularly by drought. The study of DREB2 in *Oryza sativa* showed that OsDREB2A transcript was induced within 24 h of dehydration (Dubouzet et al. 2003). The transcript level of *Sorghum bicolor* SbDREB2 increased at 1 h exposure to drought. *Populus euphratica* PeDREB2 also induced its expression under drought (Chen et al. 2009).

DREB2 thereafter can be used as a molecular marker to assist the selection technique for drought tolerance character in plants due to its activity as a master regulator of drought-responsive gene expression. However, the highly conserved AP2 domain can be deduced as considered sequence region to design specific primer in studying drought stress tolerance plant species based on the high induction in DREB2 gene expression.

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