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Characterization of Drought Resistance Gene *Dehydration Responsive Element Binding/DREB* of Local Corn (*Zea mays* L.) Cultivars in Kisar Island Maluku-Indonesia Using PCR-Sequencing

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Abstract: Plant has a certain mechanism in adaptation to drought environment. The mechanism of plant adaptation to drought can be tracked to the molecular level by the drought stress induction and expression of some genes. One of the genes that induced by drought is dehydration responsive element binding or dehydration binding protein (DREB/DBP). The aim of this research was to identify the sequence of DREB gene of six local corn cultivars in Kisar Island South West Maluku Regency. Six local corn cultivars were obtained from local farmer in Kisar Island, and one reference variety as drought tolerance varieties originated from Research Institute of Cereals were planted in three replicates with complete randomized block design. Leaf sample collection for DNA analysis was harvested at the 65 days after planting. DNA isolation was conducted with using CTAB method. After success running PCR for amplification of DREB gene, the PCR product was sent to professional company for the sequencing process. The sequence result was aligned using the Clustal W alignment from Bioedit and the homology was compared with the same gene that already been reported in the data base of genebank. When compare with the DREB sequence of accession in the gene bank, the DREB gene sequence of local corn cultivars and reference variety showed similarity except nucleotide no 9. The nucleotide was deleted. It meant that DREB gene belong to local and reference corns was mutated.
Keywords: drought, DREB genes, corn cultivars.

Introduction

Corn is one important commodity which serve as a source of carbohydrate. The increasing of corn demand sometimes is not followed by the increasing of corn production. Corn production in Maluku is still low (1.0 ton/ha) compare with national corn production in Indonesia (7 ton/ha)[1]. The decrease of corn production is caused by the most of area for corn plantation is in the marginal dry land, like Kisar Island in South West Maluku Regency Maluku province. In this regency, there are six local corn cultivars[2] that have been cultivated

by farmers for a long time. These local corn cultivars have high potential to develop, because they have ability in adaptation to drought environment[3].

Mechanism of plant adaptation to drought environment can be tracked to the molecular level by the induction and expression of some genes. One of the genes which is induced by drought is dehydration responsive element binding or dehydration binding protein (DREB/DBP)[4,5]. This DREB gene is a gene that encoding protein wich function as transcription factors namely DREB protein[4,5,6,7,8].

To investigate the potential of corn cultivars from Kisar Island South West Maluku Regency in adaptation to drought environment at the molecular level, identification and characterization of DREB gene needs to be done. Until now, there is no data related to the characterization of DREB gene of local corn cultivars was available. So, the aim of this research was to identify the sequence of DREB gene of six local corn cultivars in Kisar Island South West Maluku Regency, using PCR-sequencing method.

Material and Method

Plant material, and experimental conditions

Six local corn cultivars that widely cultivated by local farmer in Kisar Island, and one reference variety as drought tolerance varieties originated from Research Institute of Cereals were planted. At the 30 days after planting, the seedling were thinned and remained for 2 seedlings. These seedlings were grown until 65 days after planting for sample collection. At the 65 days after planting, a second leaf under the flag leaf from one selected plant was collected and used for DNA isolation. The air temperature, amount of rainfall, and the number of rainy days were measured to confirm the drought conditions during corn planting.

Laboratory experiment

The leaf samples was cut in 1x1 cm size, and kept in 70% ethanol at room temperature until used for DNA isolation. DNA isolation was conducted using the CTAB method[9,10]. As much as 0.1 g of leaf sample was ground in a mortar and 1 mL of CTAB extraction buffer was added (2 % CTAB, 5 M NaCl, 20 nM EDTA, 100 mM Tris-HCl pH 8,0 and 1 % of mercapthoethanol). The mixture then incubated on the waterbath at the 65^{o}C for 60 minutes, and centrifugated 13.000 rpm for 15 minutes.

The supernatant then separated into a new test tube and purified by addition of chloroform: isoamylalcohol (24 : 1) with equal volume to the volume of supernatant, and centrifugated at 13.000 rpm for 15 minutes at the 4^{o}C. The supernatant obtained was separated into a new test tube and added with 1 mL of cold isopropanol, and then was shaken slowly until the DNA pellet was visible. This DNA pellet then incubated in a freezer for 30 minutes an centrifugated at the 10.000 rpm for 10 minutes.

The DNA pellet was washed with 0.5 mL washing buffer (80% ethanol, 3 M Na-asetat), and centrifugated at 10.000 rpm for 10 minutes at 4^{o}C. Five hundred microlitters of 70% ethanol was added to the DNA pellet and centrifugated 10.000 rpm for 10 minutes at the of 4^{o}C. The DNA pellet was dried, then was added with 50 µL of TE buffer and stored at the –20^{o}C. The primer that was used for the amplification of DREB gene included F (5′-GCCCGATGGCATTTTAGACG-3′ , and R(5′-AACCAGGAGATTAGCACGCA-3′)[11]. The PCR amplifications were initiated by predenaturation at 94^{o}C for 5 minutes followed by 35 amplification cycles of denaturation at the 94^{o}C for 30 seconds, annealing at the 53^{o}C for 30 seconds and DNA synthesis at the 72^{o}C for 30 second, and final extension at 72^{o}C for 10 minutes. The presence of the DNA was observed using 1.5% agarose gel electrophoresis. Ladder with 1000 base pairs was used as a marker DNA size. After obtain good quality of DNA, the PCR product was sent to the First Base Laboreatories Selangor Malaysia for the sequencing process.

Data analysis

The DNA sequence was aligned using the Clustal W alignment from Bioedit and the homology was compared with the same gene that already been reported in the data base of genebank.

Result and Discussion

The Result of environmental condition

Environmental condition measured during this research including temperature which is ranging from 27.1^{0}C (Dec 2013) and 28.5-28.9^{0}C (January-March 2014). The rainfall was about 47.84 mm/week (Dec 2013), and 17-3-0.2 mm/week (January-March 2014), and the number of rainy days was 20 days on December 2013, 2 days during January and February 2014, and only one day on March 2014.

The result of DNA isolation and amplification

Amplification of DREB gene from the corn genome using ZmDBP2 primer resulting in 150 base pairs length PCR product (Fig 1). This result shows that primer used was appropriate, and allows the amplification of DREB gene in the corn genome.

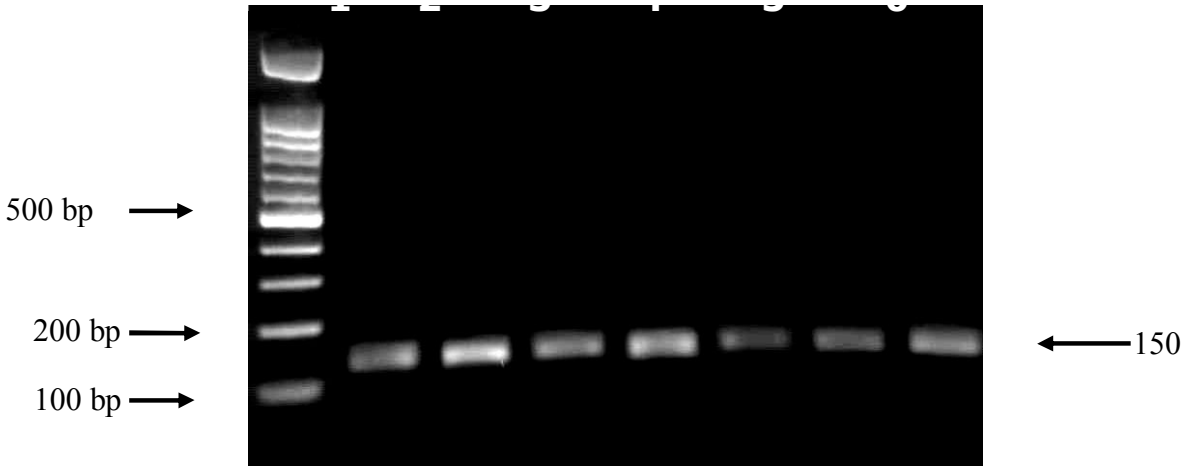

Figure 1. The result PCR product detected with 1,5% agarose gel electrophoresis M: Marker (1000 bp), 1: Rubby Brown Cob Cultivar, 2: Red Blood, 3: Waxy, 4: Early Maturing Yellow, 5: Deep Yellow, 6: White, 7: *Srikandi.*

The result of homology analysis of DREB gene

Homology analyzing of obtained sequence was conducted by BLAST application to DREB sequence that already deposited in the NCBI. Nucleotide BLAST search showed of the sequence of six local corn cultivars and one reference variety grown are highly similar (95-99% homology) with the *Zea mays* genome ZmDRE-binding protein (DBP2) mRNA (Table 1). The high sequence similarity of DREB gene fragments indicated that the fragments of genomic DNA from the tested corn was truly a DREB gene fragment. To ensure that the sequence obtained is part of the target gene, the value of identity is at least 25%[12].

Table 1. Result of homology analysis of DREB gene

Corn cultivars	discription	*E-value*	Homology (%)	Accession number of gene bank
Rubby Brown Cob	ZmDRE-bindingprotein (DBP2), mRNA	2e-36	96%	NM_001279928.1
Red Blood	ZmDRE-binding protein (DBP2),Mrna	2e-34	98%	NM_001279928.1
Waxy	ZmDRE-binding protein (DBP2), mRNA	2e-35	96%	NM_001279928.1
Early Maturing Yellow	ZmDRE-binding protein (DBP2), mRNA	4e-32	99%	NM_001279928.1
Deep Yellow	ZmDRE-binding protein (DBP2), mRNA	7e-35	96%	NM_001279928.1
White	ZmDRE-binding protein (DBP2), mRNA	2e-34	98%	NM_001279928.1
Srikandi	ZmDRE-binding protein (DBP2), mRNA	2e-34	98%	NM_001279928.1

Detection of mutation and conserved region of DREB gene fragment

To identify whether mutations were taken place and which areas were conserved (sustainable region) in the DREB gene fragment, DREB gene sequences were aligned to the same gene that is already reported in the gene bank database by the application of Clustal W alignment of Bioedit. Mutation analysis was done by comparing the sequences of each cultivar and variety to accession which has been reported by the gene bank. There is only one mutation site occurred on the six local corn cultivars (J1-J6) and reference varieties (J7) compared with two accession that has been reported in the gene banks (Figure 2).

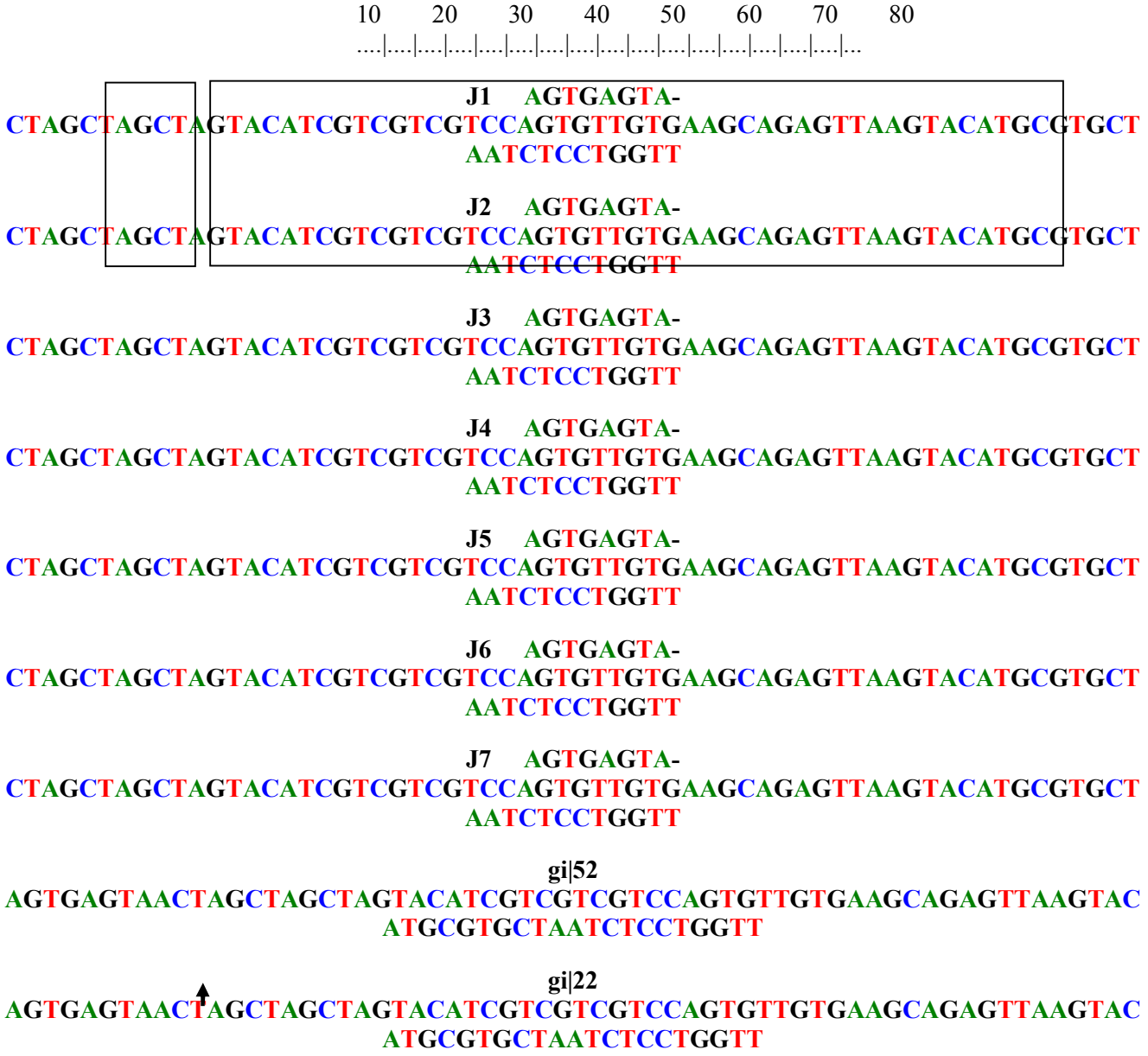

Figure 2. Results of DREB gene sequence alignments of local corn cultivars and varieties to the similar gene sequences in the gene bank. J1: Rubby Brown Cob, J2: Red Blood, J3: Waxy, J4: Early Maturing Yellow, J5: Deep Yellow, J6: White, J7: Reference variety: *Srikandi*, gi|525343462 and gi|226531635: DREB gene sequence of accession in the gene bank

It can be seen that all of local corn cultivars and the reference *Srikandi* varieties lost the nucleotide number 9. On the other hand there were no differences of DREB sequence among local corn cultivars and the reference variety. In other words, all DREB DNA region of Indonesian local corn is conserved sequences.

The DREB gene sequence, site of mutation, and type of mutation among local corn cultivars were similar to *Srikandi* as reference variety. This similarity is probably due to the mechanism of both local cultivars and reference variety in adaptation to the environmental condition. Local corn cultivars are cultivars which already adapted to the local environment for a long time, while *Srikandi* is a hybrid variety which known tolerance to drought. It might have superior gene generated from the crossbreeding between two parental with superior genetic composition[13] and this leading to it's ability in adaptation to drought stress environment. Local corn cultivars are cultivars that are formed through isolation process of genotypes change and adaptation to specific agroclimate[14], has a superior genetic traits, and can affect the cultivar resistance against environmental factors of growth[15].

Local corn cultivars and *Srikandi* reference varieties have different sequence when compared with the same gene sequences that already been reported in the Genebank. This difference might be caused by the difference in environmental conditions to grow. Kisar island with dry environmental condition could be an external signal that affect gene structure, especially to the DREB genes on local corn cultivars and *Srikandi* reference varieties.

Environmental factors such as drought can become an external signal received by an organism and integrated into the cell through a series of mechanisms of signal reception and transduction[16]. Environmental signals can cause DNA damage and leading to mutations. Deletion is one type of spontaneous mutation that took place during replication. Basically, DNA has a mechanism to repair during replication[17]. Allegedly, deletion mutation that occurs in the DREB gene sequences in local corn cultivars and *Srikandi* as a reference varieties due to severe drought, so the DNA repair mechanism can not able to recover the structure of the DNA DREB gene. In addition, the mutation in the sequence of DREB gene from local corn cultivars is a mechanism in response to their adaptation to the dry environment. Other researcher was stated that in some cases, the mutations benefit the plant because it deals with the ability of plant adaptation to environmental conditions[18].

Conclussion

Based on the results and discussion, it was concluded that the gene sequences of DREB gene belong both local corn cultivars and reference varieties in Kisar Island Southwest Maluku district Maluku Province jointly experienced deletions in the base position number 9. DREB gene sequences both on local cultivars and reference varieties are conserved sequences because it has the same sequence with accession contained in the data base of gene banks.

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