IN-SILICO Analysis of ABA-Responsive Element Binding Gene (AREB) Orthologues Base on Phylogenyand Nucleotide Frequency

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Abstract:

Background: The present research analysis of 10 AREB gene orthologous was carried out in order to determine their phylogeny, promoter region and the present of SSR motifs. ABA-responsive element binding protein (AREB) also called SIAREB was identified as gene responsible for mitigating the effects of some abiotic stresses such as water stresses (drought and flooding), cold and in some cases severe salt stress.

Materials and Methods :The AREB gene orthologous DNA sequences were downloaded from National Centre for Biotechnology Information (NCBI) database. The phylogenetic analysis was done using MEGA 6 software. However, nucleotide frequency of the genes and Maximum Likelihood were also analysed using the same software.

Results: In phylogenetic analysis, 10 orthologous of AREB gene were used to understand the evolutionary relationship among the species using neighbour joining method of MEGA 6. A tree with three main clusters was formed with first cluster containing four of the gene and the remaining two clusters containing three genes respectively. This study also analysed the frequency of nucleotide in the gene. NC 015441.3 found in Arabidopsis thaliana shows the highest total nucleotide frequency of 3840 followed by NC 003070.9 also from Arabidopsis thaliana with 2800 next is NC 0030717.7 from Solanum lycopersicum with 2743.

Conclusion: in conclusion, plant species with AREB gene share the same ancestral linage with similar function. (ii) it was identified that the phylogenetic cluster containing plant species has the highest frequencies of AREB nucleotide compared to fungal species with AREB.

Keywords: AREB Gene, orthologues, phylogeny, promoter region, simple sequence repeats.

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I. Introduction

ABA-responsive element binding protein (AREB) also called SlAREB was identified as gene responsible for mitigating the effects of some abiotic stresses such as water stresses (drought and flooding), cold and in some cases severe salt stress¹. Abiotic stress is defined as the negative impact of non-living factors on living organisms in a specific environment. The stresses include drought, salinity, low or high temperatures, and other environmental extremes. Abiotic stresses, especially hypersalinity and drought, are the primary causes of crop loss worldwide. A gene promoter composition is the Cis element found in it that will determine how and when that gene is expressed by allowing cognate transcription factors to bind and thus bring about transcription of the gene². AREB gene was identified in different plants and fungi including Solanum lycopersicum, Arabidopsis thaliana, Aspergillus species, Beauveria bassiana among others.

Orthologous are class of homologous genes that are found in different species which are diverse due to speciation of the species hosting them. Therefore, orthologous genes in different species derive from a common ancestral gene. Orthologous genes evolve in parallel with the diversification of species, they are markers of choice for the reconstruction of the evolutionary history of species using molecular phylogeny³.

Abiotic stress is one of the major aspect interfering with physiological processes of plants there by affecting the rate of metabolism and causing low yield in crops plant. Severe abiotic stress can lead to death of plant⁴. Hence the need to analyse the genes that alleviate the effects with view to enhance them where their production is low.

II. Materials and Methods

Data Mining: A total of 10 *AREB* gene orthologous were retrieved from NCBI database (<u>https://www.ncbi.nlm.nih.gov/</u>) with gene identification number. The sequence was queried against the genome sequence of the respective species using Plant Ensembl database (http://:plants.ensembl.org/index.html). parameters such as percentage identity, sequence coverage were considered⁴.

Phylogenetic Analysis: The orthologous sequence of *AREB* were aligned using multiple alignment tool of MEGA 6. Phylogenetic tree was generated using neighbour joining method (Ja'afar et al.,2020). However, nucleotide frequency of the genes and Maximum Likelihood were also analysed using the same software.

Results: *AREB* gene orthologous were found in different species at different location of the chromosome. However, it's found to performed same functions in some species and different on others. The genes were found to function as transcription factor except NC_015441.3 that function as ABA-response element binding factor (Table 1).

Table 1: AREB gene orthologous gene ID, location on chromosome and their functions.

S/No.	Gene ID	Organism	Location on chromosome	Function			
1	NC_015441.3	Solanum lycopersicum	4	ABA-response element binding factor			
2	NW_019170137.1	Aspergillus bombycis	Unknown	Transcription factor			
3	NW_015378174.1	Aspergillus nomiae	Unknown	Transcription factor			
4	NW_007930848.1	Beauveria bassiana	1	Transcription factor			
5	NC_003071.7	Arabidopsis thaliana	2	Transcription factor			
6	NC_003070.9	Arabidopsis thaliana	1	Transcription factor			
7	NW_003315664.1	Schizophyllum commune	Unknown	Transcription factor			
8	NW_002990120.1	Talaromyces stipitatus	3	Transcription factor			
9	NT_166519.1	Aspergillus niger	2	Transcription factor			
10	NW_001509762.1	Aspergillus fischeri	2	Transcription factor			

Phylogenetic Analysis

The constructed phylogenetic tree among the 10 *AREB* gene orthologous displayed 3 clusters (NC 0515441.3 gene NW015378174.1 and NW09170137.1 genes with sub clusters accommodating the rest of the genes) Figure 1.

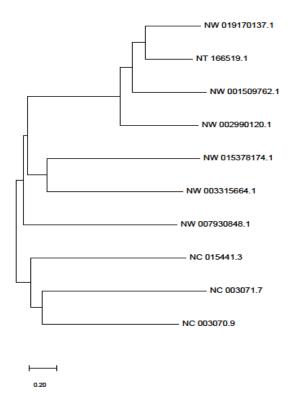


Figure 1: Phylogenetic Analysis of Orthologous AREB gene family

Nucleotide frequency of orthologous AREB gene

Table 2 shows the percentage frequency of the 10 selected orthologous of AREB gene family. The composition of the Nucleic acids (adenine, guanine, thymine and cytosine) varies in all the genes in term their frequencies. All frequencies are given in percent. (Table 2)

	T (U)	С	A	G	Total	T-1	C-1	A-1	G-1	Pos #	1 T-2	C-2	A-2	G-2	Pos #2
T-3 C-3 A-3	G-3	Pos #	3												
NW 019170137.1	19.4	30.0	24.2	26.4	1046	18.3	30.1	31.2	20.3	349	23.2	27.8	21.2	27.8	349
16.7 32.2 20.1	31.0	348													
NW 015378174.1	23.6	29.9	22.1	24.3	1996	26.7	28.1	18.6	26.6	666	20.2	31.6	23.9	24.4	665
23.9 30.1 23.9	22.1	665													
NW 007930848.1	20.8	29.5	24.5	25.2	1901	20.7	28.4	30.0	21.0	634	21.9	33.3	20.8	24.0	634
19.7 26.9 22.7	30.6	633													
NW 003315664.1	23.7	34.9	16.7	24.6	2166	21.3	36.8	18.3	23.5	722	24.2	36.6	14.8	24.4	722
25.6 31.4 17.0	25.9	722													
NW 002990120.1	29.3	23.4	24.6	22.7	1858	26.3	25.5	27.9	20.3	620	32.5	20.4	26.2	21.0	619
29.1 24.4 19.7	26.8	619													
NW 001509762.1	19.6	30.6	24.1	25.7	1044	17.5	30.2	24.1	28.2	348	19.8	31.0	29.3	19.8	348
21.6 30.5 19.0	29.0	348													
NT 166519.1	18.9	31.7	22.3	27.2	1045	19.5	32.4	29.2	18.9	349	17.8	29.3	18.7	34.2	348
19.3 33.3 19.0	28.4	348													
NC 015441.3	31.6	16.8	30.0	21.6	3840	31.8	18.0	29.4	20.9	1280	34.3	14.8	31.3	19.5	1280
28.7 17.7 29.3	24.4	1280													
NC 003071.7	33.9	18.8	28.3	19.1	2743	32.6	18.5	27.8	21.2	915	34.4	19.3	29.3	17.1	914
34.7 18.7 27.7	18.9	914													
NC 003070.9	31.7	15.9	28.5	24.0	2800	30.5	18.5	28.4	22.6	934	37.2	12.6	27.3	22.8	933
27.3 16.4 29.8	26.5	933													
Avg.	27.2	24.1	25.4	23.3	2043.	9 26.5	24.7	26.5	22.2	681	.7 28.	8 23.	5 25.	3 22.4	681.2
26.3 24.0 24.3	25.5	681													

Table 2: AREB gene orthologous, Nucleotide Frequencies

III. Discussion

Under stress conditions, a myriad of genes that function in the stress tolerance and response are induced in diverse plant species. Under such conditions, various biochemical and physiological responses, including the expression of many stress-inducible genes that function in stress tolerance and ABA accumulation, are triggered. Numerous drought stress-responsive genes have been reported, and many are induced by ABA⁵.

In phylogenetic analysis, 10 orthologous of *AREB* gene were used to understand the evolutionary relationship among the species using neighbour joining method of MEGA 6. A tree with three main clusters was formed with first cluster containing four of the gene and the remaining two clusters containing three genes respectively Figure 1. This shows the distinctness and diversity and closeness this organism are with respect to *AREB* gene. These results are in accordance with the phylogenetic tree of tomato *AREB* screening ⁴. I the first cluster three of the genes were from same genus of Aspergillus with only one from Talaromyces all have the function of transcription. Interestingly, the third cluster contain genes from plant species (*Solanum lycopersicum* and *Arabidopsis thaliana*). Similar result was reported by⁵. This shows that a gene from *Arabidopsis thaliana* could be introgressed or enhance *AREB* gene in cultivated tomato in order to control abiotic stress.

This study also analysed the frequency of nucleotide in the gene. NC 015441.3 found in *Arabidopsis thaliana* shows the highest total nucleotide frequency of 3840 followed by NC 003070.9 also from *Arabidopsis thaliana* with 2800 next is NC 0030717.7 from *Solanum lycopersicum* with 2743. The nucleotide frequencies of the orthologous *AREB* is higher in plant species compared to the fungal species. The frequency of nucleotides in a gene have a very high impact on the gene expression in an organism ⁶.

IV. Conclusion

In this study, comparative *in silico* analysis of *AREB* was performed using orthologous sequence derived from NCBI database. Based on our findings, (i) plant species with *AREB* gene share the same ancestral linage with similar function. (ii) it was identified that the phylogenetic cluster containing plant species has the highest frequencies of *AREB* nucleotide compared to fungal species with *AREB*.

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