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Genotypic screening of tomato's *AREB 1* gene for drought tolerance and computational protein structure prediction

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In this investigation, the coding sequence of the drought-stress inducible gene *AREB1* in tomato derived from cDNA indicated 100% identity with the reference gene in the NCBI PlantEnsembl database. The protein structure of the *AREB1* sequence derived from polymerase chain reaction from tomato DNA template was done using ExPASy and its protein parameter tools ProtParam. The structures of *AREB1* protein showed a MolProbity score of 1.49. Multiple sequence alignment of *AREB1* gene from 20 tomato genotypes revealed a phylogenetic tree with five clusters, each with the same evolutionary trend. The nucleotide sequence analysis showed higher similarities among the selected tomato genotypes. This indicated the conserved nature of the gene among the genotypes.

Key words: Tomato, drought, resistant, *AREB1*gene, *AREB1*protein.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a fruit cultivated and consumed worldwide. Mostly red in colour with different sizes and shapes. Though cultivated worldwide tomato is reported to be originated from Western South America (Acquaah, 2008). Tomato fruit has being classified as berry and consumed in different ways, raw, cooked, sauces, salads, and drinks (Singh et al., 2012). Tomato plants typically grow to 1-3 m (3-10 ft) in height. They are vines that have a weak stem that sprawls and typically needs support (Acquaah, 2008). The tomato sizes vary according to the cultivar, in the range of 0.5-4 inches (1.3-10.2 cm) (IPGRI, 2015). Many water deficit (Drought) stress-inducible genes have been highlighted and found to be activated by abscisic acid ABA. The *AREB-I* gene mutant family are more tolerant to ABA than are the other single and double mutants with respect to primary root growth, and it displays reduced drought tolerance (Takuya et al., 2010). AREB/ABF transcription factors are induced as the result of environmental stresses, only AREB1 is reported to be regulated by ABA-dependent phosphorylation (Fujita et al., 2005). *AREB1* needs to be activated fully for ABA (Fujita et al., 2005), Abscisic acid is a plant hormone that regulates many important processes in plant metabolism, such as seed germination and dormancy, opening and closing of stomata, abscission, and adaptation to water stress (Redenbaugh et al., 1992).

There are reports on drought resistant gene (*AREB*) in

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Table 1. Tomato accessions and their place of collection.

S/N	Accessions name/ number	Species name	Place of collection
1	NG/SA/01/10/002	*Solanum lycopersicum* L.	NACGRAB
2	NGHB/09/120	*Solanum lycopersicum* L.	NACGRAB
3	NG/AA/SEP/09/045	*Solanum lycopersicum* L.	NACGRAB
4	NHGB/09/113	*Solanum lycopersicum* L.	NACGRAB
5	NG/AA/SEP/09/044	*Solanum lycopersicum* L.	NACGRAB
6	L00170	*Solanum lycopersicum* L.	NACGRAB
7	NGHB/09/114	*Solanum lycopersicum* L.	NACGRAB
8	NG/AA/SEP/09/013	*Solanum lycopersicum* L.	FRIN
9	NG/AA/SEP/09/042	*Solanum lycopersicum* L.	FRIN
10	L00169	*Solanum lycopersicum* L.	FRIN
11	VG-004/83	*Solanum lycopersicum* L.	FRIN
12	GRC1936/04	*Solanum lycopersicum* L.	FRIN
13	GRC1925/04	*Solanum lycopersicum* L.	FRIN
14	VE-027/83	*Solanum lycopersicum* L.	FRIN
15	GR279/99	*Solanum lycopersicum* L.	FRIN
16	Mylo	*Solanum lycopersicum* L.	NACGRAB
17	Mylati	*Solanum lycopersicum* L.	NACGRAB
18	GRC1807/04	*Solanum lycopersicum* L.	NACGRAB
19	L00190	*Solanum lycopersicum* L.	NACGRAB
20	Karabola	*Solanum lycopersicum* L.	NACGRAB

tomato; however, to the best of our knowledge, no such report has been published on the *AREB* gene in a wide

range of tomato genotypes, or on the prediction of protein structure and computational protein analysis of the *AREB* gene in tomato genotypes. Plant breeding through a conventional way to improve drought resistance, in many cases, is too slow due to lack of precise molecular and genetic information on drought tolerance associated genes and their regulations. Nigeria is the largest producer of tomato in Sub-Saharan Africa, and ranks 13th in the world. Notwithstanding, the production faces challenges with storage, distribution abiotic and biotic stresses (GAIN, 2018). This is due to climate change such as increase in temperature, evaporation and drought (He et al., 2003).

MATERIALS AND METHODS

Plant materials

The seeds of the selected tomato genotypes were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), North Centre Zone, Badeggi Nigeria, Forestry Research Institute of Nigeria (FRIN), Department of Agricultural Technology, Federal College of Forestry, Jos, Nigeria and National Centre for Genetic Resources and Biotechnology (NACGRAB), Department of Plant Genetic Resources, Ibadan. Seeds of each genotype were germinated on nursery beds. After 14 days of germination fresh leaves of the seedlings were collected for DNA (Table 1).

DNA and RNA isolation

Leaves of the tomato genotype (weight 100 g) were frozen in liquid nitrogen. The leaves were ground into a fine powder in a free chilled mortar. The powder was transferred into a tube of pre-warmed CTAB buffer and the mixture was incubated at 65°C for 20 min. DNA was isolated following the CTAB protocols of DNA extraction (Singh et al., 2012).

Drought resistant gene sequence retrieval and primer design

AREB1 also known as *AREB*; *LeAREB*; *SlAREB1* gene sequence (NCBI Reference Sequence: NC_015441.3) was retrieved from the National Center for Biotechnology Information (NCBI) database. The sequence was used to design primers for the *AREB* gene amplification in the selected tomato genotype. The retrieved *AREB* gene sequence was used to design primers with the following parameters: primer length 18-30 bp, melting temperature 50-60°C, GC percentage 40-60 and product size: 160-500 bp, using Vector NTI software (Ja'afar et al., 2018).

Polymerase chain reaction (PCR) Amplification of the *AREB* Gene

Polymerase chain reaction amplifications of *AREB* gene in the selected tomato genotype was carried out using *AREB* gene specific primers in a total volume of 25 µl using a C1000 Thermal Cycler (Bio Rad, USA). Each 25 µl volume of reaction mixture contained 50 ng of genomic DNA as template, 1X Taq polymerase buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mix, 0.4 pM each of the forward and reverse primer, 1 U of Taq polymerase. The optimized condition was initial 5 min incubation at 97°C for complete

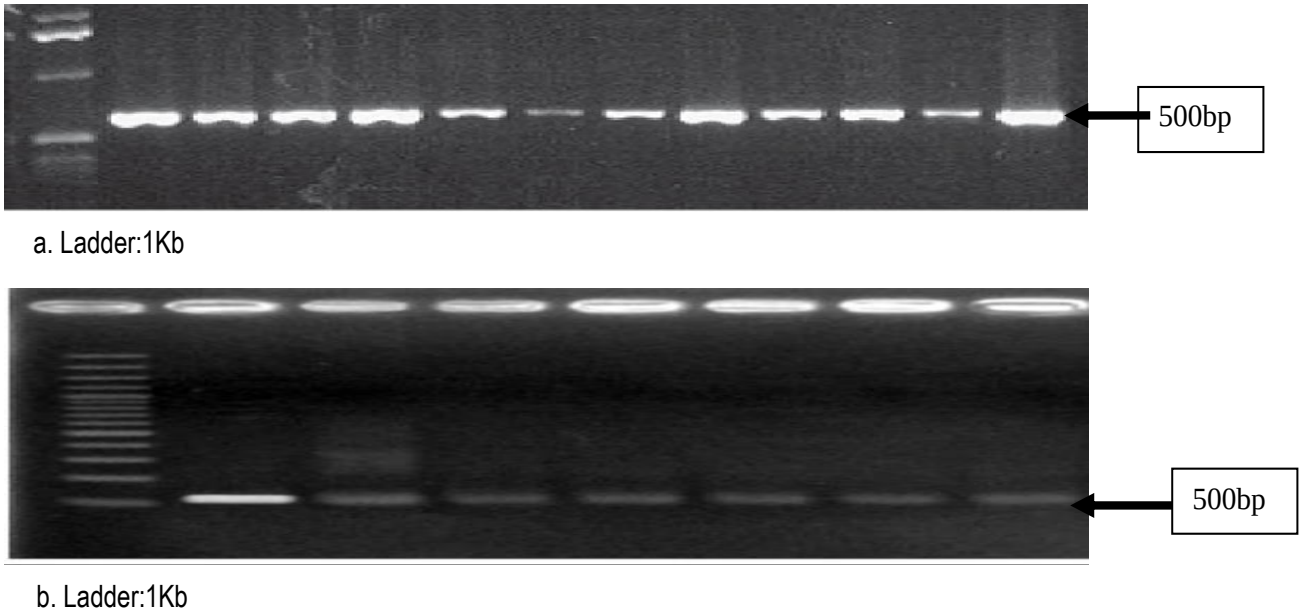

Figure 1. Polymerase chain reaction of the *AREB* gene in 19 genotypes of tomato. (a) DNA bands of NG/SA/01/10/002, NGHB/09/120, NG/AA/SEP/09/045, NHGB/09/113, NG/AA/SEP/09/044, L00170, NGHB/09/114, NG/AA/SEP/09/013, L00169, VG-004/83, GRC1936/04 and GRC1925/04, tomato genotypes PCR with *AREB 1*. (b) DNA bands of VE-027/83, Karabola, L00190, GRC1807/04, Mylati, Mylo, GR279/99, VE-027/83 and GRC1936/04 tomato genotypes PCR with *AREB 1*.

denaturation, followed by 38 cycles consisting of 94°C for 1 min, 55- 60°C (varying with the primer pair) for 1 min., 72°C for 2 min, and finally 72°C for 10 min. The experiments were repeated twice (Molla et al., 2015).

Resolving of all PCR products was performed in a vertical non-denaturing 3% Agarose gel electrophoresis system at constant 90 V with 1X TAE (Tris acetate EDTA) buffer (pH = 8.0). The gel was stained with ethidium bromide solution and visualized using a gel documentation system (Protein Simple, USA) adopting the methods of Botstein et al. (1980).

Gene sequencing and sequence submission to gene bank

The PCR products were purified with Gel Extraction Kit, the products were used for sequencing. The forward and reverse contigs was edited and joined to make a complete sequence, which was used for *in silico* analysis (Molla et al., 2015).

Sequence and phylogenetic analysis

The *AREB* gene sequences from the DNA of the selected tomato genotypes was aligned with the original reference sequence and edited for SNPs and INDEL detections. The phylogenetic relationship was designed using a molecular evolutionary genetic analysis tool (MEGA).

Computational protein analysis and structure prediction of *AREB1* protein

The open reading frame (ORF) of the AREB nucleotide sequence was translated into amino acid using ExPasy translation tool and aligned to the amino acid residue of other sequences of tomato using a multiple sequence alignment tool. The 3-D structure prediction of AREB protein was performed using SWISSMODEL program and NCBI prediction tool (Botstein et al., 1980).

Data analysis

The data obtained from plant DNA was analyzed using bioinformatics software: MEGA, SWISS model and ExPasy tools.

RESULTS

AREB gene amplification

The primers designed from the *AREB* gene of tomato showed amplification in all the twenty selected genotypes after PCR using genomic DNA of each genotype (Figure 1a and b). The PCR products were extracted and purified using gel extraction kit and then sequenced. The products were check on 1.2% agarose gel electrophoresis to view the respective sizes. All the amplified bands studied correspond to 500 bp size with the aid of 1 kb ladder (Figure 1a and b).

Gene sequencing

The amplified PCR products (Genomic DNA and cDNA derived sequences) were sequenced using next generation contigs (forward and reverse), and edited

Table 2. AREB1 gene sequences of 20 *Solanum lycopersicum* genotypes using the blast approach of the Ensembl plants database.

Name of Genotypes	Length	Score	Orientation	%ID	Chromosome no.	Location	E-value
NG/SA/01/10/002	500	200	Reverse	100.0	1	4:63589828-63590027	1.3e-108
NGHB/09/120	500	500	Reverse	100.0	1	4:63589328-63589827	0.0
NG/AA/SEP/09/045	484	484	Reverse	100.0	1	4:63588827-63589310	1.3
NHGB/09/113	500	500	Reverse	100.0	1	4:63588327-63588826	2.3e-08
NG/AA/SEP/09/044	500	283	Forward	96.0	1	4:63587757-63588039	3.7e-158
L00170	500	500	Reverse	100.0	1	4:63587257-63587756	0.3
NGHB/09/114	500	500	Forward	100.0	1	4:63586757-63587256	0.0
NG/AA/SEP/09/013	500	480	Forward	95.5	1	10:10397596-10397617	5.1
NG/AA/SEP/09/042	500	500	Forward	100.0	1	10:2354125-2354142	0.0
L00169	500	500	Forward	100.0	1	11:32688512-32689011	0.0
VG-004/83	500	500	Forward	100.0	1	11:32689012-32689561	0.0
GRC1936/04	500	500	Forward	100.0	1	11 32689562 to 32690061	0.0
GRC1925/04	500	500	Forward	100.0	1	11 32690062 to 32690561	0.0
VE-027/83	500	500	Forward	100.0	1	11 32690562 to 32691061	0.0
GR279/99	500	496	Forward	99.8	1	11 32691062 to 32691561	0.0
Mylo	500	500	Forward	100.0	1	11 32691562 to 32692061	0.0
Mylati	500	450	Forward	95.0	1	11 32692062 to 32692611	0.0
GRC1807/04	500	500	Forward	100.0	1	11 32692612 to 32693111	0.0
L00190	500	500	Forward	100.0	1	11 32693112 to 32693611	0.0
Karabola	500	482	Reverse	92.5	1	10 21263510 to 21263735	1.5e-83

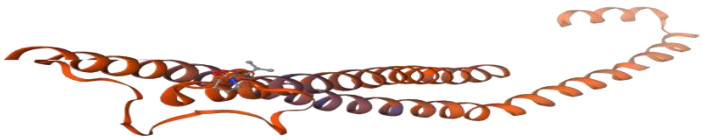

Figure 2. The predicted 3-D structure of the GC box binding domain of the *AREB1* protein of the tomato reference sequence with alpha helical structures of 2.6 amino acid residue and alphatic index of 56.66.

using Vec Screen software. All of the sequence analyses revealed a 500 bp sequence length. The genomic sequences of the *AREB1* gene from 20 tomato genotypes were aligned with a reference sequence of tomato obtained from ensemble plant (Gramene Database), using a multiple sequence alignment method. The aligned sequence revealed the presence of the conserved AREB1 gene throughout the selected genotypes (Table 2).

Computational analysis and structure prediction of tomato *AREB* genes family

The ExPASy bioinformatic tool was used to translate the DNA sequences into amino acid sequences and to construct the 3-D secondary structure of the *AREB1* protein of tomato generated using the SWISS-MODEL program (Figure 2). The structural protein properties predicted by the ProtParam tools had a MolProbity score of 1.49, and Ramachandran favored of 95.83%.

Phylogenetic relationships analysis of the *AREB1* gene among the selected tomato genotypes

The phylogenetic dendrogram (Figure 3) was generated using a Neighbor-Joining method, with MEGA software based on the data set from the tomato sequences generated from the product of PCR with *AREB1* gene primers. The results showed an optimal tree with the sum of branch lengths that equals 68.50928440. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is

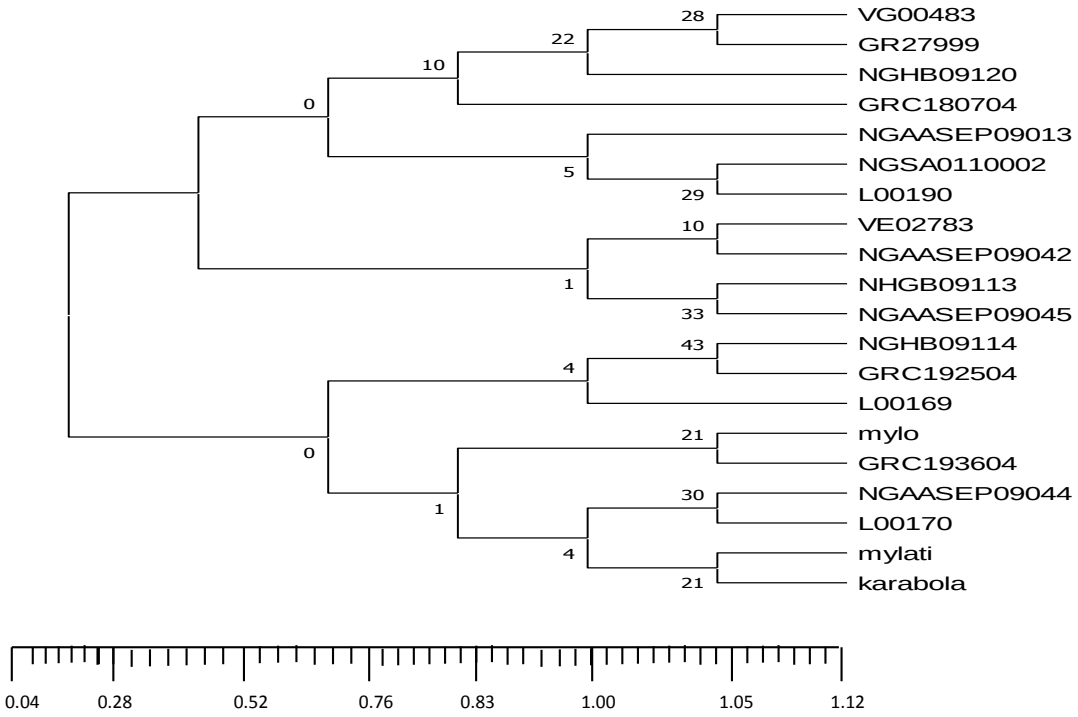

Figure 3. The phylogenetic tree based on nucleotide sequence of tomato genotypes including scale bar for base sequence distances.

shown next to the branches (Figure 3). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding (Figure 3).

DISCUSSION

Sequence alignment of the *AREB1* gene from the genomic DNA of the selected tomato genotypes was successfully done using specific primers derived from the AREB1 gene. The DNA isolated revealed a sequence with a 500-bp length, which is similar to some of the reference genes in the Ensembl Plants data base. Sakshi and Kavita (2020) reported that conventional separation by agarose gel electrophoresis results only in a single DNA band and is largely non-descriptive. However, Zhou et al. (2016) reported that the gene *ABF1* was clearly induced by drought, high salinity and ABA treatments, although its expression levels were low even under stress conditions compared with those of *AREB1, AREB2* and *ABF3*. The *AREB1* gene along with *Dreb* gene were identified as a strong genes in the drought responsive pathways in *Arabidopsis*, tomato, rice and other members of solanaceae (Alves and Setter, 2004). *AREB1* gene is found to have a very poor sensitivity for abscisic acid; and, therefore, this suggested that it could be involved in the ABA independent pathway (Barry, 2001).

The local coordinate system was defined using the main chain atoms of each amino acid, as described previously. This is the foundation of neighborhood analysis for each amino acid. The structure shows a property of free proline and glycine betaine which are the major biochemical parameters of abiotic resistance in plants. Free proline contents and glycine betaine are the signals that the plant shows in response to stress and can be used to measure the level of tolerance to a particular stress in plants (Ja'afar et al., 2018). The sequence-based phylogenetic tree generated (Figure 3) showed five distinct clusters with different bootstrap values. Those genotypes in the same cluster are found to be closely related and share a common evolutionary trend. A similar trend was reported by Molla et al. (2015) for *Oriza sativa* and *Oriza glaberrima* with some of the closely related species occupying the same cluster.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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