Research Article

Prediction of drought related transcripts in cotton (*Gossypium hirsutum*): an in-silico approach

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Abstract

Prediction of candidate transcripts using bioinformatics tools is an exciting domain which saves the time of a researcher. Drought responsive genes were identified using homology method. The known genes from sequenced organisms (like *Arabidopsis*, rice, etc.) were compared with ESTs of different crops from various databases. Total 177 genes of *Arabidopsis* and rice with known functions were retrieved from different databases. The genes were classified and their role in drought was characterized on the basis of already available literature. After classification, 110 genes were selected for further studies. mRNA sequences and accession numbers of the 110 drought tolerant genes were retrieved which were analyzed using BLAST tool against ESTs on “NCBI” databases. ESTs in different crops (like cotton, wheat, potato, maize, sorghum, etc.) were found and data regarding ESTs from cotton were retrieved. Total 9096 ESTs were analyzed from NCBI. The 26 short listed genes were analyzed for “Multiple Sequence Alignment” and aligned with ESTs which were identified earlier. They showed considerable homology with ESTs. All the genes were thoroughly searched for their novelty and finally 8 genes were reported as candidate genes responsible for drought stress tolerance.

Keyword: Bioinformatics; Cotton; Drought; ESTs; Transcripts

Introduction

Drought stress usually happens in atmospheric conditions where the water is continuously lost by the process of

evaporation and transpiration and as a result the water level in the soil is decreased. Drought is one of the most prominent natural environmental hazard which affects

large number of people directly [1, 2]. Almost all climatic regions are affected by drought [2] and every year almost half of the regions of the world are vulnerable to the threat of drought [3]. In many countries, drought stress is also an obstinate environmental obstacle. In the last few years, the disaster incidences from drought stress are pointedly increased without any reported evidences of increased drought severity [2].

Drought tolerance is observed in almost all plant species but the extent of response is variable from specie to specie and sometimes even with in the species [4]. Drought affects plants in different ways. Severe drought may result in the disruption of metabolism, seizure of photosynthesis and finally it can lead to the plants death [4]. It has been observed that drought stress plays a vital role as a limiting factor on some initial phases of plant growth and establishment [5]. Water stress hinders enlargement of cell, elongation of cell and cell division. It can reduce the growth of plant by the disturbance in many biochemical and physiological processes [6]. Identification of genes responsible for drought is an important strategy to combat the serious problem. Once the genes are identified, they can be used to develop genetically engineered plants having resistance to drought. Using bioinformatics tools, the transcripts/genes responsible for drought stress can be identified. In this study, the drought related transcripts in cotton were identified using bioinformatics tools. The procedure is less laborious, less time consuming and it requires less resources. Study of genes related to drought stress in cotton and there identification is of key importance to understand the mechanism of drought tolerance and can help in increasing the cotton production which will ultimately contribute towards the national economy.

Materials and Methods

Retrieval of Drought Tolerant genes of Different Plant Species

The previously reported drought tolerant genes from different plants i.e., *Arabidopsis thaliana*, rice wheat and soya been were searched from various scholarly articles and then their accession numbers and sequences in FASTA format were retrieved from different data bases like NCBI (www.ncbi.nlm.nih.gov/), TAIR (www.arabidopsis.org/), GRAMENE (www.gramene.org/), TIGR (www.jcvi.org/), etc.

Nucleotide BLAST of drought tolerant genes

The retrieved genes were subject to BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the identified ESTs were saved for further analysis.

Figure-1. Flow chart summary of methods used in prediction of drought related transcripts in cotton

Multiple Sequence Alignment of ESTs

Multiple Sequence Alignment was carried out at EMBL-EBI web portal (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and CUSTAL OMEGA software was used which is freely available online. The potential candidate genes/transcripts were searched in the literature for their possible homologues in cotton. Only the novel genes/transcripts not identified previously were reported in this article. The detailed scheme of the methodology is given in **Figure-1**.

Results

Retrieval of Genes

The known drought tolerant genes were retrieved from various plant species including *Arabidopsis thaliana*, *Hordeum vulgare*, *Glycine max*, *Solanum lycopersicum*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, etc. Total 177 genes were retrieved, cataloged and their functions were listed. On the basis of their functions and involvement in drought stress tolerance mechanism, 110 genes were short listed and included in present study (**Table-1**).

Sequence Alignment and ESTs analysis

After nucleotide and protein BLAST of 110 genes, the 9096 ESTs were identified in cotton (*Gossypium hirsutum*). The number of ESTs against various plant species identified in cotton are being shown in **Figure-2**. Maximum 429 cotton ESTs were identified in genes of *Glycine max* origin followed by 273 in *Triticum aestivum*, 181 in *Solanum lycopersicum* and 179 in *Arabidopsis thaliana*. ESTs in other plant species were also found in cotton.

Multiple Sequence Alignment of cotton ESTs

Multiple sequence alignment of the EST's from cotton was carried out with the respective genes and sequence similarities were found. Total of 26 genes were aligned and their sequence similarities were found.

On the basis of significant similarities, 19 genes were short listed as potential novel genes in cotton. Genes which were aligned with cotton were then searched in literature and 8 genes were found not to be known previously in cotton (**Table-2**).

Discussion

Identification of genes/transcripts using bioinformatics tools is very popular now days. Because of the availability of large amount of information and complete or partial genome sequence of *Arabidopsis thaliana*, *Hordeum vulgare*, *Glycine max*, *Solanum lycopersicum*, *Triticum aestivum*, *Zea mays* and *Oryza sativa*, which could be used to identify novel genes. New gene discovery approach requires searching of whole genome sequence for homologous sequences of known genes or known gene families by *in silico* methods. Recent researches prove the proficiency of this *in silico* technique of finding new genes [7, 8]. All the nucleotide and protein sequences of 143 were aligned using BLASTn and BLASTp respectively. Out of these 110 genes 26 genes aligned with cotton genome which proved the presence of drought tolerant genes in cotton.

In this study, we report 8 novel genes which are supposed to play important role in drought stress tolerance in cotton. These genes are known for the said function in other plants and are well characterized. ABF3 is identified in *Arabidopsis* which is responsible for transcription activation [9]. It is also known as a DNA binding protein gene and is a transcription factor. STRS1 is also identified in *Arabidopsis thaliana* and is known as a stress response suppressor [10]. It is involved in DNA replication. SEP2 is a stress enhanced protein and is identified in *Arabidopsis thaliana* [11]. WAPX1 is reported in *Triticum aestivum* and codes for ascorbate peroxidase enzyme which catalyse the conversion of H₂O₂ to H₂O [12].

Figure-2. Cotton ESTs identified against genes already known in different plant species

Table-1: Genes responsible for drought tolerance in different Plants species

S. No.	Gene Name	Accession No.	Source	S. No.	Gene Name	Accession No.	Source
1.	HSA32	NM_118250.4	<i>Arabidopsis thaliana</i>	2.	HRD	DQ446604.1	<i>Arabidopsis Thaliana</i>
3.	DREB2A	NM_001036760.1	<i>Arabidopsis thaliana</i>	4.	rice adc	AY604047.1	<i>Oryza sativa</i>
5.	ABF3	NM_001036708.3	<i>Arabidopsis thaliana</i>	6.	SB	JN661889.1	<i>Oryza sativa</i>
7.	STRS1	NM_102933.2	<i>Arabidopsis thaliana</i>	8.	OsMPG1	JN408497.1	<i>Oryza sativa</i>
9.	ERD15	NM_201929.2	<i>Arabidopsis thaliana</i>	10.	MPG2	AF062466.1	<i>Cucumis melo</i>
11.	LOS4	NM_115171.3	<i>Arabidopsis thaliana</i>	12.	OsMPG3	JN408499.1	<i>Oryza sativa</i>
13.	SEP2	NM_127766.2	<i>Arabidopsis thaliana</i>	14.	OsGRF5	BK004860.1	<i>Oryza sativa</i>
15.	AKIN11	NM_202645.2	<i>Arabidopsis thaliana</i>	16.	OsAE115	OS04G48510	<i>Oryza sativa</i>
17.	ROP10	NM_114673.2	<i>Arabidopsis thaliana</i>	18.	OsGRF8	BK004863.1	<i>Oryza sativa</i>
19.	HOS10	NM_179418.1	<i>Arabidopsis thaliana</i>	20.	NFYB12	AK227164.1	<i>Arabidopsis Thaliana</i>
21.	ERD15	NM_180019.2	<i>Arabidopsis thaliana</i>	22.	DST	GQ178286.1	<i>Oryza sativa</i>
23.	ERD15	NM_201928.2	<i>Arabidopsis thaliana</i>	24.	AtHDG11	NM_105996.3	<i>Arabidopsis thaliana</i>
25.	ERD15	NM_201929.2	<i>Arabidopsis thaliana</i>	26.	OsTIR1	EU400583.1	<i>Oryza sativa</i>
27.	DREB2B	NM_001036760.1	<i>Arachis hypogaea</i>	28.	OsAFB2	NM_113593.2	<i>Arabidopsis thaliana</i>
29.	DREB2A	AB007790.1	<i>Arabidopsis thaliana</i>	30.	OsmIR393	NM_101152.4	<i>Arabidopsis thaliana</i>
31.	ltp-BR1	U88090.1	<i>Hordeum vulgare</i>	32.	WR1	M94959.1	<i>Triticum aestivum</i>
33.	PR-10	AY220734.1	<i>Hordeum vulgare</i>	34.	OsCY20-2	NM_121315.2	<i>Arabidopsis thaliana</i>
35.	HSP90	AY325266.1	<i>Hordeum vulgare</i>	36.	bZIP23	BT029022.1	<i>Arabidopsis thaliana</i>
37.	PHGPX	AB096704.1	<i>Hordeum vulgare</i>	38.	RD22-like	NM_122472.3	<i>Glycine max</i>
39.	CHIA1	AF202731.1	<i>Glycine max</i>	40.	RD29B-like	NM_001198228.1	<i>Glycine max</i>
41.	MYB92	DQ822903.1	<i>Glycine max</i>	42.	ARR5	NP_190393.1	<i>Arabidopsis thaliana</i>
43.	MYB177	DQ822925.1	<i>Glycine max</i>	44.	ARR7	NM_101763.2	<i>Arabidopsis thaliana</i>
45.	STI	X79770.1	<i>Glycine max</i>	46.	ARR15	NM_106147.1	<i>Arabidopsis thaliana</i>
47.	DD4/62	AJ289155.1	<i>Glycine max</i>	48.	ARR22	NM_180180.2	<i>Arabidopsis thaliana</i>
49.	DREB3	AF506825.1	<i>Solanum lycopersicum</i>	50.	NHX1	AF056190.1	<i>Arabidopsis thaliana</i>
51.	rps4	YP_514854.1	<i>Solanum lycopersicum</i>	52.	SOS1	NM_126259.3	<i>Arabidopsis thaliana</i>
53.	RbcL	YP_514860.1	<i>Solanum lycopersicum</i>	54.	P5CS1	NM_001202786.1	<i>Arabidopsis thaliana</i>
55.	WESR4	AB011445.1	<i>Triticum aestivum</i>	56.	RD29A	NM_124610.2	<i>Arabidopsis thaliana</i>
57.	Cht2	AF112963.1	<i>Triticum aestivum</i>	58.	AtPCS1	NM_120297.3	<i>Arabidopsis thaliana</i>
59.	Cht4	AF112963.1	<i>Triticum aestivum</i>	60.	ZmSNAC1	EU963406.1	<i>Zea Mays</i>
61.	GST1	AF184059.1	<i>Triticum aestivum</i>	62.	ABCG22	XM_641139.1	<i>Dictyostelium discoideum</i>
63.	GST1	AF387085.1	<i>Triticum aestivum</i>	64.	PYL5	NM_120626.2	<i>Arabidopsis thaliana</i>
65.	Tat1l	AY077702.1	<i>Triticum aestivum</i>	66.	KfVP1	FJ176929.1	<i>GRAPE</i>
67.	WAPX1	AF387739.1	<i>Triticum aestivum</i>	68.	CBF2	NM_118679.1	<i>Arabidopsis Thaliana</i>
69.	GST	AY377972.1	<i>Triticum aestivum</i>	70.	CBP60g	NM_001203479.1	<i>Arabidopsis Thaliana</i>
71.	WCOR410	L29152.1	<i>Triticum aestivum</i>	72.	AtLOS5	NM_101519.2	<i>Arabidopsis Thaliana</i>
73.	Chi 2	AB029935.1	<i>Triticum aestivum</i>	74.	SnRK2	NM_001203118.2	<i>Arabidopsis Thaliana</i>

75.	Chi 3	AB029936.1	<i>Triticum aestivum</i>	76.	AtNCED3	NM_112304.2	<i>Arabidopsis Thaliana</i>
77.	hsp18a	EU952864.1	<i>Zea mays</i>	78.	LTl65	NM_124609.3	<i>Arabidopsis Thaliana</i>
79.	gst1	M16901.1	<i>Zea mays</i>	80.	RNS1	NM_126351.2	<i>Arabidopsis Thaliana</i>
81.	gpa1	X07157.1	<i>Zea mays</i>	82.	AtDiB	NM_130216.2	<i>Arabidopsis Thaliana</i>
83.	hsp 17.2	X65725.1	<i>Zea mays</i>	84.	AtDi19	NM_104107.4	<i>Arabidopsis Thaliana</i>
85.	glu1	U25157.1	<i>Zea mays</i>	86.	AtD121	NM_117683.4	<i>Arabidopsis Thaliana</i>
87.	hsp22	EU961071.1	<i>Zea mays</i>	88.	AtDr4	NM_105993.2	<i>Arabidopsis Thaliana</i>
89.	dbf3	AF450481.1	<i>Zea mays</i>	90.	CBF4	NM_124578.1	<i>Arabidopsis Thaliana</i>
91.	hsp18f	X54076.1	<i>Zea mays</i>	92.	Atlea3	NM_001203153.1	<i>Arabidopsis Thaliana</i>
93.	hsp26	EU964533.1	<i>Zea mays</i>	94.	AtP5CS2	NM_115419.4	<i>Arabidopsis Thaliana</i>
95.	OsNAC10	JQ974964.1	<i>Oryza sativa</i>	96.	AtProT1	NM_001084556.1	<i>Arabidopsis Thaliana</i>
97.	ERA1	AF214106.1	<i>Arabidopsis Thaliana</i>	98.	AtAPX1	AY094002.1	<i>Arabidopsis Thaliana</i>
99.	LEA3	DQ056621.1	<i>Arabidopsis Thaliana</i>	100.	AtSOS1	NM_126259.3	<i>Arabidopsis Thaliana</i>
101.	DREB1A	EF523125.1	<i>Arabidopsis Thaliana</i>	102.	AtNHX1	NM_122597.2	<i>Arabidopsis Thaliana</i>
103.	OsDREB2A	AB007790.1	<i>Arabidopsis Thaliana</i>	104.	Hua1	NM_112103.3	<i>Arabidopsis thaliana</i>
105.	DREB1C	EF523053.1	<i>Arabidopsis Thaliana</i>	106.	TaWRKY2	EU665430.1	<i>Triticum aestivum</i>
107.	DREB1B	AB007788	<i>Arabidopsis Thaliana</i>	108.	TaWRKY19	EU665428.1	<i>Triticum aestivum</i>
109.	rd29A	L22567.1	<i>Arabidopsis Thaliana</i>	110.	DI21	X78585.1	<i>Arabidopsis Thaliana</i>

Table-2: Novel candidate genes predicted for drought tolerance in cotton (*Gossypium hirsutum* L.)

S. No.	Gene Name	Accession No.	Origin	Gene Function
1.	AFB3	NM_001036708.3	<i>Arabidopsis thaliana</i>	Abscisic acid responsive elements-binding factor-3, DNA binding/ protein, transcription factor, transcription activator
2.	STRS1	NM_102933.2	<i>Arabidopsis thaliana</i>	Stress response suppressor-1, ATP-dependent helicase
3.	SEP2	NM_127766.2	<i>Arabidopsis thaliana</i>	Stress enhanced protein
4.	ltp-BR1	U88090.1	<i>Hordeum vulgare</i>	Nonspecific lipid transfer protein
5.	WAPX1	AF387739.1	<i>Triticum aestivum</i>	Ascorbate peroxidase
6.	ERA1	AF214106.1	<i>Arabidopsis thaliana</i>	Protein farnesyltransferase subunit beta
7.	AtProT1	NM_001084556.1	<i>Arabidopsis thaliana</i>	Proline transporter
8.	TaWRKY2	EU665430.1	<i>Triticum aestivum</i>	WRKY19 transcription factor

It is regulated in response to biotic and abiotic stress and is responsible to protect plant cell against adverse environmental conditions. ltp-BR1, ERA1, AtProT1 and TaWRKY2 were also predicted in this study which play role in drought stress tolerance in various plants [13-16].

Due to availability of large amount of sequence data in the various databases, it is very easy to predict the functions of some particular fragment of nucleotides. Genome sequencing made it even more reliable. It is easy, time saving, reliable and also saves resources. Number of genes has been identified by using *in silico* approaches. Here, we also report the identification of 8 drought tolerant genes in cotton by gene homology approach using various bioinformatics tools.

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