



Full Length Article

Genetic Characterization of Different Tomato Varieties on the Basis of *atpB* Gene Promoter

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Abstract

Several chloroplast genes have been studied so far, among them is single copy large *atpB* gene, which encodes β -subunit of ATP synthase. ATP synthase is present in membranes of mitochondria, chloroplast and prokaryotes having primary function of ATP synthesis. With the aim to gain discernment into the functions and regulation, promoter of *atpB* gene (~1000bp) from five selected tomato varieties (Moneymaker, Nagina, Punjab chuara, Riogrande and Roma) was amplified, sequenced and analyzed. The sequence analysis was done using BLASTn, MEGA5 and PLACE/Web Signal Scan. Functional significance of each regulatory element was also studied by using already available data. The *atpB* gene promoter sequence data from the varieties has been submitted to GenBank. Succinctly it can be recognized that *atpB* gene promoter sequences from five tomato varieties have shown variation and genetic diversity and ensure a wide range of common and unique *cis*-regulatory elements having variance in their copy number and location. These elements are functionally known to be significant in gene regulation and metabolism. © 2013 Friends Science Publishers

Keywords: Tomato; *atpB* promoter; *Cis*-regulatory elements

Introduction

Tomato (*Solanum lycopersicum* L., formerly called *Lycopersicon esculentum* Miller) is an economically and nutritionally important crop worldwide and is intensively studied model system for genetic studies in plants. Various resources are accessible now for its research, which can lead to uprising in evaluation of tomato biology (Barone *et al.*, 2008). Many studies have been done using different genes to examine its genetic diversity such as RAPD (Carelli *et al.*, 2006), RFLP (Asamizu and Ezura, 2009), AFLP and SSR (Garcia-Martinez *et al.*, 2006; Benor *et al.*, 2008).

Complete sequence analysis of tomato is being under process and many genes have been sequenced so far (Mueller *et al.*, 2009). Chloroplast gene sequences have been widely utilized as genetic markers for plant and algal phylogenetic studies (Clegg, 1993). Among them *rbcl*, *atpB*, *matK* remained in the limelight for many years (Graham and Olmstead, 2000a, b). *atpB* gene of chloroplast has an ample sum of information with conserved evolutionary rate, therefore it is being used by scientists for comparative and phylogenetic studies (Savolainen *et al.*, 2000). At the same time, many databases specific for genomes and several bioinformatics tools have been developed (Jansen *et al.*, 2005). In this context, several phylogenetic issues and problems can be solved using chloroplast genome sequences and genome scale datasets (Goremykin *et al.*, 2005).

In the current study, promoter region of chloroplast *atpB* gene from five different tomato varieties was used to establish genetic diversity among them and to identify, compare and analyze functional importance of its regulatory elements.

Materials and Methods

Plant Material and Genomic DNA Isolation

Young fresh leaves of five different varieties of tomato (*Solanum lycopersicum* L.) namely Moneymaker, Nagina, Punjab chuara, Riogrande and Roma were collected from National Agriculture Research Centre, Pakistan and DNA extraction was done by CTAB method (Richards, 1997).

Primer Designing and Amplification of *atpB* Promoter Region

A pair of primers was designed by using *atpB* gene promoter from chloroplast DNA sequence of *Nicotiana tabacum* available at NCBI GenBank (www.ncbi.nlm.nih.gov) using Primer 3 (version 4.0) (<http://primer3.sourceforge.net/>). The pair of primers might let PCR amplification of ~1000bp. The sequence of primers is; *atpBP* Forward: 5' CCAGAAGTAGTAGGATTGATTCTCA 3' *atpBP* Reverse: 5' TCTTCAGGTGGAAGTCCAGGTT 3' PCR reaction was performed in 25 μ L reaction mixture, which

contained 10 x PCR buffer, 25 mM MgCl₂, 2 mM dNTPs, 25 pM forward and reverse primer, nanopure water, 30-50 ng/μL template DNA and eventually 1.5 U *Taq* polymerase (MBI Fermentas) using PCR MultiGene Thermal Cycler (Labnet). PCR conditions used for amplification were pre-PCR denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 sec, annealing at 61°C for 40 sec, extension at 72°C for 45 sec and final extension at 72°C for 20 min was done. Amplification was confirmed by running it on 1.5 % agarose gel in 0.5 x TAE buffer. Confirmed PCR amplified products were purified using JET quick PCR Product Purification Spin Kit (Genomed) according to standard procedures.

Sequencing of the Amplified Product

Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit by Beckman and Coulter CEQ (8800) was employed for sequencing as per manufacturer's instructions. Sequences obtained were submitted to GenBank for getting accession numbers.

Sequence Analysis

The sequence analysis was completed by means of BLASTn (Altschul *et al.*, 1990), MEGA5 (Tamura *et al.*, 2011) and PLACE/Web Signal Scan (Prestridge, 1991; Higo *et al.*, 1999). The varieties were compared by using the information concerning the presence of regulatory elements in each variety, their copy number and proposed functions of these regulatory elements.

Results and Discussion

Sequences from all studied tomato varieties were submitted to Genbank for getting accession numbers (Table 1).

Phylogenetic Analysis

The sequences revealed by sequencing were matched by using BLASTn (Altschul *et al.*, 1990). Chloroplast *atpB* promoter sequence from Moneymaker showed 98 % identity with the chloroplast genome of *S. lycopersicum* (Accession No. AM087200.3), while Nagina showed 100% identity with *Nicotiana forgetiana* plastid *rbcL* gene intron (AM286780.1). Chloroplast *atpB* promoter sequences from Punjab chuara, Riogrande and Roma showed 100 %, 93% and 82% identity with the *N. undulata* chloroplast genome (JN563929.1), tobacco chloroplast *atpase* gene (b and e) and flanks (K00507.1) and *N. sylvestris* chloroplast DNA (AB237912.1), respectively.

Based on sequenced data, phylogenetic relationship among five tomato varieties was also evaluated. A phylogenetic tree was deduced and genetic diversity was investigated by using Neighbor Joining distance data method (Saitou and Nei, 1987). The bootstrap consensus tree (Felsenstein, 1985) was inferred from 500 replicates and the percentage of replicate trees in which the associated

taxa clustered together are shown next to the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. The phenogram revealed two clusters denoted by cluster 1 and cluster 2 (Fig. 1). Overall genetic distance and nucleotide substitution rate of 0.05 indicating close genetic relationship and similarity among them. Moneymaker and Riogrande being the members of cluster 1 and Punjab chuara and Roma reside in cluster 2 indicating that they are genetically more closely related both having bootstrap value 100. However, Nagina was found to show genetic deviation and placed separately (Fig. 1).

Pairwise distance that is estimate of evolutionary divergence between sequences was also assessed (Table 2). The analysis exhibited evolutionary divergence values ranging from 0.251-0.425 for *atpB* gene promoter with mean value of 0.369. As a whole, the values indicated that sequences are genetically similar with less divergence.

Identification of Cis-acting Elements

With the help of PLACE database using Web Signal Scan (Prestridge, 1991; Higo *et al.*, 1999), the putative *cis*-regulatory elements existing in the *atpB* gene promoter sequence of each tomato variety were traced. Maps of regulatory elements were constructed manually for both the positive and negative strands of *atpB* promoter independently for each tomato variety (Figs. 2-6).

Comparative Analysis of Regulatory Elements among Studied Tomato Varieties Based on *atpB* Promoter Sequences

Based on data obtained, common regulatory elements (found in *atpB* gene promoter from all the varieties)

Table 1: Accession numbers of the sequences of chloroplast *atpB* gene promoter from five different tomato varieties

Tomato Variety	Accession No.
<i>Solanum lycopersicum</i> var. <i>moneymaker</i>	JQ657819
<i>Solanum lycopersicum</i> var. <i>nagina</i>	JQ657820
<i>Solanum lycopersicum</i> var. <i>Punjab chuara</i>	JQ657821
<i>Solanum lycopersicum</i> var. <i>riogrande</i>	JQ657822
<i>Solanum lycopersicum</i> var. <i>roma</i>	JQ657823

Table 2: Sequence analysis commencing *atpB* gene promoter from selected tomato varieties on the basis of pairwise distance calculation

Tomato Variety	1	2	3	4	5
Moneymaker	1.000				
Nagina	0.351	1.000			
Punjab chuara	0.403	0.407	1.000		
Riogrande	0.251	0.410	0.425	1.000	
Roma	0.356	0.412	0.319	0.356	1.000

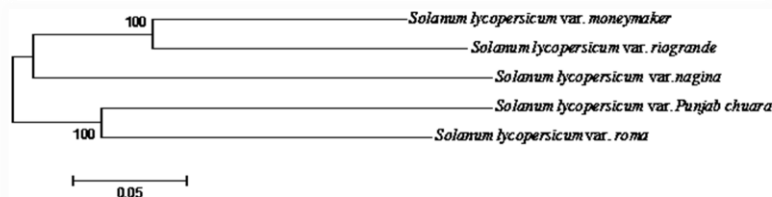


Fig. 1: Phenogram deduced commencing *atpB* gene promoter from the selected tomato varieties presenting genetic divergence among them along with evolutionary distance (0.05) and bootstrap values

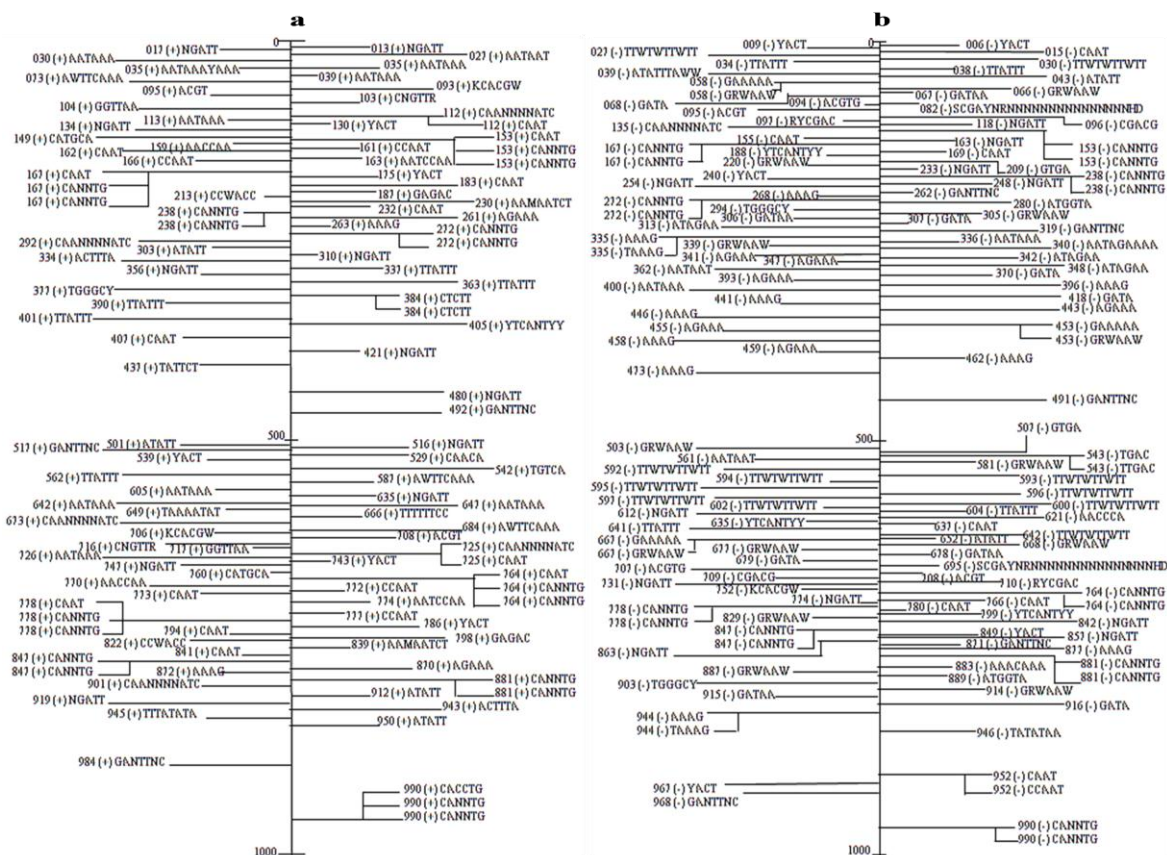


Fig. 2: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Moneymaker

(Table 3) and unique regulatory elements (found in one variety, but absent in rest of the tomato varieties) (Table 4) were identified. Copy number and functions (already reported) of each element were also checked as well (Table 3-4).

Common Regulatory Elements

Thirty three common *cis*-regulatory elements among *atpB* gene promoters from selected tomato varieties having varying numbers of copies were identified from both the strands by PLACE database. These regulatory elements were found to play significant role in a variety of metabolic

course of actions. The identified common elements includes regulatory elements involved in pollen maturation, light, CO₂ and stress responsiveness, elements linked with transcription and mRNA poly adenylation, elements associated with phenylpropanoid biosynthesis, nitrogen fixation and non-symbiotic hemoglobin related elements, root and guard cell specific regulatory elements. Moreover, regulatory elements involved in plant pathogen defense signaling, assimilation of nitrogen, elements associated with seed development, flowering and circadian rhythms were also found. Varying number of these elements has been identified in the studied tomato *atpB* promoter region (Table 3).

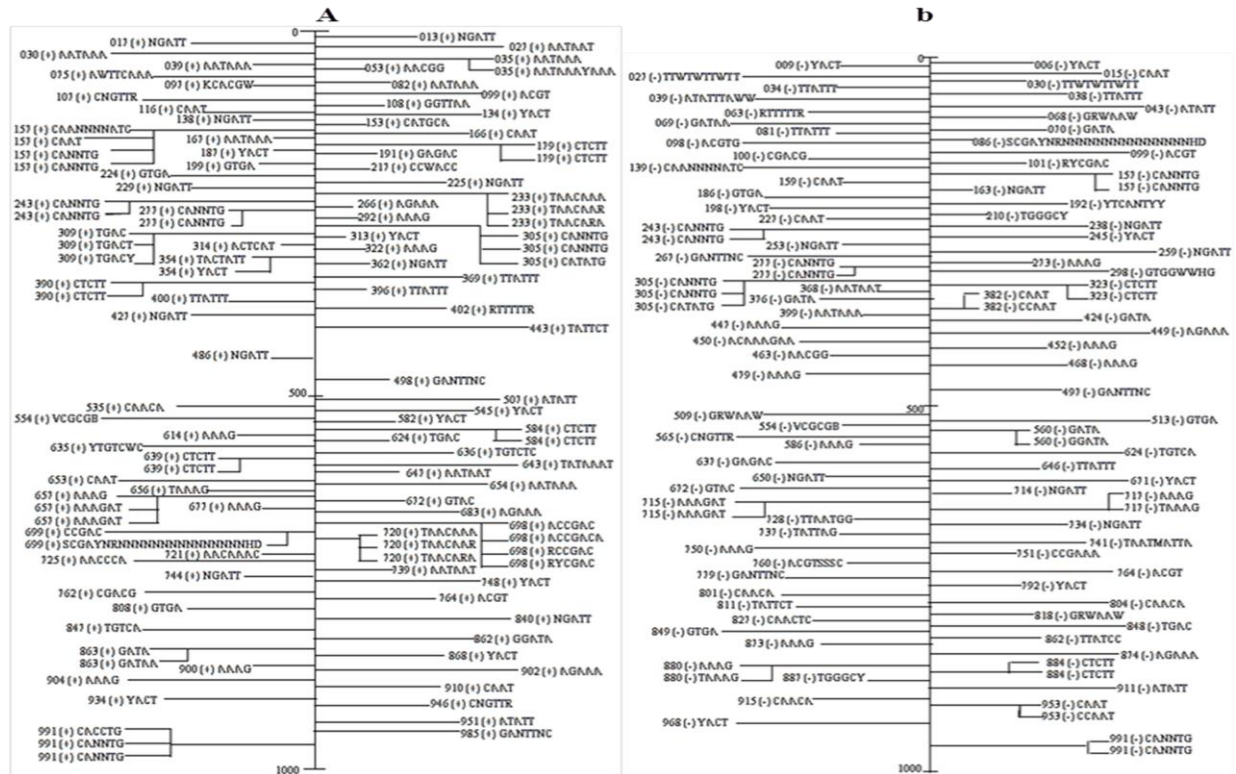


Fig. 3: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Nagina

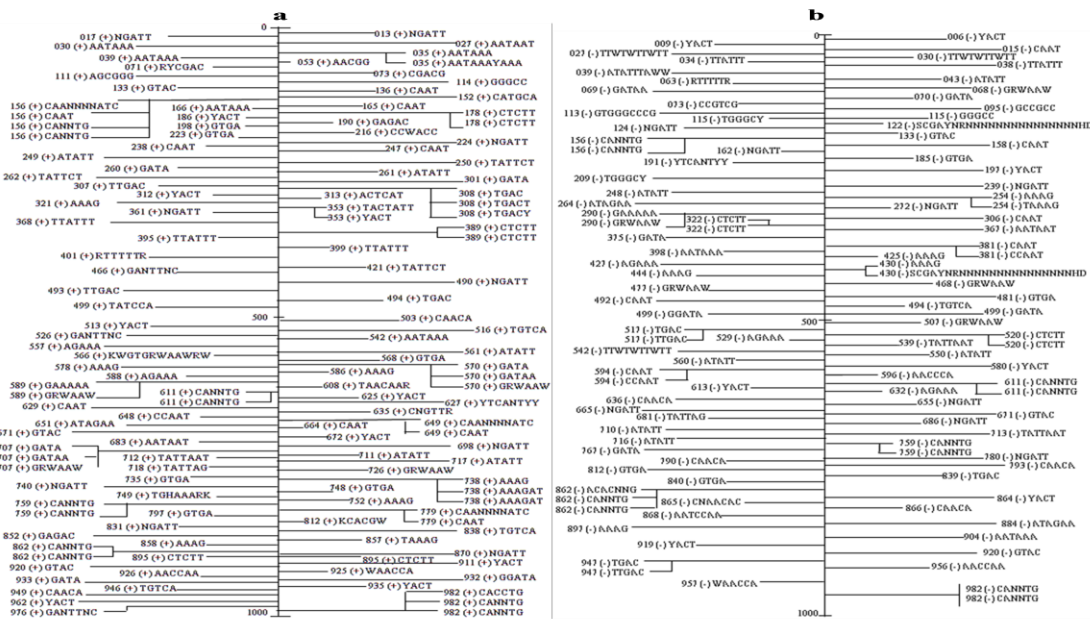


Figure 4: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Punjab chuara.

Fig. 4: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Punjab chura

Table 3: Comparative analysis of common regulatory elements identified in the sequence of *atpB* gene promoter from selected tomato varieties (S1: Moneymaker, S2: Nagina, S3: Punjab chuara, S4: Riogrande, S5: Roma)

Signal Sequence	Factor's Name	Copy Number					Functions
		S1	S2	S3	S4	S5	
TATTCT	-10PEHVPBDB	1	2	3	2	1	Involved in the expression of the plastid <i>psbD</i> which encodes chlorophyll-binding protein of photosystem II (Thum <i>et al.</i> , 2001).
NGATT	ARR1AT	22	17	17	17	21	NGATT is an <i>ARR1</i> -binding element, a response regulator (Sakai <i>et al.</i> , 2000).
TGTCA	BIHDIOS	1	2	4	1	2	TGTCA motif is the DNA sequence of the homeodomain transcriptional factors (Luo <i>et al.</i> , 2005).
CAAT	CAATBOX1	20	10	15	21	18	Promoter consensus sequence was found in <i>legA</i> seed storage protein (Shirsat <i>et al.</i> , 1989).
YACT	CACTFTPPCA1	10	16	16	15	16	Regulatory element engaged in C4 photosynthesis (Gowik <i>et al.</i> , 2004).
RYCGAC	CBFHV	2	2	1	1	1	Involved in regulation of cold responsive genes (Xue, 2002).
CCAAT	CCAATBOX1	5	2	3	5	3	Found in the promoter of heat shock protein genes and also regulate flowering of <i>Arabidopsis</i> (Wenkel <i>et al.</i> , 2006).
CAANNNNATC	CIACADIANLELHC	6	2	3	6	4	Essential for circadian expression of tomato LHC Gene (Piechulla <i>et al.</i> , 1998).
AAAG	DOFCOREZM	12	18	11	8	17	A core site required for binding of <i>Dof</i> proteins in maize (Yanagisawa, 2000).
CANNTG	EBOXBNNAPE	18	10	10	14	8	Consensus binding site of RRE important in flavonoid production along with other elements (Hartmann <i>et al.</i> , 2005).
GATA	GATABOX	6	5	9	11	2	Implicated in light-dependent and nitrate-dependent control of transcription (Reyes <i>et al.</i> , 2004).
GRWAWW	GT1CONCENSUS	14	3	9	8	11	Consensus GT-1 binding site in many light-regulated genes (Zhou, 1999).
GANTTC	EECCRAHI	8	5	3	6	9	Involved in CO ₂ -responsive transcriptional activation of <i>Cah1</i> coding a periplasmic carbonic anhydrase in <i>Chlamydomonas reinhardtii</i> (Kuchro <i>et al.</i> , 2003).
GTGA	GTGANTG10	2	6	10	3	7	Promoter of the tobacco late pollen gene <i>g10</i> (Rogers <i>et al.</i> , 2001).
GATAA	IBOXCORE	4	2	3	4	1	Involved in light regulated transcription (Terzaghi and Cashmore, 1995).
YTCANTYY	INRNTPSADB	4	1	2	2	5	Light-responsive transcription of <i>psaD</i> depends on these (Nakamura <i>et al.</i> , 2002).
AATAAAYAAA	MARABOX1	1	1	1	2	2	A-box found in scaffold attachment region (SAR), or matrix attachment region (MAR) (Gasser <i>et al.</i> , 1989).
TTWTWTTWTT	MARTBOX	11	2	3	3	6	A-box found in scaffold attachment region (SAR), or matrix attachment region (MAR) (Gasser <i>et al.</i> , 1989).
CANNTG	MYCCONSENSUSAT	18	10	10	14	8	Regulates the transcription of CBF/DREB1 genes in the cold in <i>Arabidopsis</i> (Chinnusamy <i>et al.</i> , 2004).
CTCTT	NODCON2GM	1	6	5	4	1	Controlling the root nodule-specific soybean leghemoglobin <i>lbc3</i> gene (Stougaard <i>et al.</i> , 1990).
CTCTT	OSE2ROOTNODULE	2	5	3	2	2	Regulatory elements involved in nitrogen fixation (Vieweg <i>et al.</i> , 2004).
AATAAA	POLASIG1	10	7	7	10	11	Poly A signal is found in <i>legA</i> gene of pea (Joshi, 1987).
AATAAT	POLASIG3	3	4	3	2	2	Consensus sequence for plant poly adenylation signal (Joshi, 1987).
AGAAA	POLLEN1LELAT52	8	5	5	5	7	Required for pollen specific expression (Filichkin <i>et al.</i> , 2004).
SCGAYNRNNNNN	PRECONSCRHSP70A	2	2	2	2	1	Involved in induction of <i>HSP70A</i> gene by both MgProto and light (Von Gromoff <i>et al.</i> , 2006).
NNNNNNNNNNH	CAACA	1	4	6	2	4	CAACA is a binding consensus sequence of <i>Arabidopsis</i> transcription factor, RAV1 (Kagaya <i>et al.</i> , 1999).
CACCTG	RAV1BAT	1	1	1	1	1	CACCTG is a binding sequence of <i>Arabidopsis</i> transcription factor, RAV1 (Kagaya <i>et al.</i> , 1999).
ATATT	ROOTMOTIFTAPOX1	6	4	11	7	3	A root motif is found in both promoters of <i>rolD</i> of <i>Agrobacterium</i> rhizogenes (Elmayan and Tepfer, 1995).
ATATTTAWW	SEF1MOTIF	1	1	1	1	1	Involve in embryo development (Lessard <i>et al.</i> , 1991).
TGGGCY	SITEIATCYTC	3	2	2	2	1	Over represented in the promoters of nuclear genes involved in oxidative phosphorylation (Welchen and Gonzalez, 2006).
TAAAG	TAAAGSTKST1	2	3	2	2	5	Guard cell specific regulatory element (Plesch <i>et al.</i> , 2001).
TTATTT	TATABOX5	10	7	5	9	6	Involved in assimilation of nitrogen (Edwards and Coruzzi, 1989).
TGAC	WRKY7IOS	1	3	5	3	7	W-box promoter elements to which specific proteins bind e.g. the WRKY proteins (Xie <i>et al.</i> , 2005).

Unique *cis*-regulatory Elements

The *cis*-regulatory elements identified by PLACE database have shown the presence of unique elements in the promoter region of chloroplast *atpB* gene of the studied tomato varieties (Table 4). However, the copy number of these unique elements varies and was found to be diverse. Among the five varieties, promoter of *atpB* gene from Nagina has shown largest number (15) of these unique elements. On the contrary, in promoter of *atpB* gene from Moneymaker and Riogrande least number (3 each) of the unique elements have been found. Six unique elements were identified in

each *atpB* gene promoter sequences from Punjab chuara and Roma. On the whole thirty three unique elements were found in the present study. These might play similar functions as presented by earlier studies; however their role *in vivo* needs to be checked before making any final conclusion.

Conclusion

Genetic characterization of five selected tomato varieties has been done on the basis of chloroplast *atpB* promoter region. Phylogenetic analysis was done to find genetic

Table 4: Comparative analysis of unique regulatory elements identified in the sequence of *atpB* gene promoter from selected tomato varieties (S1: Moneymaker, S2: Nagina, S3: Punjab chuara, S4: Riogrande, S5: Roma)

Signal Sequence	Factor's Name	Copy Number					Functions
		S1	S2	S3	S4	S5	
AAACAAA TTTTTCC	ANAERO1CONSENSUS PYRIMIDINEBOXHVEPB1	1	-	-	-	-	Involved in the fermentative pathway (Mohanty <i>et al.</i> , 2005). Responsible for the degradation of seed endosperm storage proteins (Cercos <i>et al.</i> , 1999).
AATAGAAAA AACAAAC	SURE1STPAT21 AACACOREOSGLUB1	1	-	-	-	-	Involved in metabolic regulation of potato storage gene (Grierson <i>et al.</i> , 1994). Involved in controlling the endosperm-specific expression (Wu <i>et al.</i> , 2000).
ACGTSSSC TGCTCTC	ABREOSRAB21 ARFAT	-	1	-	-	-	Involve in regulation of abscisic acid-induced transcription (Busk <i>et al.</i> , 1997). Involved in auxin regulated expression (Goda <i>et al.</i> , 2004).
CAACTC	CAREOSREPI	-	1	-	-	-	Cis-acting elements essential and adequate for gibberellin-upregulated proteinase expression in rice seeds (Sutoh and Yamauchi, 2003).
CATATG VCGCGB	CATATGGMASUR CGCGBXAT	-	2	-	-	-	Involved in auxin responsiveness (Xu <i>et al.</i> , 1997). Involved in ethylene signaling, abscisic acid signaling, and light signal perception (Yang and Poovaiah, 2002).
ACCGAC RCCGAC	DRE2COREZMRAB17 DRECRTCOREAT	-	1	-	-	-	Stress response related cis-regulatory element (Dubouzet <i>et al.</i> , 2003). Core motif of DRE/CRT cis-acting element, identified in many genes in <i>Arabidopsis</i> (Suzuki <i>et al.</i> , 2005).
TAATMATTA	HD2IP2ATATHB2	-	1	-	-	-	Through morphological changes during shade avoidance responses (Ohgishi <i>et al.</i> , 2001).
ACCGACA CCGAC	LTREATLTI78 LTRECOREATCOR15	-	1	-	-	-	Show different roles in the low temperature stress response (Nordin <i>et al.</i> , 1993). Involve in cold induction of <i>BN115</i> gene from winter <i>Brassica napus</i> LTRE (Jiang <i>et al.</i> , 1996).
YTGTWCW TTATCC	SEBFCONSSTPR10A SREATMSD	-	1	-	-	-	Defense-specific regulatory element (Boyle and Brisson, 2001). Control gene expression during origination of axillary bud development in <i>Arabidopsis</i> (Tatematsu <i>et al.</i> , 2005).
GTGGWWHG TTAATGG	SV40COREENHAN WUSATAG	-	1	-	-	-	SV40 core enhancer affecting transcription (Weiher <i>et al.</i> , 1983). Target sequence of WUS in the intron of AGAMOUS gene (Kamiya <i>et al.</i> , 2003).
AGCGGG	BS1EGCCR	-	-	1	-	-	Needed for vascular expression of the cinnamoyl CoA reductase gene and for protein-DNA complex development (Lacombe <i>et al.</i> , 2000).
GTGGGCCCCG GCCGCC	GCBP2ZMGAPC4 GCCCORE	-	-	1	-	-	Required for heterologous anaerobic gene expression (Geffer <i>et al.</i> , 2000). Found in many pathogen-responsive genes and has been shown to play role as ethylene-responsive element (Chakravarthy <i>et al.</i> , 2003).
KWGTGRWAAWRW	GT1MOTIFPSRBCS	-	-	1	-	-	Required for light-dependent transcriptional activation of the <i>rbcs-3A</i> gene (Villain <i>et al.</i> , 1996).
CCGTGC TATCCA	HEXAMERATH4 TATCCAOSAMY	-	-	1	-	-	Motif of <i>Arabidopsis thaliana</i> histone H4 promoter (Chaubet <i>et al.</i> , 1996). Mediate sugar and hormone regulation of alpha-amylase gene expression during cereal grain germination (Chen <i>et al.</i> , 2006).
GATAAG TAAGTG	IBOX MYB2AT	-	-	-	1	-	Binding site of <i>LeMYB1</i> that act as a transcriptional activator (Rose <i>et al.</i> , 1999). Binding site for <i>ATMYB2</i> that is involved in regulation of genes that are responsive to water stress (Urao <i>et al.</i> , 1993).
YAACKG	MYB2CONSENSUSAT	-	-	-	2	-	MYB recognition site found in the promoters of the dehydration-responsive genes (Abe <i>et al.</i> , 2003).
TTGACC	ELRECOREPCRPI	-	-	-	-	1	Consensus sequence of elements W1 and W2 of parsley PR1-1 and PR1-2 promoters, required for elicitor responsiveness (Rushton <i>et al.</i> , 1996).
GTTAGGTT	MYB26PS	-	-	-	-	1	Cis-elements in the promoter regions of several phenylpropanoid biosynthetic genes (Uimari and Strommer, 1997).
MACCWAMC	MYBPLANT	-	-	-	-	1	Consensus sequence related to box P in promoters of phenylpropanoid biosynthetic genes (Tamagnone <i>et al.</i> , 1998).
CCTTTT	PYRIMIDINEBOXOSRAMY1A	-	-	-	-	1	Involved in sugar repression, found in promoter of alpha-amylase gene (Morita <i>et al.</i> , 1998).
ATGGTATT	S1FSORPL21	-	-	-	-	1	May play a role in down regulating RPL21 promoter activity (Lagrange <i>et al.</i> , 1993).
CTGACY	WBOXNTCHN48	-	-	-	-	1	Involved in elicitor-responsive transcription of defense genes in tobacco (Yamamoto <i>et al.</i> , 2004).

diversity among these selected varieties. In a nutshell it can be deduced that the promoter region of chloroplast *atpB* gene, in the selected varieties of tomato (*Solanum lycopersicum* L.) have shown divergence and distinct variations in the *cis*-regulatory elements that proved (from already reported studies) to have vital impact on crop yield as they are involved in the regulation of thousands of genes including metabolic and signaling pathways to combat environmental stresses. However, simply the presence of such

sequences cannot guarantee their role in respective gene regulation and this data can be checked *in vivo* for functional analysis before making final conclusion.

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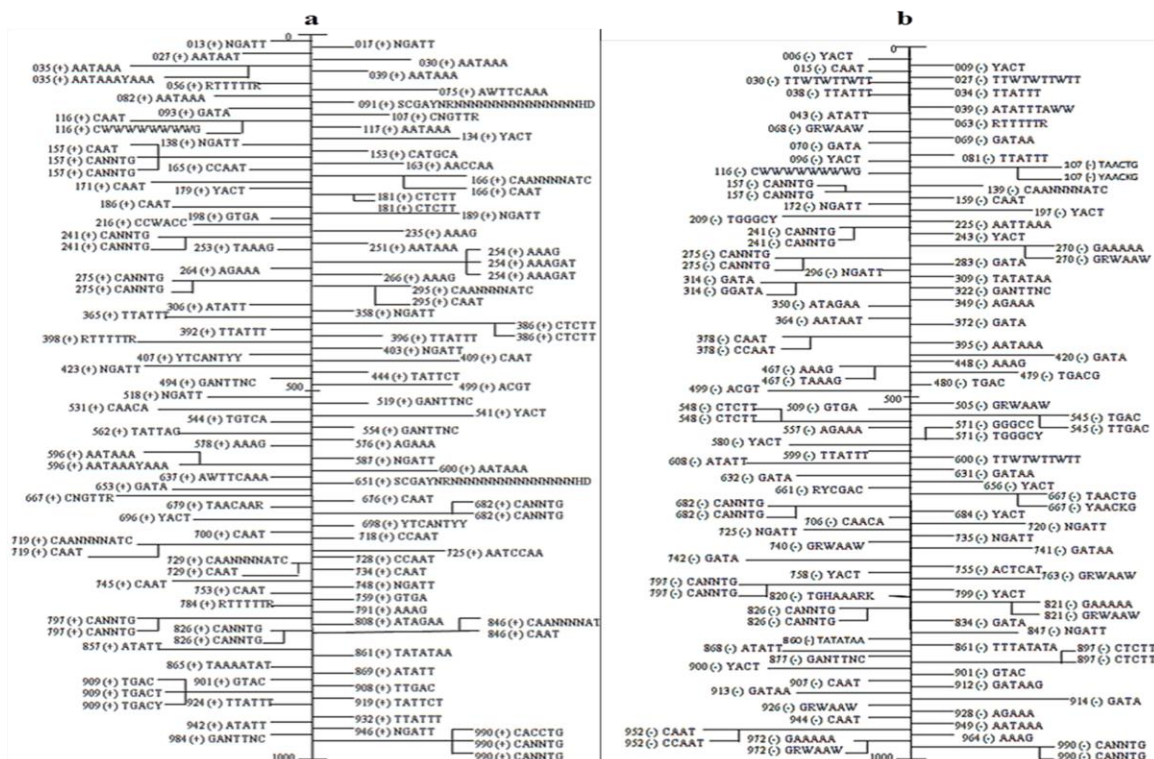


Fig. 5: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Riogrande

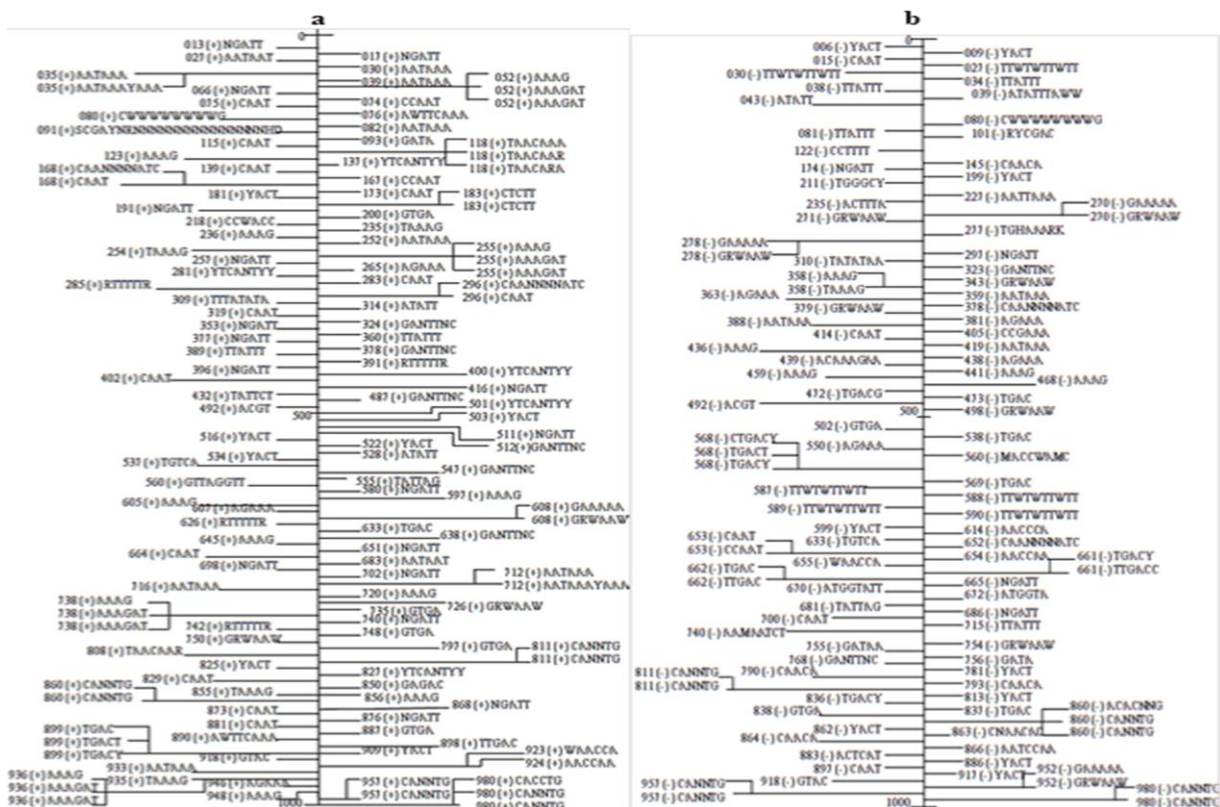


Fig. 6: Mapping of *cis*-regulatory elements identified in the positive (a) and negative (b) strands of chloroplast *atpB* gene promoter from Roma

References

- Abe, H., T. Urao, T. Ito, M. Seki, K. Shinozaki and K. Yamaguchi-Shinozaki, 2003. *Arabidopsis*AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell*, 15: 63–78
- Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman, 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215: 403–410
- Asamizu, E. and H. Ezura, 2009. Inclusion of tomato in the genus *Solanum* as "*Solanum lycopersicum*" is evident from phylogenetic studies. *J. Jpn. Soc. Hortic. Sci.*, 78: 3–5
- Barone, A., M.L. Chiusano, M.R. Ercolano, G. Giuliano, S. Grandillo and L. Frusciante, 2008. Structural and functional genomics of tomato. *Int. J. Plant Genomics*, Article ID 820274, 12p
- Benor, S., M.Y. Zhang, Z.F. Wang and H.S. Zhang, 2008. Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *J. Genet. Genomics*, 35: 373–379
- Boyle, B. and N. Brisson, 2001. Repression of the defense gene PR-10a by the single-stranded DNA binding protein SEBF. *Plant Cell*, 13: 2525–2537
- Busk, P.K., A.B. Jensen and M. Pages, 1997. Regulatory elements in vivo in the promoter of the abscisic acid responsive gene rab17 from maize. *Plant J.*, 11: 1285–1295
- Carelli, B.P., L.T.S. Gerald, F.G. Grazziotin and S. Echeverrigaray, 2006. Genetic diversity among Brazilian cultivars and landraces of tomato *Lycopersicon esculentum* Mill. revealed by RAPD markers. *Genet. Resour. Crop. Ev.*, 53: 395–400
- Cercos, M., A. Gomez-Cadenas and T.H.D. Ho, 1999. Hormonal regulation of a cysteine proteinase gene, EPB-1, in barley aleurone layers: cis and trans- acting elements involved in the co-ordinated gene expression regulated by gibberellins and abscisic acid. *Plant J.*, 19: 107–118
- Chakravarthy, S., R.P. Tuori, M.D. Dascenzo, P.R. Fobert, C. Despres and G.B. Martin, 2003. The tomato transcription factor Pit4 regulates defence-related gene expression via GCC box and non-GCC box cis-elements. *Plant Cell*, 15: 3033–3050
- Chaubet, N., M. Flenet, B. Clement, P. Brignon and C. Gigot, 1996. Identification of cis-elements regulating the expression of an *Arabidopsis* histone H4 gene. *Plant J.*, 10: 425–435
- Chen, P.W., C.M. Chiang, T.H. Tseng and S.M. Yu, 2006. Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of alpha-amylase genes. *Plant Cell*, 18: 2326–2340
- Chinnusamy, V., K. Schumaker and J.K. Zhu, 2004. Molecular genetics perspectives on cross-talk and specificity in abiotic stress signalling in plants. *J. Exp. Bot.*, 55: 225–236
- Clegg, M.T., 1993. Chloroplast gene sequences and the study of plant evolution. *Proc. National Academy of Sciences of the United States of Amer.*, 90: 363–367
- Dubouzet, J.G., Y. Sakuma, Y. Ito, M. Kasuga, E.G. Dubouzet, S. Miura, M. Seki, K. Shinozaki and K. Yamaguchi-Shinozaki, 2003. OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought, high salt and cold responsive gene expression. *Plant J.*, 33: 751–763
- Edwards, J.W. and G.M. Coruzzi, 1989. Photorespiration and light act in concert to regulate the expression of the nuclear gene for chloroplast glutamine synthetase. *Plant Cell*, 1: 241–248
- Elmayan, T. and M. Tepfer, 1995. Evaluation in tobacco of the organ specificity and strength of the rolD promoter, domain A of the 35S promoter and the 35S2 promoter. *Transgenic Res.*, 4: 388–396
- Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39: 783–791
- Filichkin, S.A., J.M. Leonard, A. Monteros, P.P. Liu and H. Nonogaki, 2004. A novel endo-beta-mannanase gene in tomato LeMAN5 is associated with anther and pollen development. *Plant Physiol.*, 134: 1080–1087
- Garcia-Martinez, S., L. Andreani, M. Garcia-Gusano, F. Geuna and J.J. Ruiz, 2006. Evolution of amplified length polymorphism and simple sequence repeats for tomato germplasm fingerprinting: Utility for grouping closely related traditional cultivars. *Genome*, 49: 648–656
- Gasser, S.M., B.B. Amati, M.E. Cardenas and J.F.X. Hofmann, 1989. Studies on scaffold attachment sites and their relation to genome function. *Int. Rev. Cytol.*, 119: 57–96
- Geffers, R., R. Cerff and R. Hehl, 2000. Anaerobiosis-specific interaction of tobacco nuclear factors with cis-regulatory sequences in the maize GapC4 promoter. *Plant Mol. Biol.*, 43: 11–21
- Goda, H., S. Sawa, T. Asami, S. Fujioka, Y. Shimada and S. Yoshida, 2004. Comprehensive comparison of auxin-regulated and brassinosteroid regulated genes in *Arabidopsis*. *Plant Physiol.*, 134: 1555–1573
- Goremykin, V.V., B. Holland, K.I. Hirsch-Ernst and F.H. Hellwig, 2005. Analysis of *Acorus calamus* chloroplast genome and its phylogenetic implications. *Mol. Biol. Evol.*, 22: 1813–1822
- Gowik, U., J. Burscheidt, M. Akyildiz, U. Schlue, M. Koczor, M. Streubel and P. Westhoff, 2004. Cis-Regulatory elements for mesophyll-specific gene expression in the C4 plant *Flaveria trinervia*, the promoter of the C4 phosphoenol pyruvate carboxylase gene. *Plant Cell*, 16: 1077–1090
- Graham, S.W. and R.G. Olmstead, 2000a. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *Amer. J. Bot.*, 87: 1712–1730
- Graham, S.W. and R.G. Olmstead, 2000b. Evolutionary significance of an unusual chloroplast DNA inversion found in two basal angiosperm lineages. *Curr. Genet.*, 37: 183–188
- Grierson, C., J.S. Du, M.T. Zabala, K. Beggs, C. Smith, M. Holdsworth and M. Bevan, 1994. Separate cis sequences and trans factors direct metabolic and developmental regulation of a potato tuber storage protein gene. *Plant J.*, 5: 815–826
- Hartmann, U., M. Sagasser, F. Mehrtens, R. Stracke and B. Weisshaar, 2005. Differential combinatorial interactions of cis-acting elements recognized by R2R3-MYB, BZIP and BHLH factors control light-responsive and tissue-specific activation of phenylpropanoid biosynthesis genes. *Plant Mol. Biol.*, 57: 155–171
- Higo, K., Y. Ugawa, M. Iwamoto and T. Korenaga, 1999. Plant cis-acting regulatory DNA elements (PLACE) database. *Nucleic Acids Res.*, 27: 297–300
- Jansen, R.K., L.A. Raubeson, J.L. Boore, C.W. De Pamphilis, T.W. Chumley, R.C. Haberle, S.K. Wyman, A.J. Alverson, R. Peery and S.J. Herman, 2005. Methods for obtaining and analyzing whole chloroplast genome sequences. *Methods Enzymol.*, 395: 348–384
- Jiang, C., B. Lu and J. Singh, 1996. Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from winter *Brassica napus*. *Plant Mol. Biol.*, 30: 679–684
- Joshi, C.P., 1987. Putative polyadenylation signals in nuclear genes of higher plants: A compilation and analysis. *Nucleic Acids Res.*, 15: 9627–9640
- Kagaya, Y., K. Ohmiya and T. Hattori, 1999. RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Res.*, 27: 470–478
- Kamiya, N., H. Nagasaki, A. Morikami, Y. Sato and M. Matsuoka, 2003. Isolation and characterization of a rice WUSCHEL-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. *Plant J.*, 35: 429–441
- Kucho, K., S. Yoshioka, F. Taniguchi, K. Ohyama and H. Fukuzawa, 2003. Cis-acting elements and DNA-binding proteins involved in CO₂-responsive transcriptional activation of Cah1 encoding a periplasmic carbonic anhydrase in *Chlamydomonas reinhardtii*. *Plant Physiol.*, 133: 783–793
- Lacombe, E., J. Van Doorselaere, W. Boerjan, A.M. Boudet and J. Grima-Pettenati, 2000. Characterization of cis-elements required for vascular expression of the cinnamoyl CoA reductase gene and for protein-DNA complex formation. *Plant J.*, 23: 663–676
- Lagrange, T., B. Franzetti, M. Axelos, R. Mache and S. Lerbs-Mache, 1993. Structure and expression of the nuclear gene encoding for the chloroplast ribosomal protein L21: Developmental regulation of a house-keeping gene by alternative promoters. *Mol. Cell Biol.*, 13: 2614–2622
- Lessard, P.A., R.D. Allen, F. Bernier, J.D. Crispino, T. Fujiwara and R.N. Beachy, 1991. Multiple nuclear factors interact with upstream sequences of differentially regulated beta-conglycinin genes. *Plant Mol. Biol.*, 16: 397–413

- Luo, H., F. Song, R.M. Goodman and Z. Zheng, 2005. Up-regulation of *OsBIHD1*, a rice gene encoding BELL homeodomain transcriptional factor, in disease resistance responses. *Plant Biol.*, 7: 459–468
- Mohanty, B., S.P. Krishnan, S. Swarup and V.B. Bajic, 2005. Detection and preliminary analysis of motifs in promoters of anaerobically induced genes of different plant species. *Ann. Bot.*, 96: 669–681
- Morita, A., T. Umemura, M. Kuroyanagi, Y. Futsuhara, P. Perata and J. Yamaguchi, 1998. Functional dissection of a sugar-repressed alpha-amylase gene (*RamylA*) promoter in rice embryos. *FEBS Lett.*, 423: 81–85
- Mueller, L.A., L.R. Klein, S.D. Tanksley, J.J. Giovannoni, R. White, J. Vrebalov, Z. Fei, J. van Eck, R. Buels, A.A. Mills, 2009. A snapshot of the emerging tomato genome sequence. *Plant Genome*, 2: 78–92
- Mueller, L.A., S.D. Tanksley, J.J. Giovannoni, J. Van Eck, S. Stack, D. Choi, B.D. Kim, M. Chen, Z. Cheng and C. Li, 2005b. The tomato sequencing project, the first cornerstone of the International Solanaceae Project (SOL). *Comp. Funct. Genomics*, 6: 153–158
- Mueller, L.A., T.H. Solow, N. Taylor, B. Skwarecki, R. Buels, J. Binns, C. Lin, M.H. Wright, R. Ahrens and Y. Wang, 2005a. The SOL genomics network. A comparative resource for Solanaceae biology and beyond. *Plant Physiol.*, 138: 1310–1317
- Nakamura, M., T. Tsunoda and J. Obokata, 2002. Photosynthesis nuclear genes generally lack TATA-boxes: A tobacco photosystem I gene responds to light through an initiator. *Plant J.*, 29: 1–10
- Nei, M. and S. Kumar, 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York
- Nordin, K., T. Vahala and E.T. Palva, 1993. Differential expression of two related, low-temperature-induced genes in *Arabidopsis thaliana* (L.) Heynh. *Plant Mol. Biol.*, 21: 641–653
- Ohgishi, M., A. Oka, G. Morelli, I. Ruberti and T. Aoyama, 2001. Negative auto regulation of the *Arabidopsis* homeobox gene *ATHB-2*. *Plant J.*, 25: 389–398
- Piechulla, B., N. Merforth and B. Rudolph, 1998. Identification of tomato *Lhc* promoter regions necessary for circadian expression. *Plant Mol. Biol.*, 38: 655–662
- Plesch, G., T. Ehrhardt and B. Mueller-Roeber, 2001. Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. *Plant J.*, 28: 455–464
- Prestridge, D.S., 1991. SIGNAL SCAN: A computer program that scans DNA sequences for eukaryotic transcriptional elements. *Comput. Appl. Biosci.*, 7: 203–206
- Reyes, J.C., M.I. Muro-Pastor and F.J. Florencio, 2004. The GATA family of transcription factors in *Arabidopsis* and rice. *Plant Physiol.*, 134: 1718–1732
- Richards, E.J., 1997. Preparation of plant DNA using CTAB. In: *Short Protocol in Molecular Biology*, Vol. 2, pp: 10–11. Ausubel, F.B., R.E. Kingston, D.D. Moore, J.G. Siedman, J.A. Smith and K. Struhl (eds.). Wiley Biology
- Rogers, H.J., N. Bate, J. Combe, J. Sullivan, J. Sweetman, C. Swan, D.M. Lonsdale and D. Twell, 2001. Functional analysis of cis-regulatory elements within the promoter of the tobacco late pollen gene *g10*. *Plant Mol. Biol.*, 45: 577–585
- Rose, A., I. Meier and U. Wienand, 1999. The tomato I-box binding factor LeMYBI is a member of a novel class of Myb-like proteins. *Plant J.*, 20: 641–652
- Rushton, P.J., J.T. Torres, M. Parniske, P. Wernert, K. Hahlbrock and I.E. Somssich, 1996. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *EMBO J.*, 15: 5690–5700
- Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406–425
- Sakai, H., T. Aoyama and A. Oka, 2000. *Arabidopsis* ARR1 and ARR2 response regulators operate as transcriptional activators. *Plant J.*, 24: 703–711
- Savolainen, V., M.W. Chase, S.B. Hoot, C.M. Morton, D.E. Soltis, C. Bayer, M.F. Fay, A.Y. De Bruijn, S. Sullivan and Y.L. Qui, 2000. Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. *Syst. Biol.*, 49: 306–362
- Shirsat, A., N. Wilford, R. Croy and D. Boulter, 1989. Sequences responsible for the tissue specific promoter activity of a pea legumin gene in tobacco. *Mol. Gen. Genet.*, 215: 326–331
- Stougaard, J., J.E. Jorgensen, T. Christensen, A. Kuhle and K.A. Marcker, 1990. Interdependence and nodule specificity of cis-acting regulatory elements in the soybean leghemoglobin *lbc3* and *N23* gene promoters. *Mol. Gen. Genet.*, 220: 353–360
- Sutoh, K. and D. Yamauchi, 2003. Two cis-acting elements necessary and sufficient for gibberellin-upregulated proteinase expression in rice seeds. *Plant J.*, 34: 636–645
- Suzuki, M., M.G. Ketterling and D.R. McCarty, 2005. Quantitative statistical analysis of cis-regulatory sequences in ABA/VP1- and CBF/DREB1-regulated genes of *Arabidopsis*. *Plant Physiol.*, 139: 437–447
- Tamagnone, L., A. Merida, A. Parr, S. Mackay, F.A. Culianez-Macia, K. Roberts and C. Martin, 1998. The AmMYB308 and AmMYB330 transcription factors from antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. *Plant Cell*, 10: 135–154
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.*, 28: 2731–2739
- Tatematsu, K., S. Ward, O. Leyser, Y. Kamiya and E. Nambara, 2005. Identification of cis-elements that regulate gene expression during initiation of axillary bud outgrowth in *Arabidopsis*. *Plant Physiol.*, 138: 757–766
- Terzaghi, W.B. and A.R. Cashmore, 1995. Light-regulated transcription. *Annu. Rev. Plant Phys.*, 46: 445–474
- Thum, K.E., M. Kim, D.T. Morishige, C. Eibl, H.U. Koop and J.E. Mullet, 2001. Analysis of barley chloroplast *psbD* light-responsive promoter elements in transplastomictobacco. *Plant Mol. Biol.*, 47: 353–366
- Uimari, A. and J. Strommer, 1997. Myb26: a MYB-like protein of pea flowers with affinity for promoters of phenylpropanoid genes. *Plant J.*, 12: 1273–1284
- Urao, T., K. Yamaguchi-Shinozaki, S. Urao and K. Shinozaki, 1993. An *Arabidopsis* MYB homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. *Plant Cell*, 5: 1529–1539
- Vieweg, M.F., M. Fruhling, H.J. Quandt, U. Heim, H. Baumlein, A. Puhler, H. Kuster and M.P. Andreas, 2004. The promoter of the *Vicia faba* L. leghemoglobin gene *Vilb29* is specifically activated in the infected cells of root nodules and in the arbuscule-containing cells of mycorrhizal roots from different legume and non-legume plants. *Mol. Plant Microbe In.*, 17: 62–69
- Villain, P., R. Mache and D.X. Zhou, 1996. The mechanism of GT element-mediated cell type-specific transcriptional control. *J. Biol. Chem.*, 271: 32593–32598
- Von Gromoff, E.D., M. Schroda, U. Oster and C.F. Beck, 2006. Identification of a plastid response element that acts as an enhancer within the *Chlamydomonas* HSP70A promoter. *Nucleic Acids Res.*, 34: 4767–4779
- Weiher, H., M. Konig and P. Gruss, 1983. Multiple point mutations affecting the simian virus 40 enhancer. *Science*, 219: 626–631
- Welchen, E. and D.H. Gonzalez, 2006. Over representation of elements recognized by TCP-Domain transcription factors in the upstream regions of nuclear genes encoding components of the mitochondrial oxidative phosphorylation machinery. *Plant Physiol.*, 141: 540–545
- Wenkel, S., F. Turck, K. Singer, L. Gissot, J. Le Gourrierec, A. Samach and G. Coupland, 2006. CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. *Plant Cell*, 18: 2971–2984
- Wu, C., H. Washida, Y. Onodera, K. Harada and F. Takaiwa, 2000. Quantitative nature of the Prolamin-box, ACGT and AACA motifs in a rice glutelin gene promoter: Minimal cis-element requirements for endosperm-specific gene expression. *Plant J.*, 23: 415–421
- Xie, Z., Z.L. Zhang, X. Zou, J. Huang, P. Ruas, D. Thompson and Q.J. Shen, 2005. Annotations and functional analyses of the rice WRKY gene super family reveal positive and negative regulators of abscisic acid signaling in aleurone cells. *Plant Physiol.*, 137: 176–189
- Xu, N., G. Hagen and T. Guilfoyle, 1997. Multiple auxin response modules in the soybean SAUR 15A promoter. *Plant Sci.*, 126: 193–201

- Xue, G.P., 2002. Characterization of the DNA-binding profile of barley HvCBF1 using an enzymatic method for rapid, quantitative and high-throughput analysis of the DNA-binding activity. *Nucleic Acids Res.*, 30: 77
- Yamamoto, S., T. Nakano, K. Suzuki and H. Shinshi, 2004. Elicitor-induced activation of transcription via W box-related *cis*-acting elements from a basic chitinase gene by WRKY transcription factors in tobacco. *Biochim. Biophys. Acta*, 1679: 279–287
- Yanagisawa, S., 2000. Dof1 and Dof2 transcription factors are associated with expression of multiple genes involved in carbon metabolism in maize. *Plant J.*, 21: 281–288
- Yang, T. and B.W. Poovaiah, 2002. A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants. *J. Biol. Chem.*, 277: 45049–45058
- Zhou, D.X., 1999. Regulatory mechanism of plant gene transcription by GT-elements and GT-factors. *Trends Plant Sci.*, 4: 210–214

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