Exploring Cis-Regulatory Elements for Abiotic and Biotic Stresses in Plants

Sainath K[1]*, Praveen K[3], Akshaya M[2], Eshwar[1], J V Yashwanth[1] and Yashashwini M[1]

[1]*Department of Genetics and Plant Breeding, UAS, Raichur*
[2]*Department of Genetics and Plant Breeding, UAS, Dharwad*
[3]*Department of Agricultural Microbiology, UAS, Bangalore*

Corresponding Author
Sainath K
Email: sainathkarbhari98@gmail.com

OPEN ACCESS

Keywords

Unmethylated, Enhancer, Cis Regulatory Modules, Salicylic Acid, Transcription Initiation Rate

How to cite this article:

Sainath, K., Praveen, K., Akshaya, M., Eshwar., Yashwanth, J. V. and Yashashwini, M. 2025. Exploring Cis-Regulatory Elements for Abiotic and Biotic Stresses in Plants. *Vigyan Varta* 6 (12): 1-6.

ABSTRACT

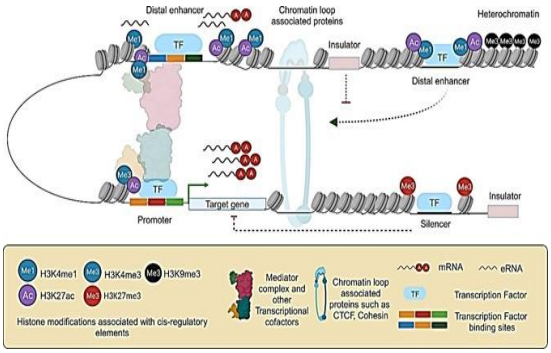
Plants encounter a wide range of abiotic and biotic stresses throughout their life cycle, necessitating precise regulation of gene expression for adaptation and survival. Gene expression is primarily controlled by specific genomic sequences known as cis-regulatory elements (CREs), which serve as binding sites for transcription factors and cis-regulatory modules (CRMs), which are clusters of CREs that include promoters, enhancers, silencers, and insulators. CRM's are stretches of DNA, usually 100-1000 DNA base pairs in length, where a number of transcription factors can bind and regulate expression of nearby genes and regulate their transcription rates. Cis-regulatory elements (CREs) are short DNA motifs in gene promoters that act as molecular switches, controlling stress-responsive gene expression through transcription factor (TF) binding. In abiotic stress, pathways such as ABA-dependent (AREB/ABF) and ABA-independent (DREB/CBF, NAC and MYB/MYC) regulate gene activation, as exemplified by the 116 to 2 bp promoter region of HRE2 in Arabidopsis bound by HAT22/ABIG1. Under biotic stress, CREs coordinate defence via., salicylic acid (SA), masonic acid (JA), and ethylene (ET) signaling, with NPR1 interacting with TGA TFs to activate pathogenesis-related genes and systemic acquired resistance.

INTRODUCTION

The plants are exposed to many abiotic and biotic stresses during the course of life. Gene expression is primarily governed by certain genomic sequences, which are specifically targeted by transcription factors. The process of selective activation of a different subset of genes is key to cell differentiation, survival and functional diversity. The genome wide identification and characterization of cis-regulatory elements (CRE's) and cis regulatory modules (CRM's) that influence the expression of protein-coding and long non-coding RNA (*lnc*RNA) genes is central to the complex pattern of gene expression regulation and how they function to coordinate responses to developmental and environmental cues is of paramount importance to plant biology.

Cis-regulatory elements (CRE's) are the individual transcription factor binding sites which are 6-10 base pair long, while Cis-regulatory modules (CRM's) are the assemblies of CRE's and include promoter, transcriptional enhancers, silencers and insulator elements. These are the regions of non-coding DNA which regulate the transcription of neighboring genes. CRE's are vital components of genetic regulatory networks, which in turn control morphogenesis, the development of anatomy and other aspects of embryonic development, studied in evolutionary developmental biology (Schmitz *et al.,* 2022)

CRE's are found in the vicinity of the genes that they regulate. CRE's typically regulate gene transcription by binding to transcription factors. A single transcription factor may bind to many CRE's, and hence control the expression of many genes (pleiotropy). The Latin prefix 'cis' means "on the side", *i.e.* on the same molecule of DNA as the gene(s) to be transcribed.



The systematic discovery of CRM's and CRE's they are composed of is the first step in the engineering and rewiring of existing regulatory networks to optimize plant growth and development, enhance stress resilience or generate plant products.

CRM'S CHARACTERISTICS

CRM's are stretches of DNA, usually 100-1000 DNA base pairs in length, where a number of transcription factors can bind and regulate expression of nearby genes and regulate their transcription rates. They are labelled as cis because they are typically located on the same DNA strand as the genes they control as opposed to trans, which refers to effects on genes not located on the same strand or farther away, such as transcription factors. CRE's are often but not always upstream of transcription site. CRE's contrast with trans-regulatory elements (TRE's). TRE's code for transcription factors. (**Long *et al.* 2016)**

In plants, the DNA of vast majority of CRM's appears stably unmethylated in a tissue independent manner and these unmethylated regions (UMR's) are enriched in accessible chromatin, histone acetylation (HAc), TF-DNA interactions**.** Much of the rest of the genome is methylated, including subset of genes and transcriptionally silenced transposable elements**.** Therefore, UMRs likely encompass the vast majority of CRM's

within plant genomes, independent of their activity. (Villar *et al.* 2014**)**

CLASSIFICATION OR MAJOR TYPES OF CRM'S

Gene expression in eukaryotes is a multiplex phenomenon, involving the TF's mediated activation and repression of genes in response to various environmental and developmental stimuli (**Oka *et al.* 2017).** Various types of CRE's are as follows,

1) **CORE PROMOTER:** Minimal sequence region that is needed to direct initiation of transcription, usually spanning 50-100 bp around the TSS.

- They are characterized by presence of TATA box, CAAT box which serves as binding sites for RNA polymerase II, accessible chromatin, H3K4me3, HAc, H2A.Z, a lack of DNA methylation, the binding of general transcription factors.

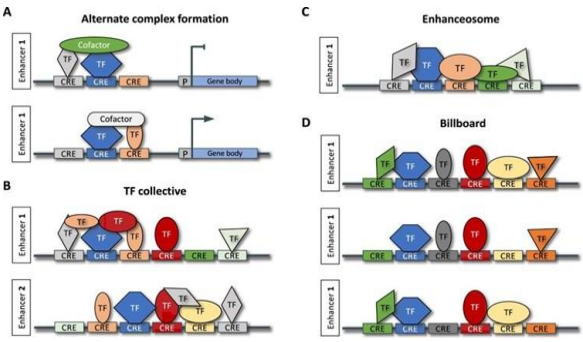
- In plants TATA box is absent and its activity is carried out by CAAT box and TC elements.

2) **ENHANCERS:** These are the DNA sequences that when bound by specific TF's and co- factors, increases the transcription initiation rate and thereby the expression of target genes in tissue-, developmental stage and/or condition specific manner. They can be located more than 1 Mbp away from their target gene, the average distance depends on size of the genome.

- The first distal enhancer discovered in plants was the maize b1 (booster **1)** hepta-repeat enhancer, located ~100-kbp upstream of the TSS of the b1 gene.

- The most distal enhancer so far described in plants is Distal Cis-Element **(DICE),** which is required for high expression of maize bx1 (benzoxazinless 1) gene ~140 kbp away.

Models for enhancer organization;



(a)Alternative complex formation: TF's can function both as transcription activators and repressors, depending on the proteins (co-activators and co-repressors) they interact with.

(b) TF collective model: This model is characterized by co-operative DNA binding, which can be achieved by many different mechanisms, and by proteins as well as DNA serving as a scaffold for the binding of TF's. It allows for flexible CRE arrangements, resulting in distinct regulatory outputs (exemplifiied by the two enhancers shown)

(c)Enhanceosome model: In the enhanceosome model, the binding of the various TFs to the respective CRE's must occur in a specific order and orientation, following a particular grammar.

(d) Billiboard enhancer: In the billiboard model, the composition as well as the position and orientation of CRE's within an enhancer is preserved. The regulatory output differs depending on the expression and activity level of TF's that can bind the CRE's.

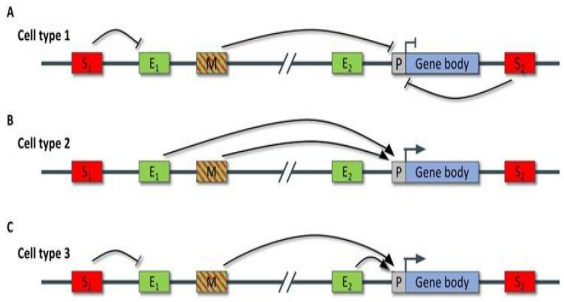
3) **SILENCERS:** A DNA sequence that, when bound by specific TFs and cofactors, actively decrease the expression of target

genes. A silencer might silence a gene directly, or indirectly by silencing enhancers.

4) **INSULATORS:** An element located between CRM's and core promoters that, when bound by the appropriate proteins, prevents the activation or silencing of potential target genes by these CRM's. Such insulators are not known to exist in plants.

5) **MULTIFUNCTIONAL SEQUENCE ELEMENT:** DNA element that exhibits more than one of the above properties at different times or conditions, or in different cells, e.g. enhancers in one cell type can function as silencers in other cell types and vice versa.

Model explaining mechanism of action of CRM's:



A. In cell type 1, Silencer element 1 represses an enhancer, while multifunctional sequence element and silencer element 2 repress promoter activity.

B. In cell type 2, Enhancer element 1 works cooperatively with a multifunctional sequence element to activate gene expression.

C. In cell type 3, silencer element 1 repress the upstream enhancer 1, whereas the multifunctional sequence element activates the gene in concert with the promoter proximal enhancer (enhancer 2).

S; Silencer, E; Enhancer, M; Multifunctional sequence element, P; Promoter.

Role of Cis-Regulatory Elements in Plant Stress Responses

1. Central Role in Stress Regulation

Cis-regulatory elements (CREs) are short DNA sequences located in gene promoters that act as molecular switches, regulating gene expression under various stress conditions. They serve as binding sites for transcription factors (TFs) that activate or repress stress-responsive genes.

2. Response to Abiotic Stresses

Abiotic stresses such as drought, salinity, temperature extremes, and osmotic imbalance trigger signal transduction pathways that lead to the activation of TFs binding to specific cis-elements.

ABA-dependent pathways: Involve AREB/ABF regulons that bind to ABA-responsive elements (ABREs). ABA-independent pathways: Include DREB/CBF, NAC, and MYB/MYC regulons that control stress-related gene expression without ABA signaling.

Example: Seok *et al.,* (2022) identified a 116 to 2 bp promoter region of HRE2 in Arabidopsis as a key hypoxia- and salt stress–responsive region bound by HAT22/ABIG1 (HD-Zip II TF).

3. Response to Biotic Stresses

Under biotic stress (pathogen attack), CREs and TFs coordinate defense gene activation through salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling.

Localized hypersensitive response (HR) and systemic acquired resistance (SAR) are triggered by recognition of pathogen signals.

NPR1 plays a central role: SA accumulation changes cellular redox potential, converting inactive NPR1 oligomers into active monomers that move into the nucleus and interact with TGA-type bZIP TFs.

These TGA factors bind to SA-responsive cis-elements in pathogenesis-related (PR) gene promoters, initiating SAR. (Johnson *et al.* 2003)

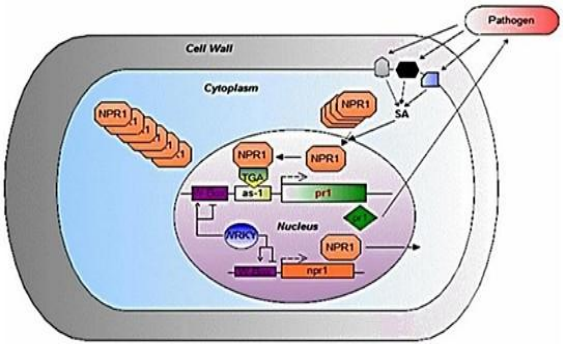
4. Transcription Factors and Regulatory Networks

Multiple TF families act through specific cis-elements to regulate stress-responsive genes:

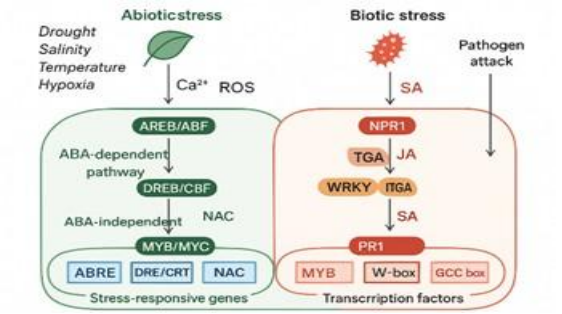
Abiotic stress: DREB/CBF, NAC, MYB, AREB/ABF, and ERF families.

Biotic stress: TGA, WRKY (binding to W-box elements), and ERF proteins such as OsEREBP1 (rice), GmERF5/GmERF113 (soybean), and SIERF (potato).

These TF–CRE interactions create an integrated transcriptional network enabling stress perception, signaling, and adaptation.



Overall view depicting an abiotic and abiotic stress tolerance in plant by different mechanism



CONCLUSION

Cis-regulatory elements (CREs) play a pivotal role in gene expression networks that enable plants to perceive and respond to diverse abiotic and biotic stresses. These short DNA sequences act as molecular switches, integrating environmental and developmental cues through their interaction with transcription factors. Recent advances in genomics, transcriptomics, and computational biology have accelerated the identification and functional annotation of stress-responsive CREs across various plant species. Understanding the complexity and specificity of these regulatory modules provides valuable insights into the adaptive mechanisms of plants under stress conditions.

Integrating CRE analysis with genome editing tools, such as CRISPR/Cas-based promoter engineering, offers promising avenues for developing stress-resilient crops. Future research should focus on validating CRE functions experimentally, exploring their combinatorial interactions, and constructing comprehensive regulatory networks linking CREs, transcription factors and target genes.

REFERENCES

Johnson, C., Boden, E. and Arias, J., 2003, Salicylic acid and NPR1 induce the recruitment of trans-activating TGA factors to a defense gene promoter in Arabidopsis. *The Plant Cell*, 15(8): 1846-1858.

Long, H.K., Prescott, S.L. and Wysocka, J. (2016), Ever-changing landscapes: transcriptional enhancers in development and evolution. Cell, 167(5): 1170–1187.

Oka, R., Zicola, J., Weber, B., Anderson, S.N., Hodgman, C., Gent, J.I., Wesselink, J.J., Springer, N.M., Hoefsloot, H.C., Turck, F. and Stam, M., 2017, Genome-wide

mapping of transcriptional enhancer candidates using DNA and chromatin features in maize. *Genome Biol.* 18(1): 137.

Schmitz, R. J., Grotewold, E. and Stam, M., 2022, Cis-regulatory sequences in plants: their importance, discovery, and future challenges. *The plant cell*, 34(2): 718-741.

Seok, H.Y., Tran, H.T., Lee, S.Y. and Moon, Y.H., 2022, AtERF71/HRE2, an Arabidopsis AP2/ERF transcription factor gene, contains both positive and negative cis-regulatory elements in its promoter region involved in hypoxia and salt stress responses. *"Int. J. Mol. Sci.*, 23(10): 5310.

Villar, D., Flicek, P. and Odom, D.T. (2014), Evolution of transcription factor binding in metazoans. *Trends Genet.,* 30(9): 509–519.