

# ROLE AND FUNCTION OF ETHYLENE RESPONSE FACTOR IN DIFFERENT PLANTS UNDER MULTIPLE BIOTIC AND ABIOTIC STRESSES

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## ABSTRACT

Abiotic and biotic stresses are the causes of drastic changes in plants growth and development. These stresses effect crop production and quality, thus result is in economic lose and food insecurity. Many factors play vital role in regulating growth of plants along with developmental pathways during biotic and abiotic stresses. Transcription factors are proteins that control physiological, developmental and stress responses in plants. Ethylene response factors belong to the biggest family of transcription factors, known to participate in various stress tolerance like drought, heat, salt and cold. They are significant regulators of plant gene expression. The objective of this review is to present how ethylene response factor family proteins became the focus of stress tolerance as well as the development and growth of plants.

## INTRODUCTION

Plants are susceptible to several types of environmental stresses throughout their life cycle. These adverse environmental conditions, specifically high salt concentration, temperature, soil moisture affect the growth, development, yield and geological distribution of cultivated crops across the world and decrease possible crop productivity approximately to 70% (1).

Biotic stresses for example weeds, diseases and insects occur at variable extent which may not be relevant in a particular developmental stage but decrease plant productivity (2). One of the main objective of crop breeding program is development of stress tolerant crop (3). Several

widespread abiotic stresses reported to take place around the world are salinity (4), drought (5), water logging, extreme temperatures (6), also nutrient deficiency (7).

A variety of defense mechanism like maintenance of membrane integrity, generation of antioxidants, hormone regulation, stimulation of stress proteins and carbon fixation rate have been found to work in plant. Various mechanisms in plant are governed involving particular genes for these stresses(8) and help plants to protect against a series of outside stresses during morphological adaptation (9). A large number of genes with diverse functions are induced or repressed when a plant is undergo to abiotic stresses. These genes encode for proteins which could be divided into two groups. One of them is primary group comprises functional proteins that is key enzymes for osmolytes biosynthesis like proline and sugar alcohols, molecular chaperones, and membrane transporters which are directly related to defense of plants from abiotic stress. The other group contains proteins that are regulatory in nature and control signal transduction and stress receptive gene expression and consists of a variety of transcription factors and protein kinases (10).

Transcription factors (TFs) that are regulatory proteins have a central part in initiating defense gene expression (11). They interact through *cis*-acting components present in promoter region of various stress regulated genes and consequently start cascades or entire system of genes that perform together increased tolerance to various stresses at a time. Stress responsive transcription factors are strong tools for genetic engineering because their overexpression can result in either upregulation or downregulation of entire collection of genes under their control. A large number of transcription factors participate in plant stress tolerance and regulate plant responses to diverse stresses. In *Arabidopsis* more than 1500 genes have reported to encode a various transcription factors (12). Some of the transcription factors related to plant stress response are categorized into numerous large families for example AP2/ERF, NAC, bZIP, zinc-finger, MYB, MYC and WRKY (13). Ethylene responsive factor is the member of large gene family the APETALA2/Ethylene-Responsive Factor superfamily. This superfamily also include AP2as well as RAV family(12). This superfamily has characteristic AP2/ERF domain, which contains near about sixty to seventy amino acids. The families of AP2/ERF superfamily defined like, proteins of AP2 family have AP2/ERF domains which are two repeated domains, and proteins of ERF family consist of one domain that is AP2/ERF, whereas proteins of RAV family have a B3 domain. The ethylene response factor family is further divided into two distinctive subfamilies, one is CBF/DREB subfamily, second is ERF subfamily (14). The ERF region initially identified as conserved element in four proteins characterized in *Nicotiana*

*tabacum*. These proteins are distinctive ethylene responsive element binding proteins, named ERF 1, 2, 3, 4 also found to interact with consensus promoter sequence GCC box which play role in ethylene responsive transcription of genes (15). The ERF subfamily proteins were classified into six sets, B-1 to B-6. Nakano *et al.*, (16) studied the expression of various genes of ERF subfamily in various plants and their biological roles.

The ethylene responsive factors also governed by feedback mechanism and may be ethylene dependent or ethylene independent. Apart from regulation mechanism, ERFs expressions are regulated post-transcriptionally via micro RNAs, while expressions of miRNA are also regulated through ERFs. Ethylene responsive factors have found to involve as activator or various genes while it may act as repressor of various genes during of plants defense response averse to biotic stress condition (17). Ethylene responsive factors also defense role in fungal and bacterial diseases responses as well as during viral infection in plants (18). SERF1 overexpression enhanced tolerance of salinity through ROS stimulated MAPK indicating flow (19). TaERF1 in wheat was appeared in the direction of improve easiness to abiotic, also biotic stress conditions (20). AP2/EREBP family which are plant-specific TFs exposed by *Arabidopsis* mutational analysis, that were liable for enhance wax substance, decline stomatal density, changed cuticle premises in addition to the connected important raise in tolerance of drought *Arabidopsis* (21).

Ethylene response factors (ERF) are associated in stress tolerance in a number of species of plants (22), it was revealed that *GhERF2*, *GhERF3* and *GhERF6* can proceed as significant frequent components of various signaling pathways receptive to abiotic and biotic stresses. To advance identify with their activity, the development of transgenic plants overexpressing these genes is ongoing (23).

### **Discovery of Ethylene response Factor**

ERFs previously named ethylene response binding proteins denoted by ERE binding proteins. The ethylene responsive element proteins earliest extracted from plant *Nicotiana tabacum* (15). ERFs consist of a domain, AP2 DNA-binding region, as of a plant-definite fabulous family of 122 TFs in *Arabidopsis*. In genome of tomato, near 85 gene of ethylene responsive factors family protein stay uncharacterized (24). It is described that in various plants ERF domains product be present within several regulatory genes (25, 26)but is not present in fungi, yeast or mammalian (27). In the most recent decade, to a large extent, consideration have been accentuated on AP2/ERF large family. The hypothesis elucidates as the lateral gene movement

of HNH-AP2 endonuclease originated from bacteria or virus into the host plant might bring about regenerate the AP2/ERF great family (28).

### **Functional Diversity in Plants**

Up to now, Ethylene responsive TFs have been recognized as well as distinguished from numerous species of plants for example *Arabidopsis* (16), poplar (29), cotton (30), soybean (31), barley (32), maize (33), cucumber (34), apple, wheat (35), rice (36), sorghum (37), *Medicago truncatula* (38), also potato (39). Overall 147 genes in *Arabidopsis*, encoding AP2/ERF TFs, transcription factors named ERF encoded by 122 of these 147. ERF TFs of *Arabidopsis* may be divided into 12 groups different from each other, found on phylogenetic analysis, these groups named as I to X, VI-L, Xb-L (16). Functional diversity of ethylene response factor in different plants is given in table 1.

### **Structure**

The ERF TFs take part in plants development procedures, in response to environmental stresses and hormone signaling (39). The ethylene response factor family has been comprehensively studied in recent times. In AP2/ERF domain  $\beta$ -sheet, 2 conserved amino acid residues distinguished ERF transcription factors, residues are 14th Ala as well as 19<sup>th</sup> Asp (14). ERF TFs allocate a conserved 58–59 amino acid domain (the ethylene response factor domain) and encompass sturdy capability to bind an extensive sort of CREs within promoter of mark genes. From the *cis*-regulatory elements, DRE/CRT and GCC-box are the two chief DNA-binding elements (40). Most stimulatingly, numerous studies expose ERF proteins interconnect by DRE/CRT motif, a *cis*-acting element which acts in response to osmotic stress or cold (41), they build up defense by interrelating among stress-regulated genes (42).

The  $\alpha$ -helix as well as  $\beta$ -sheet of ERF region identify the GCC box (called core *cis*-acting element AGCCGCC) of objective DNA on the ER promoter region (43). Pathogenesis-related (PR) genes are expressed by these GCC box elements (44). Additionally, the minority conservative elements of other than AP2 region grant transcriptional repression or activation in favor of particular gene, for example ERF connected amphiphilic oppression element that is EAR: DLNxxP (45), LWSY element (30) in addition to B3 oppression domain motif that is BRD:RLFGV (46). ERF protein either has homology resemblance with identified DNA joining proteins nor has fundamental zinc finger or leucine zipper elements, which propose ERF is a

set of novel DNA binding proteins (43). Figure 1 gives classification and highlights functional association among AP2/ERFs in a variety of abiotic stress responses plants.

### **Role of ethylene response factor**

Ethylene responsive factors take part essential regulatory tasks in various developmental processes and stress responses in plants. Genes of ERF family in an extensive array of plant types, were categorized into 8 groups and 14 subgroups on the base of gene structures, conserved motifs, phylogenetic relationships, and biological functions. ERF genes, particularly in groups II as well as VII, may be utilized like contenders to develop crop resistance, for the reason that overexpression of ERF genes increases various disease resistance along with tolerance to drought, salt, freezing also in transgenic plant. The inclusive testing of phylogenetic correlations, conserved pattern, moreover physiological functions is beneficial in highlighting the biological roles of genes of ERF family in signal transduction, gene regulation along with defense response below stress environments (11).

ERF TFs have been revealed to be concerned in the regulation of expression of lipids, cell wall constituents, pathogenesis related genes, biosynthesis genes, basic type defense related genes, chitinase, osmotin, more over  $\beta$ -1,3-glucanase encoding genes (28). Few of them are exposed to be concerned with in the control of plant reactions to biotic, also abiotic pressures by either activating or repressing abscisic acid responsive genes (47). Such as AtERF4 over-expression plant was a smaller amount of responsive to ABA suppressed root enlargement that entails negative control of ABA also ethylene reactions (48). The expression of ABA-responsive genes are repressed by AtERF7 that binds to the GCC box (49). ERF111 or ABR1 takes part as a negative controller of ABA reactions throughout seed germination, ABA and stress regulated gene expression (50) wherever as in Arabidopsis ABA hypersensitivity is conferred by transgenic plant, overexpressing AtERF13 (51). AtERF15 exposed on the way to proceed as activist controller of ABA reactions (52). In contrast, ABA may also bring about the few ERF genes expression such as the expression of tomato ERF gene JERF1/3, cotton ERF gene GbERF in addition to tobacco ERF gene NtCEF1 has been revealed to be evoked by ABA (53). Sub group IX in IXERF subfamily accommodates 4 tiny ERFs through amino acids, varied as of 131-139. These tiny ethylene response factors named ERF95, ERF96, ERF97, ERF98. Additionally toward AP2/ERF area, these ethylene response factor have unfamiliar role pattern termed CMIX-1 (16). In the group of these, ERF95 in addition called ethylene and salt inducible1 (ESE1), furthermore ERF98 have been exposed to concerned with the control of

salt tolerance (54). In the past ERF97 termed AtERF14, has been revealed to control plants protection (55). In recent times ERF96 also has been revealed to regulate plant defense response (56).

## Role of ERF during Stress

ERFs are concerned during the parameter of plant growth, expansion, metabolism as well as plant reactions to ecological stimulus like abiotic and biotic stresses (57). The ERF transcription factors are participated in the ethylene signaling pathway as well as directing in the direction of the expression of a variety of protection-associated genes, for example pathogenesis-related genes (40), abiotic stress receptive genes (58). The existence of a GCC-box or a dehydration responsive element/C-repeat element (DRE/CRT, CCGAC) situated in the promoter sequences of defense-related genes are involved in characterization of these genes (14). Figure 2 shows stress related genes expression, their role under stresses

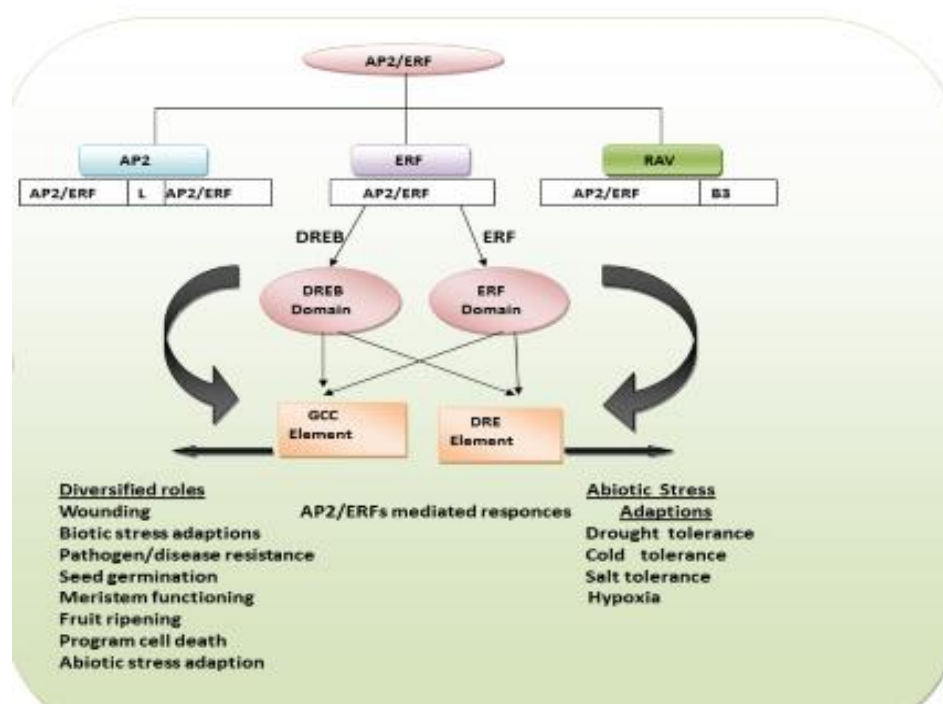


Figure 1: AP2/ERF transcription factors in plants, their classification and their functions has also been reported.

Table 1: Examples of ERF family proteins involved in regulation of multiple abiotic stress responses in different plants.

ERF Gene	Identified in plant species	Function	References
<i>TaERF3</i>	<i>Triticum aestivum</i> (Wheat)	Drought and salinity tolerance	(79)
<i>TaERF1</i>	<i>Triticum aestivum</i> (Wheat)	Drought, salt, and low temperature tolerance	(20)
<i>AtERF019</i>	<i>Arabidopsis</i> (Mouseear cress)	Drought tolerance, smaller stomata aperture, and lower transpiration rate	(80)
<i>DREB1A</i>	<i>Arabidopsis</i> (Mouseear cress)	Delayed water stress symptoms	(81)
<i>SodERF3</i>	<i>Saccharum officinarum</i> (Sugarcane)	Drought and osmotic tolerance	(82)
<i>OsERF4a</i>	<i>Oryza sativa</i> (Rice)	Enhanced drought tolerance	(83)
<i>AtERF7</i>	<i>Arabidopsis</i> (Mouseear cress)	AtERF7 binds to the GCC box and acts as a transcriptional repressor in ABA and drought stress responses.	(84)
<i>OsERF3</i>	<i>Oryza sativa</i> (Rice)	EAR motif in OsERF3 is required to transcriptionally regulate ethylene biosynthesis and drought tolerance.	(38)
<i>OsERF4a</i>	<i>Oryza sativa</i> (Rice)	OsERF4a improves drought tolerance through the repression of a downstream suppressor of stress response gene, Sir2	(83)
<i>GmERF6</i>	<i>Oryza sativa</i> (Rice)	GmERF6 functions as an EAR repressor to downregulate expression of <i>AtKin1</i> , <i>AtPR3</i> and <i>AtRD22</i> in transgenic <i>A. thaliana</i>	(85)
<i>SIERF36</i>	<i>Solanum lycopersicum</i> (Tomato)	The EAR motif in SIERF36 controls early flowering and senescence and is responsible for reduction of stomatal density and photosynthesis	(86)
<i>OsERF71</i>	<i>Oryza sativa</i> (Rice)	Positive regulator in Drought salinity	(87)
<i>GhERF70D</i>	<i>Gossypium herbaceum</i> (Levant cotton)	Positive regulator in cold stress	(88)
<i>GhDREB56A</i>	<i>Gossypium herbaceum</i> (Levant cotton)	Positive regulator in heat stress	(88)
<i>GhDREB7A</i>	<i>Gossypium herbaceum</i> (Levant cotton)	Drought, heat, and cold	(88)
<i>AtERF019</i>	<i>Arabidopsis</i> (Mouseear cress)	Drought	(89)
<i>BaERF14</i>	<i>Brachypodium distachyon</i> (Purple false brome)	Drought, salt, cold	(90)
<i>BaERF118</i>	<i>Brachypodium distachyon</i> (Purple false brome)	Negative regulator in cold stress	(90)
<i>ERF109</i>	<i>Nicotiana tabacum</i> (Tobacco)	Positive regulator in Salt	(91)
<i>MeERF17</i>	<i>Manihot esculenta</i> (Cassava)	Positive regulator in drought	(92)
<i>ZmCBF3</i>	<i>Zea mays</i> (Corn)	Negative regulator in Cold	(93)
<i>StERF1</i>	<i>Solanum tuberosum</i> (Potato)	Positive regulator of Salt, drought, and hormonal like methyl jasmonate	(94)
<i>Sl-ERF.B.3</i>	<i>Solanum lycopersicum</i> (Tomato)	Positive or Negative regulator of Heat, cold and flood, drought	(95)
<i>LeERF3b</i>	<i>Solanum lycopersicum</i> (Tomato)	Positive regulator of Drought, desiccation, and low temperature	(96)

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## **Abiotic Stress**

Environmental stresses that are nutrient deficiency, drought, salinity, extreme temperatures and chemical toxicity, leads to a chain of physiological, morphological, molecular, and biochemical changes which negatively have an effect on plant development, yield and survival all-inclusive (59); (60), in response to those stresses plants develop mechanisms. Plant feedbacks are multifarious, including extensive metabolic and transcriptional events (61). The molecular and physiological studies of environmental stresses have proposed a familiar network of several signaling tracks that mediate salt, extreme temperatures and drought stress responses in plants (62). In abiotic stress responses the DREBs proteins are most extremely considered ethylene response factor. Elements of the subfamily *DREB1/CBFs* are quickly induced in retort to chilly stress and get better tolerance to freezing when expressed ectopically, (63). Whereas, RNAi lines of *Arabidopsis* for *CBF1* or *CBF3* have decreased cold tolerance (64). For freezing tolerance *CBF2* associated by quantitative trait locus (65), point out that *DREB1/CBF* genes are cold activated and in *Arabidopsis* are liable to be main monitor of reaction to wintry stress. Furthermore in *Arabidopsis* *CBF2* abnormal gene communication has been revealed to retard leaf senescence, indicating that *CBFs* promotes extremely cold temperature tolerance (66).



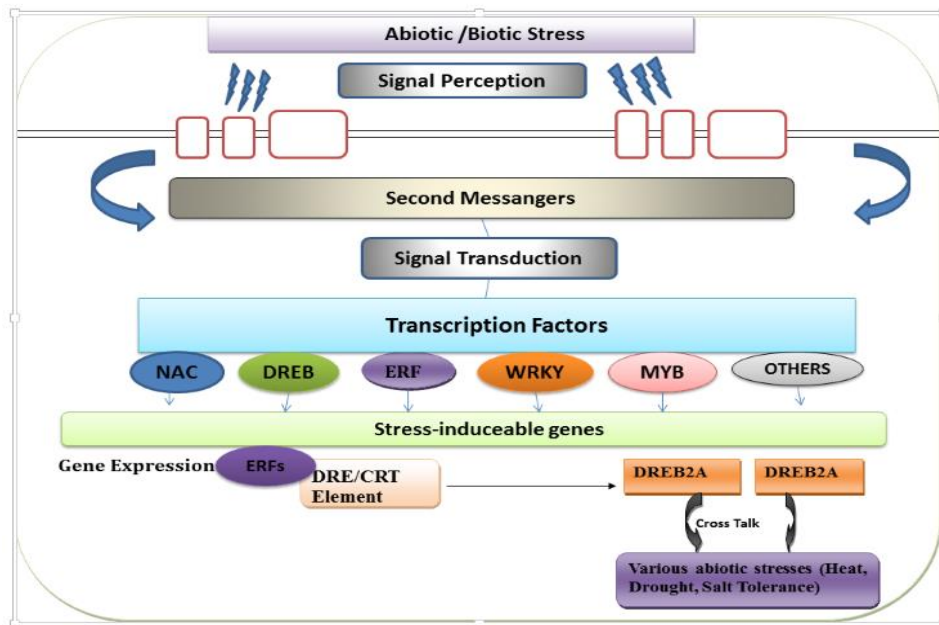


Figure 2: A model of process of abiotic or biotic stress tolerance in plants.

The subgroup of DREB2 contains eight elements in *Arabidopsis* and more homologs in the genetic material of numerous angiosperm families. Along with the components of the DREB2 sub division, *Arabidopsis* DREB2A as well as DREB2B were induced in loss of water, heat and elevated saltiness in an ABA independent way (67). The abnormal gene expression of a constitutively dynamic structure of DREB2A demonstrates developed forbearance to famine, heat and elevated salt stresses (67, 68) whereas DREB2A are further susceptible to temperature Shock (67). From rice or *Arabidopsis* several *ERF-VII* genes are exposed to be participated with in response to hypoxia as well as submergence (69). In *Arabidopsis* RAP2.12 are constitutive ERF-VII factors, they are recommended to proceed as prime triggers for the molecular feedback to insufficiency of oxygen (70); (71), that is then comforted at the oxygen deprivation activated ethylene response factors HRE1 also HRE2 (72). In submerged deep water rice, SK1 and SK2 motivate internodes elongation to effect clearance of the water level (73), whereas Sub1A promotes a quiescent strategy that allows carbohydrate saving and improves tolerance after flash-flood (74). A number of *ERF* genes from various plants have been exposed to confer various stress tolerance when expressed ectopically (75). This ‘unspecific’ effect may be explained by the activation of tolerance pathways which reduce a common stress including oxidative bursts generated as results of the primary stresses. Otherwise constitutive ERF expression could set the plant in a common alert state that fasten or expand the response when a specific stress is applied (28).

## Biotic Stress

ERFs are entailed in biotic stress responses, ethylene response factor proteins initially extracted the same as transcription factors (TFs) which connect to promoter regions of stress-responsive genes. ERF genes assessed to date, are provoked through biotic stresses, consist of wounding, pathogen induced stresses, such as transcription of basic type pathogenesis-related (PR) genes, defense-related genes, chitinase, osmotin, and b-1,3-glucanase activated by numerous ERFs. On the other hand, the group of target genes regulated by each ERF has not been fully discovered. ERF1 as well as its homologs be associated to ERF-IX group, are possibly the ERF transcription factors whose participation in pathogen reaction in *Arabidopsis* has been most widely distinguished (76), as well a number of proteins of ERF regulate ethylene biosynthesis (77). Tobacco OPBP1 when expressed ectopically in transgenic rice increases resistance to pathogens (78).

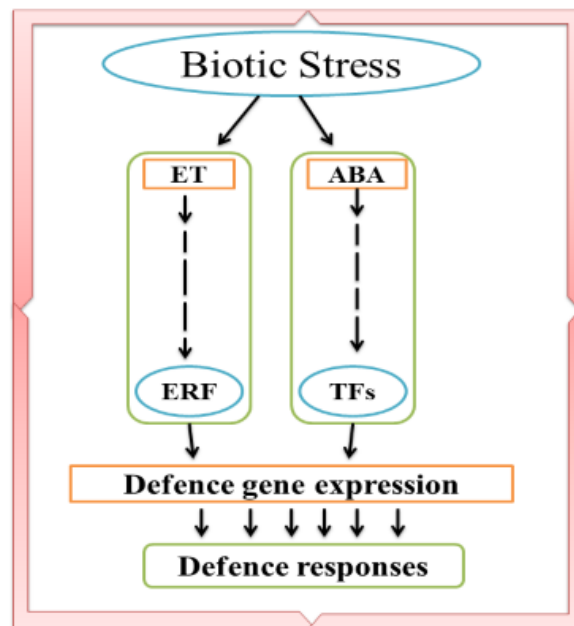


Figure 3: Hormonal signalling and expression of genes in biotic stresses.

## Conclusion

The existing information about various roles of AP2/ERFs recommends that the members of this large super family can be utilized for improving stress tolerance for better crop yields. The members of this family can be employed in improving nutritional quality of grown varieties.

Up to now extensive advancement has been done to know the functions of AP2/ ERFs in response to various stresses. To develop multiple stress tolerant crops the members of this superfamily have been used. The plants genome sequencing projects have facilitated for recognition of ethylene response factors in different plants. It is stated that the proteins of ERF family has ability to confer resistance against numerous stresses in crop plant.

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