The Role of Transcription Factors in Plant Immunity

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**Abstract**

Transcription factors (TFs) are proteins that recognize and bind to specific DNA elements to control the level of transcription of their target genes. TFs play a number of critical roles in the plant immune response by regulating the expression of defense-related genes, and also participate in crosstalk and feedback loops with key plant defense hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). A number of plant TF families have been identified as having immune functions, including the WRKY, bHLH, bZIP, and ERF TF families, which interact with the SA and JA/ET pathways to enhance resistance to biotrophic and necrotrophic pathogens, respectively. While many of these TFs are well-characterized, the advent of new biotechnologies has allowed for more in-depth analysis of the complex networks in which these TFs function.

**Introduction**

The plant immune system is typically viewed as a two-layered response. Of these, the first layer is a general response which acts across the plasma membrane. This is accomplished by membrane-bound pattern recognition receptors (PRRs) that bind to foreign molecules termed pathogen-associated molecular patterns (PAMPs) in a process known as pattern-triggered immunity (PTI) [1]. This layer of the immune response is relatively non-specific, as it does not recognize distinct pathogens themselves, is weaker, and is employed more frequently [2]. The other layer acts in the interior of the cell, making use of the protein products of resistance (R) genes, which have nucleotide binding and leucine rich repeat domains (termed NLR genes) that recognize specific pathogen effectors, resulting in effector-triggered immunity (ETI) [1]. ETI can be understood as a stronger, more robust version of the immune response than PTI, though they share many of the same characteristics [3]. This specificity is accomplished by having an NLR protein that recognizes, either directly or indirectly, a specific effector of the pathogen, a molecule secreted directly into the cell [4]. It has recently been reported that PTI and ETI are mutually dependent and both are required for effective resistance [5,6].

For PTI and ETI to be effective in generating an immune response, however, transcription factors (TFs) are critical. By binding to specific DNA sequences, TFs can play roles in gene expression, repression, and the establishment of protein-protein interactions [7]. These functions are largely mediated by the regulation of mRNA transcription, a process that often changes drastically upon the introduction of external stresses such as the encounter of pathogens [8]. By fulfilling these regulatory functions, TFs allow the plant immune response to be deployed in a manner that rapidly reacts to the pathogen and transmits this distress signal efficiently through the cell: TFs are responsible for reprogramming the plant transcriptome, thereby forgoing typical cellular processes to prioritize a defense response [8]. Because this requires thousands of genes to be reprogrammed rapidly, transcription cofactors (TCFs), which do not have the capability to bind DNA, but instead interact with TFs, are also required. Necessarily, these TFs and TCFs must function cooperatively in a network to control gene expression [8].

In plants, TF genes usually make up a significantly larger portion of the genome than in most animals—for instance, between 6% and 10% of all *Arabidopsis thaliana* (more familiarly known as simply “Arabidopsis”) genes encode TFs compared to the 5% in humans [9]. There are several predominant families of TFs notable for their roles in immune response management and regulation. The largest of these is the WRKY superfamily, which was named for its conserved motif of amino acids, WRKYGQK, and was identified based on the ability of WRKY proteins to bind to a DNA motif known as the W box. External stimuli such as bacteria, viruses, fungi, and physical wounding of plants have been demonstrated to upregulate the production of WRKY proteins, which in turn activate various genes, acting in distinct waves [10]. WRKY proteins are known to specifically interact with DNA through a zinc-finger-type domain, allowing access to the major groove of the DNA [11]. WRKY genes first originated in early eukaryotes, but evolved and expanded significantly in plants, creating the WRKY superfamily with TFs of many diverse roles and functions [12]. In fact, it is a general trend that transcription factor families in plants have significantly higher expansion rates than those in animals [13].

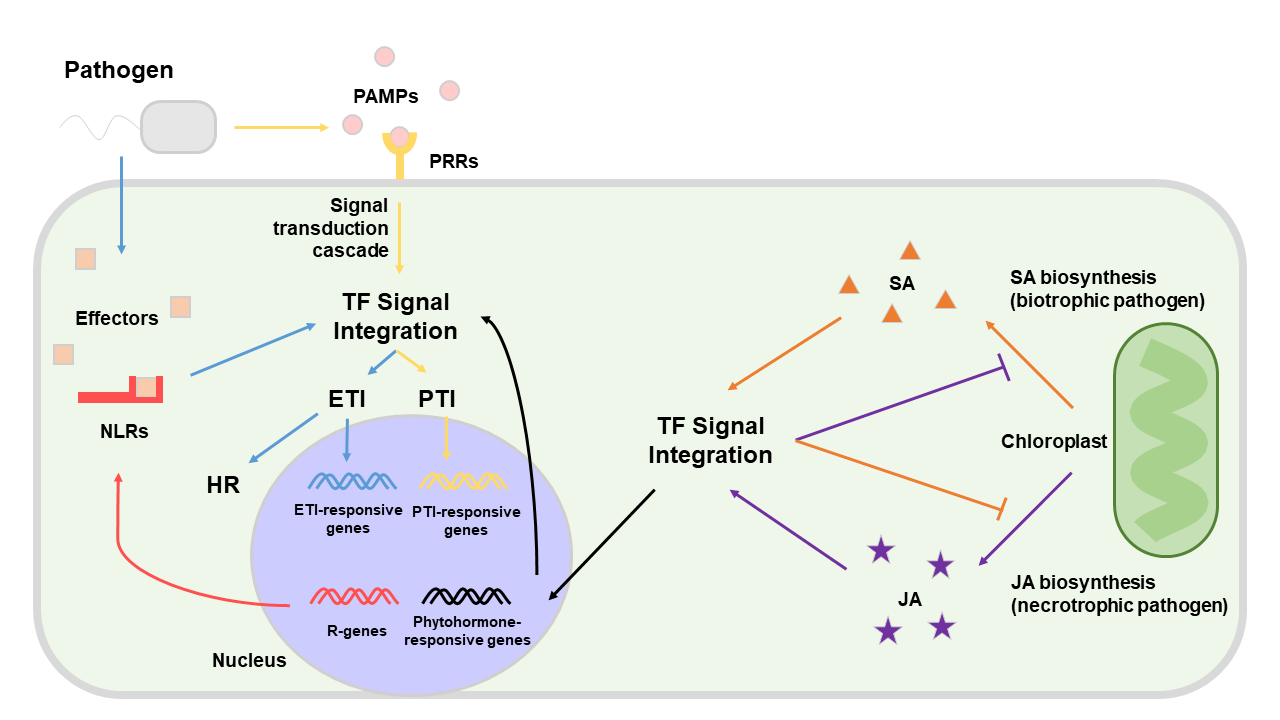
Often, plant hormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are important in triggering the reprogramming of the plant transcriptome by inducing regulators such as WRKY TFs [14]. SA and JA are also known to induce other families of transcription factors with known roles in immunity, such as the TGA family, which is responsible for reactivating genes that have been suppressed by SA [15]. Another class of plant TFs, ethylene-responsive factors (ERFs), also play an important role in plant stress responses to pathogens [16].

The above examples illustrate the complex web of plant TFs, TCFs, and the hormones that induce them, which all serve to coordinate a fast, effective response to pathogenic stresses. By better understanding these connections, the mechanisms of plant immunity can be further elucidated, which can allow for the development of new strategies for improving the ability of plants to resist pathogens. Plant disease exacerbates food insecurity in a world where 800 million people do not have consistently sufficient access to food, and the associated pathogens continue to evolve, rendering resistance achieved through breeding inadequate [17]. In addition to its effects on the availability of food, plant disease also causes significant detriments to the agricultural economy, causing millions of dollars of loss [18]. An understanding of the genetic intricacies of the plant immune response is required to keep pace with the evolution of pathogens, and this review seeks to provide a detailed portrayal of the role transcription factors play in this response.

**Transcription and Hormone Signaling in the Plant Immune Response**

To a large extent, the efficacy of the plant immune response depends on the ability of the cell to rapidly reprogram its transcriptome upon encountering a pathogen [19]. This is due to the constant molecular dialog within a plant that is responsible for prioritizing either growth or the immune response at different times and locations within the plant. This balance needs to be carefully maintained based upon the cellular expense of the immune response: at a minimum, the plant will need to expend energy and resources to combat a pathogen, and at its most extreme, the plant will activate its hypersensitive response (HR), which results in rapid programmed cell death [20]. This necessitates very high specificity of when and to what extent immune responses are employed, especially the HR, depending on factors such as pathogen type and quantity, and even environmental factors such as light and temperature [21].

Maintaining such stringent control over the HR requires intricate regulation of ETI, and a central component of this is mediated by the chloroplast, which is responsible for producing defense-signaling molecules such as SA and JA [22]. This role of the chloroplast is shown below in Figure 1, which situates phytohormone signaling within the plant immune response. In pathogen defense, there are two central signaling pathways, one dependent on SA and the other independent of SA, instead depending on JA and ET [23]. The SA-mediated pathway has roles in both PTI and ETI, and furthermore has been shown to contribute to systemic acquired resistance (SAR) [24]. SAR allows resistance to spread through a plant’s tissues from the infected region to distal areas, and the resistance conferred is long-lasting and broad-spectrum [25]. In general, the SA-mediated signaling pathway can be understood to act against biotrophic pathogens, which keep the host alive while absorbing nutrients, such as *Pseudomonas syringae* [26]. In contrast, the JA/ET pathway contributes to resistance against necrotrophic pathogens, which kill host cells before extracting nutrients, such as *Botrytis cinerea* [27].



**Figure 1**: An overview of the plant immune system. Here, TFs act as critical nodes to integrate signals from PRRs, NLRs, and phytohormones while facilitating crosstalk between these pathways.

These two pathways show considerable crosstalk and are thought to be mutually synergistic at low intercellular concentrations and antagonistic at higher concentrations [28]. This can be seen in the following example where a plant is infected with *P. syringae*: because this is a biotrophic pathogen, the SA pathway is activated, but this in turn has been shown to increase the plant’s susceptibility to *Alternaria brassicae*, a necrotrophic pathogen [29]. This is because when triggered, the SA pathway represses the JA/ET pathway at the transcriptional level. This repression is mediated in the cytosol by a regulatory protein termed the nonexpressor of pathogenesis-related genes 1 (NPR1), which is also responsible for the activation of pathogenesis-related (PR) genes in the nucleus [30].

The activation and regulation of the SA and JA/ET pathways relies heavily on the plant TFs involved in regulating biosynthesis of these hormones—often, when a TF gene is knocked out, inappropriate activation of one of these pathways is observed. Additionally, signal transduction between TFs and plant hormones is assisted by mediator proteins—proteins that function in a complex to act as transcriptional coactivators—that also interact with NPR1 [19], and TFs have been shown to interact with one another, even across TF families [31]. Looking at the complexity of these interactions, understanding plant immune regulation can seem to be quite a daunting task; however, the more interactions that can be teased out, the clearer the overall picture of the immune response becomes, and better manipulation of it will be possible.

**The WRKY Superfamily of Plant TFs**

WRKY proteins are found in a characteristically wedge-shaped domain (named “the -wedge”) which also contains a zinc-finger region for interaction with the major groove of DNA [11]. These TFs contain either one or two of these WRKY domains, and they target a specific region of DNA known as the W-box element (C/TTGACT/C) [14]. In addition to abiotic stresses and insect herbivory, WRKYs have known roles in regulating responses to bacterial [32], fungal [33], and viral [34] pathogens. WRKYs are a very large family of TFs with up to 100 members found in the model organism, Arabidopsis [10].

Many PR genes contain W-box motifs in their promoters and are regulated by WRKYs. In many instances WRKYs are self-regulating, as many WRKY genes themselves contain W-box elements in their promoters [35]. WRKY factors, especially in the context of their immune functions, have been studied most extensively in the model systems of rice (*Oryza sativa*) and Arabidopsis through gain-of-function and loss-of-function studies [36]. Several of the most critical and representative WRKY functions are outlined here.

A key WRKY in integrating SA and JA signals in Arabidopsis is WRKY70, a TF which is activated by SA and repressed by JA, meaning that it is activated in response to biotrophic pathogens [37]. WRKY70 is a fairly unique member of the TF family, with the next closest match to any other WRKY being WRKY54, which also increases resistance to biotrophic pathogens [38], with only a 43% amino acid identity match [39]. WRKY70 acts downstream the proteins NPR1 (an SA receptor), which induces WRKY70 production, and COI1 (a JA receptor), which represses WRKY70 production, and is responsible for integrating these two signals to elicit the appropriate response without altering the biosynthesis of either of these hormones itself [37]. This allows WRKY70 to specifically control the production of either SA- or JA-responsive defense proteins based upon the kind of pathogen that is present, a critical point of signal integration.

It is characteristic of many WRKYs that they mediate resistance to either biotrophic or necrotrophic pathogens while increasing susceptibility to the other. A representative example of this phenomenon is Arabidopsis WRKY33, which, in coordination with other WRKYs, contributes to resistance to the necrotizing pathogen *B. cinerea* by suppressing the SA pathway and upregulating the JA/ET pathway [40]. As a counterexample, Arabidopsis WRKY46 works with other WRKYs to increase resistance to the biotrophic pathogen *P. syringae* by upregulating the SA pathway and suppressing the JA/ET pathway [41].

It may seem counterintuitive for these TFs to act as negative regulators in any capacity, especially in proteins that are rapidly synthesized at the onset of the immune response. However, when considering the repercussions of eliciting a response unnecessarily (e.g., implementing the JA/ET pathway when a biotrophic pathogen is detected or the SA pathway when a necrotroph is present), the plant faces increasing consequences with the severity of the response. For instance, plants that spontaneously undergo the HR have been shown to have lower fertility and stunted growth [42], and even maintaining the capability for resistance surveillance by R genes in the plant comes at a great cost [43].

A characteristic that has made studying WRKY immune roles challenging is that there are many redundancies and codependences within the WRKY family that require analyzing double and even triple mutants to decipher. An excellent example of this is seen within the network of WRKY18, WRKY40, and WRKY60. It was found that while mutants with single knockouts of these genes exhibited little to no change in resistance to any pathogen, analysis of double and triple knockouts revealed enhanced resistance to *P. syringae* and increased susceptibility to *B. cinerea* [44], indicating that these genes are either codependent or redundant in upregulating the JA/ET pathway. This example is important to bear in mind while screening putative immunity-associated genes, as single knockouts may not always reveal a gene’s role in the immune response.

**bHLH TFs**

Basic helix-loop-helix (bHLH) transcription factors are another large family of TFs observed in plants, with over 100 members occurring in Arabidopsis [45], several of which have demonstrated immunity-related functions. The general structure of a bHLH TF consists of a basic, hydrophilic DNA-binding domain (DBD) attached to two hydrophobic α-helices connected by a linker [46]. Typically these TFs act as a dimer, and the HLH structure allows for this [47]. These bHLH factors recognize a sequence known as the E-box (CANNTG) [48], and this allows them to have distinct roles in not only immunity, but also growth, light signal transduction, and other stress responses [49].

One common way in which bHLH factors can influence the immune response is through interactions with the Mediator complex, which plays a critical role in transcriptional regulation. An example of this is the Arabidopsis TF MYC2, a bHLH protein that interacts with the MED25 Mediator subunit to enhance *B. cinerea* resistance via the JA/ET pathway [50]. Similarly, the bHLH protein FAMA interacts with the MED8 subunit as a further contribution to *B. cinerea* resistance [51]. Much like WRKYs, many bHLH factors participate in crosstalk with either the SA or JA/ET pathways to transduce signals [52].

Another key role taken on by a bHLH TF is in the ever-important tradeoff faced by plants between growth and immunity. Analysis of the Arabidopsis HBI1 TF has shown that this protein serves as a regulatory node that integrates signals related to both growth and environmental stresses [53]. Overexpression and knockout assays of HBI1 demonstrated its inhibition of growth arrest, R gene expression, and pathogen resistance, meaning that its regulation and crosstalk can have a significant impact on the decision to fight a pathogen or continue to grow [53].

TFs in the bHLH family are often regulated by post-translational modifications (PTMs) such as ubiquitination by E1, E2, and E3 ligases [9]. Upon polyubiquitination, proteins are degraded by the proteasome; however, reversal by deubiquitinating enzymes can protect proteins from degradation [54]. This allows for ubiquitin to alter the function of TFs by monoubiquitination: monoubiquitination of bHLHs is often required for binding the necessary target promoters to activate gene transcription [55]. The regulation of bHLH factors by PTMs allows, consequently, for the downstream regulation of immune responses, especially through the Mediator complex.

**bZIP TFs**

Basic leucine zippers (bZIPs) are yet another large TF family in plants that are implicated in stress responses including pathogen response. The structure of a bZIP, similarly to a bHLH, is often a heterodimer that contains a basic, hydrophilic DBD, and this is attached to a coiled coil of α-helices stabilized by a heptad leucine (or other hydrophobic residue) repeat [56]. The DBD recognizes the ACGT core motif of *cis*-acting DNA elements such as the A- (TACGTA), C- (GACGTC), G- (CACGTG), and H-boxes (CCTACC) [57]. bZIPs have been found in the genomes of many plants, including Arabidopsis [57], tomato (*Solanum lycopersicum*) [58], corn (*Zea mays*) [59], grapevine (*Vitis vinifera*) [60], and others.

Compared to other TF families, less is known about the bZIPs [61]; however, they have several demonstrated roles in plant immunity. Arabidopsis bZIP10, for instance, is a key basal defense protein that promotes cell death via the HR [62]. Regulated in a mutually antagonistic relationship by the LSD1 protein, bZIP10 contributes to successful pathogen response by binding G- and C-box elements [63].

While many studies have been conducted on immune-related bZIPs in model organisms such as Arabidopsis, relatively few bZIPs have been studied in commercially important plants. An example can be seen in soybean (*Glycine max*), where the G/HBF-1 bZIP TF targets G- and H-box elements to prepare a plant for pathogen attack by increasing the production of lignin, phytoalexins, and SA [64]. While model organisms provide an excellent resource to lay the groundwork for understanding the roles of bZIPs and TFs in general, more studies will ultimately need to be conducted to seek out practical applications of this area of research.

The TGA TFs, a subfamily of the bZIP family named for their TGACG sequence recognition, have also been demonstrated to play key roles in mediating the immune response, often through interactions with NPR1, a key part of the SA pathway. These interactions occur inside the nucleus of the cell: NPR1 is imported by a nuclear pore protein, and once inside, interacts with TGA factors, allowing them to bind to SA-responsive genes [65]. In order to minimize any negative feedback in the SA pathway from the accumulation of NPR1 in the nucleus, NPR1 is ubiquitinated during its interaction with its TGA partner, subsequently allowing for degradation by the proteasome [66].

There are 10 TGA factors currently known in the Arabidopsis genome, and of these, seven have been shown to play roles in the SA pathway [57]. TGA2 and TGA3, for example, have demonstrated strong binding affinities for NPR1, indicating that SA-induced PR-gene expression proceeds via these TGA factors [67]. Furthermore, TGA transcription factors are also thought to contribute to biotrophic pathogen immunity via SAR [68].

**AP2 / ERF TFs**

The Apetala 2 (AP2) / ethylene responsive factor (ERF) TF family, named for its role in flower development and ethylene responses, has many roles in plant development; however, immune functions are also observed in the ERF subfamily [69]. AP2 factors have been found to recognize the GCC-box element (AGCCGCC) found in many PR genes with a novel β-sheet binding domain [70]. As their name would suggest, ERF TFs typically interact with the JA/ET signaling pathway when triggered by pathogens.

In Arabidopsis, ERFs have been demonstrated to act as both activators (ERF1, ERF2, ERF5) and repressors (ERF3, ERF4, and ERF9) of the transcription of JA/ET-interactive genes [71,72], highlighting their importance in defense against necrotrophic pathogens. It is important to note that ERFs that repress transcription are often themselves repressed by other families of TFs. Arabidopsis DEAR1, for instance, represses ERF9, a protein that normally decreases resistance to *B. cinerea* [73]. This allows for the DEAR1 repressor to increase resistance to necrotrophic fungi not by activating the JA/ET pathway, but by repressing one of its repressors.

ERFs have been demonstrated to be important in ET/JA signal integration [74], including several ERFs that increase basal disease resistance to necrotrophic pathogens in overexpression assays such as Arabidopsis PDF1.2 [75]. It would be anticipated for TFs that are positively regulated by JA/ET to interact antagonistically with the SA pathway; however, interestingly, it has been observed that this may not be the case, as the Pti4, Pti5, and Pti6 tomato ERFs, when expressed in Arabidopsis, actually activate expression of SA-regulated genes PR1 and PR2 [76]. ERF proteins are often regulated with PTMs such as phosphorylation. An example is Pti4, which has enhanced binding affinity to its target sequence upon phosphorylation [77].

**Conclusions and Looking Forward**

Many plant transcription factors are well understood and characterized within the context of the immune response network. The most complete picture of this can be seen in the WRKY family of Arabidopsis, where a large subset of these TFs has been identified as having immune roles and interacting with plant hormones such as SA and JA, other regulatory genes such as NPR1 and COI1, and even one another, although other families such are becoming increasingly well understood. In completing this picture of the immune response and its interactions with other plant regulatory systems, the ability of the plant science community to address and improve factors such as disease resistance, yield, and resistance to abiotic stresses will improve drastically.

New biotechnologies are allowing for even more in-depth understanding of TFs that will further advance the field. The basis for much of the current understanding of plant TFs is found in knockout and overexpression experiments. While these have served the plant community incredibly well, they have drawbacks: they are often fairly time consuming to complete for large numbers of putative genes, and it is easy to miss immune-related genes due to the nature of their frequent codependences and redundancies. Newer technologies such as ChIP-seq and RNA-seq provide a more efficient, higher throughput alternative for these types of studies [78]. Additionally, protein-binding microarrays provide a similarly high-throughput approach for identifying TF binding specificity to tease out relationships that may otherwise go unnoticed [79]. Furthermore, while the vast majority of TF studies have been carried out in Arabidopsis as a model organism, perhaps these will also facilitate the study of transcriptional regulation in other plants.

These types of detailed findings that situate genes encoding TFs within the big picture of plant immunity will very likely give rise to future projects in genomic engineering by utilizing new technologies to manipulate these genes more precisely than ever before. The CRISPR/Cas9 system, for instance, has already seen extensive use in plant systems [80], and even greater feats of genomic editing will soon be possible as the Prime Editing system becomes streamlined for use in plant cells [81]. By knowing, for instance, the targets of the full set of TFs that are implicated in bacterial speck of tomato, it would be possible to use a tool like Prime Editing to adapt the genome to maximize the response and avoid crop damage. This is just one of countless possibilities that these new technologies could enable, ensuring that the future of understanding and manipulating the plant immune system is rife with promise.

# **Summary Table**

**Table 1**: A summary table of all referenced TFs in order of discussion. Each TF is notated with the findings of the cited paper regarding the resistance/susceptibility that each provides toward biotrophic or necrotrophic pathogens.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Family** | **Organism** | **Biotrophic Resistance** | **Necrotrophic Resistance** | **Citation** |
| WRKY70 | WRKY | *A. thaliana* | + | - | [37] |
| WRKY54 | WRKY | *A. thaliana* | + | - | [38] |
| WRKY33 | WRKY | *A. thaliana* | - | + | [40] |
| WRKY46 | WRKY | *A. thaliana* | + | - | [41] |
| WRKY18 | WRKY | *A. thaliana* | + | - | [44] |
| WRKY40 | WRKY | *A. thaliana* | + | - | [44] |
| WRKY60 | WRKY | *A. thaliana* | + | - | [44] |
| MYC2 | bHLH | *A. thaliana* | - | + | [50] |
| FAMA | bHLH | *A. thaliana* | - | + | [51] |
| HBI1 | bHLH | *A. thaliana* | - | - | [53] |
| bZIP10 | bZIP | *A. thaliana* | - | - | [62] |
| G/HBF-1 | bZIP | *G. max* | + | - | [64] |
| TGA2 | bZIP | *A. thaliana* | + | - | [15] |
| TGA3 | bZIP | *A. thaliana* | + | - | [82] |
| ERF1 | ERF | *A. thaliana* | - | + | [74] |
| ERF2 | ERF | *A. thaliana* | - | + | [71] |
| ERF5 | ERF | *A. thaliana* | - | + | [71] |
| ERF3 | ERF | *A. thaliana* | + | - | [72] |
| ERF4 | ERF | *A. thaliana* | + | - | [72] |
| ERF9 | ERF | *A. thaliana* | + | - | [73] |
| PDF1.2 | ERF | *A. thaliana* | - | + | [75] |
| Pti4 | ERF | *S. lycopersicum* | + | - | [77] |
| Pti5 | ERF | *S. lycopersicum* | + | - | [76] |
| Pti6 | ERF | *S. lycopersicum* | + | - | [76] |

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