REVIEW ARTICLE

Targeting transcription factors for plant disease resistance: shifting paradigm

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Transcription factors (TFs) are regulatory proteins that have the ability to alter targeted gene expression either solely by themselves or as a part of the protein complex. Many such TFs have significant regulatory role in plant defence. Being master switches for gene regulation, they become the unique candidates for targeting functional hub and dynamic networks and nodes of different defence signalling pathways in plant. Of many approaches transgenic overexpression or down-regulation of TFs is widely adopted, mainly to characterize their vital role in disease resistance; however their practical utilization remains limited in breeding programmes. Alternatively, exogenous application of synthetic chemicals/biocontrol agents is also efficient to regulate their expression, but not successful in the field. Hence, the focus has now shifted towards synthetic promoters (SPs) and synthetic transcription factors (STFs) to modulate gene expression. They have greater advantages over the natural ones for their target sequence-specificity, speed, and precise activity. Therefore, manipulation of plant defence regulatory networks by utilizing SPs or STFs represents a new era for synthetically modified crops without negative aspects of the existing biotechnology. This dynamic shift in approach from conventional to modern, transgenic to non-transgenic for manipulating plant defence is discussed in this article, with the aim of their commercial application in crop improvement.

Keywords: Transcription factors, plant defence, peptide interference, synthetic promoters, gene expression.

DISEASE resistance is the economically cheapest and environmentally safest strategy for plant health management. Developing host resistance through genetic enhancement is a cumbersome process that needs identification of resistant gene(s) followed by their transfer to an elite cultivar through either conventional or molecular breeding approaches. However, these approaches are largely constrained due to limited genetic variation in the existing gene pool. On the other hand, crops modified by transgenic approach generally carry foreign genes; so their commercialization is frequently prevented due to human health and environmental safety concerns. Some degree of race-specific monogenic resistance can be achieved, but such resistance is not durable in the long run. Therefore, the primary focus of plant scientists has now shifted towards broad spectrum resistance. In this regard, alternative strategies need to be explored in a more efficient manner. One possible way is to target the 'regulators' of defence-related genes for broad spectrum resistance. These regulators, including transcription factors (TFs) are crucial for modulating the plant defence pathway against multiple pathogens and thus, targeting these regulators/TFs will be useful for enhancing disease resistance[1].

TFs are proteins which can bind to DNA at specific sequences, specifically in the promoter region of the targeted genes and regulate the binding efficiency of RNA polymerase to carry forward the process of transcription, and thereby enhancing (as an activator) or repressing (as a repressor) their expression. A typical plant TF may have a DNA-binding domain (DBD), an oligomerization site, a transcription regulation domain and a signals localization site[2], with a few exceptions. The regulatory action of any TF protein family is determined by its regulatory domain. Variability in regulatory domains governs distinct actions of intra-family TFs, i.e. activation or repression[3]. They may function alone or together with other regulatory proteins. Thus the crosstalk between several members of a TF family is important for reprogramming gene expression. Based on functionality, they are categorized into two groups, viz. general TFs (essential for the initiation of transcription)[4] and specific TFs (regulating a particular set of genes at defined condition in tissue-specific and/or developmental stage-specific manner). The specific TFs regulate the specific recruitment of general TFs, RNA polymerase and other transcriptional elements for binding to the regulatory site. Usually in the plant, certain groups of specific TFs are involved in biotic stress response and regulate either positively or negatively the expression of defence-related genes. By modulating their function, the signal transduction pathways associated with pattern-triggered immunity

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(PTI) and effector-triggered immunity (ETI) can be targeted to acquire defence in plants. Thus, in this article, emphasis is laid on the different approaches to target the defence-related TFs, so that their regulation would be desirable and broad spectrum resistance can be achieved.

Transcription factors in plant defence

In the last decade, substantial progress has been achieved to explore the significance of TFs in regulating growth and development in plants. So far, a squillion plant TFs have been discovered, and structurally and functionally characterized. Among them, certain specific groups (TFs family), viz. APETALA 2 (AP2) and ethylene–responsive–element (ERF), WRKY, basic leucine zipper (bZIP), myeloblastosis (MYB), myelocytomatosis (c-MYC)/basic helix–loop–helix (bHLH), NAC (NAM, ATAF and CUC), Whirly, homeobox (HB) proteins, etc. are found to be associated with transcriptional reprogramming of plant defence response (Supplementary Table 1). Considerable efforts have been made to study their potential role in the activation and repression of gene regulation. All these TFs are responsive to pathogen signals (PAMPs or DAMPs) perceived by the host PRRs (pattern recognition receptors), which activate an array of downstream signalling pathways (MAP kinase, CDPK, etc.) and ultimately lead to the phosphorylation of TFs. The signal will then translocate into the nucleus via transportation of TFs. Inside the nucleus, TFs bind together specifically with the *cis*-element of defence genes to regulate their expression via either activation or repression.

Targeting transcription factors for disease resistance

So far, research on plant TFs is mainly restricted to their identification and functional characterization. However, studies on their utilization as a therapeutic for host resistance is still lacking. Now the most relevant question arises: how can we target transcriptional regulators? There are various approaches through which available information on TFs can be used to improve plant defence (Figure 1). These are categorized into two groups. First, approaches involve an exogenous way to target TFs through application of chemicals and bioagents. However, these approaches enhance host defence to a limited extent in the field. Moreover, they are mostly temporary to regulate genes and need continuous application to sustain against diseases. Recently, with the advancement of molecular tools, the functionality of TFs can be altered by genetic modification via random mutation, complementation (transgenic approach), gene silencing (pre- and post-transcriptional) and genome editing. Application of these tools makes it possible to target plant defence in different ways, like overexpression of positively regulating TFs, down-regulation of negatively regulating TFs, and transcriptional engineering of defence metabolic pathway for activation of 'synthetic' plant defence. These approaches can be exploited for various crop improvement programmes.

Targeting transcription factors via exogenous application

Modulating the expression of transcription factors by elicitor molecules: Literally, any biological, chemical or physical factors that can elicit plant defence are considered as elicitors[5]. However, in practical sense, biochemicals stimulating plant defence response are called elicitors[6]. They are of diverse origin, and classified into natural and synthetic elicitors. Natural elicitors originate from diverse biological sources like plants, algae, microbes, etc., whereas synthetic elicitors are chemically derived compounds that have the ability to induce plant immunity in the absence of stress signals[7–9]. Such defence-inducing synthetic elicitors have already been extensively studied and exploited commercially. In spite of structural dissimilarity, they functionally mimic the natural elicitors and can bind to the respective receptors for inducing defence[6].

Nowadays, such defence activators have been commercially exploited either as synthetic functional analogs of plant molecules (benzothiadiazole, probenazole, β-amino butyric acid, isonicotinic acid, thiadiazole-derivatives, imprimatins, sulphonamides, etc.) or the synthetic version of MAMP/PAMP molecules, including synthetic oligosaccharides (like chitin oligosaccharides, chitosan oligosaccharides, β-glucan oligosaccharides from fungal origin; oligogalacturonides of plant cell wall), synthetic peptides (like Pep13 of oomycetes origin, $AXY^{S}22$, elf18, flg22 of bacterial origin), synthetic lipopeptides and lipopolysaccharides, etc.[10–12]. These synthetic molecules bind to specific recognition receptors and activate the downstream signalling pathway to regulate a diverse set of TFs (WRKY, TGA, NAC, etc.) which in turn regulate defence gene expression for the generation of reactive oxygen species (ROS), pathogenesis-related (PR) proteins, etc.[13]. These synthetic elicitor molecules would be a novel alternative to their natural counterparts[14,15]. In this way, modulating the expression of plant defence by external application of synthetic chemical elicitors is a breakthrough for sustainable disease management.

Induced expression of defence regulators by microbial bioagents: It is a well-established fact that during pathogen infection, the transcriptional switch from plant growth to defence is a requisite for prioritizing defence over growth[16], and induction of defence always compensates with the reduction in plant growth and yield. To avoid such loss, microbial bioagents can be deployed that

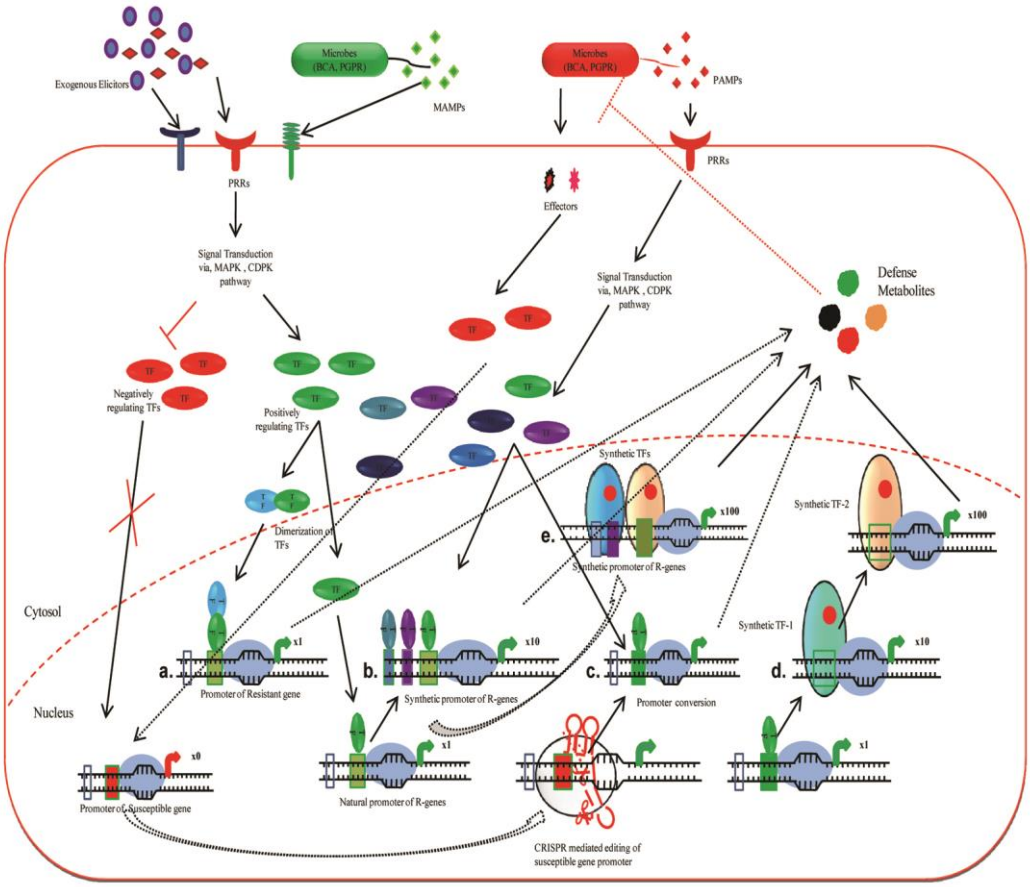

Figure 1. Different approaches for targeting transcription factors (TFs) for plant defence. a, Targeting defensive responsive TFs via exogenous application of elicitors and microbial bioagents. b, Use of synthetic promoters for overexpression of positively regulating TFs or other defence-related genes. c, CRISPR-mediated editing of promoter sequence of negatively regulating TFs. d, Engineering defence metabolic pathway by synthetic transcription factors (STFs). e, Combine application of synthetic promoters and STFs.

can simultaneously regulate both plant growth and defence. Basically, chemical signals of microbial origin regulate TFs of diverse genes to target an array of plant metabolic pathways and reprogram plant growth as well as plant defence concurrently. This has been exemplified in many plant–microbial interactions. Following priming with the biocontrol agent, activation of TFs is evident in many plants. Among them, WRKY is the most responsive for plant defence[17,18]. Experimental studies reveal that *Trichoderma* induces a diverse set of WRKY TFs at different phases of root colonization. At the early stage, *Trichoderma* sp. induces the expression of *AtWRKY60*, *AtWRKY40* and *AtWRKY18* gene in *Arabidopsis*, which function as the negative regulators of salicylic acid (SA)-dependent defence signaling pathways[19]. It became a positive feedback for jasmonic acid (JA)-mediated defence, regulated by *AtWRKY8* during the early phase[20]. While in the late phase, activation of *AtWRKY70* and *AtWRKY54* induces SA-mediated defence gene expression and suppresses JA-mediated defence-gene expression. In this way, WRKY TFs become the key regulators in plant defence via SA–JA cross-talk[20,21]. This activation is achieved through plant biopriming with different microbial bioagents. The nature of TFs involved in any interactome is highly host as well as bioagent-specific. As *WRKY20* was found to enhance disease resistance in *Brassica* sp. when primed with *Bacillus amyloliquefaciens* strain 5113 (ref. 22). Similarly, *Trichoderma* induces *WRKY33* in *Phaseolus vulgaris* to confer resistance against necrotrophs[23]. Thus TFs regulate SA-responsive defence genes to confer systemic acquired resistance in plants against pathogens. In this way, bioagents can be employed to fine-tune the transcriptional reprogramming between plant growth and defence.

Induced systemic resistance (ISR) is also an important part of induced defence in plants, and is mostly tiggered by plant growth promoting rhizobacteria (PGPR) and other beneficial microorganisms. It involves upregulation of defence genes that harness a cascade of defence pathways, mainly mediated via JA and ethylene (ET) signalling. Here MYC2, MYB72 and NPR1 TFs act as the central regulator of rhizobacteria-induced ISR. Rhizobacteria

elicit the expression MYC2 and MYB72 to induce ISR in *Arabidopsis*[24,25]. MYC2 regulates JA signalling, whereas MYB72 acts upstream to ethylene signalling in ISR, and both are required for early signalling of ISR[25,26]. Like PGPR, other beneficial microbes like mycorrhiza, and endophytes can be used to sensitize plant defence. Application of *Pseudomonas fluorescence, Bacillus* sp., etc. for biopriming plants is evidence for systemic induction of plant immunity. Induced expression of defence using microbial repository would be a sustainable and multi-targeting option for controlling biotic stress.

Targeting transcription factors via endogenous modification

Overexpression of positively regulating transcription factors: Among different endogenous modification-based approaches, overexpression of positively regulating TFs is most widely used. It offers over-regulation of defence responsive genes that in turn suppress pathogens. Sometimes overexpressed TFs may compensate for pathogen effectors attacking directly the host TF and prevent the hijacking of host metabolic system by pathogens. For this, unmodified TFs are overexpressed using either a strong constitutive promoter, or a tissue-specific/stage-specific promoter.

In the majority of cases, overexpression controlled by strong constitutive promoters (viz. CaMV35S) is most successfully applied for disease resistance (Supplementary Table 2). For example, constitutive overexpression of regulatory genes like *Osmyb4* leads to enhanced resistance in plants against fungal, bacterial and viral pathogens via activation of PR proteins, phenolics[27]. Usually, a key regulatory node in the defence pathway is targeted for overexpression. However, constitutive overexpression of such key regulators may have a pleiotropic effect in transgenic plants, causing undesirable plant phenotypes. Therefore, targeting multiple TFs for overexpression would be an alternative. However, ectopic gene expression using these strategies often results in hypermorphic phenotypic expression. When overexpressed, TFs are sufficiently able to confer ectopic expression of multiple defence genes to draw dramatic phenotypes. However, the constitutive expression of host resistance may compensate for yield loss, even if the disease is absent.

To mitigate this problem, TFs should be expressed only when and where they are needed. Herein, tissue-specific/growth stage-specific/environmental condition-specific expression of TFs will likely provide integral engineering disease-resistant plants. This can be achieved only when targeted TF genes express under a desirable synthetic promoter. These synthetic promoters can be designed by engineering the *cis*-regulatory sequences of a native promoter for efficient regulation of targeted TFs. These TFs have specific *cis*-regulatory elements, viz. GCC-like elements, as-1 element, G-box, T-box, W-box, etc. (Supplementary Table 1). Multimeric oligomerization of W-box, box D or GCC elements (box-S) induces *in planta* gene expression against pathogens[28]. Further, combining two or more such *cis*-regulatory elements confers broad spectrum defence against multiple pathogens[29]. Transforming plants with a TF gene regulated by pathogen-inducible synthetic promoter(s) is the key to success[30]. Several such pathogen-derived elicitor-induced synthetic promoters are available for selecting stage/tissue/pathogen-specific gene expression for host defence[31].

Interfering negatively regulating transcription factors: Besides positively regulating TFs, a large number have also been identified to regulate plant defence negatively. For example, WRKY11 and WRKY17 negatively regulate the basal defence against bacterial pathogens[32]. During pathogen infection, expression of negatively regulating TFs becomes higher to suppress the defence responsive genes. Sometimes their expression is promoted by the effector to attenuate host defence and create favourable environment for the pathogen. Interfering with the activity of such negatively regulating TFs would be another novel strategy for enhancing plant defence. This can easily be employed by inactivation of targeted TF genes at pre-transcriptional and post-transcriptional level. Pre-transcriptional down-regulation of TF genes can be achieved in two ways, i.e. by gene mutation and using TF-targeted DNA decoy. Mutation of TF-encoding gene is commonly followed to alter DNA binding specificity and regulatory activity of the respective protein[33]. Most of the mutation techniques adopted so far have been random. Hence, ZFN (zinc-finger nucleases), TALEN (transcription activator-like effector nucleases) and CRISPR/Cas9 (clustered regularly interspaced spacer palindromic repeat)-based sequence-specific nucleases can be exploited for targeted genome editing. These nucleases were designed for sequence-specific binding, followed by targeted genome editing[34,35]. Among them, ZFN and TALEN are chimeric nucleases consisting of a programmable DNA-binding domain (DBD) fused with a non-specific nuclease (Fok1), functioning as a dimer[36]. Due to their highly repetitive nature, these protein chimeras are difficult to design[37]. Moreover, their DNA binding and subsequent DNA cleavage is often not specific, and off-target effects result in undesirable mutation. Whereas, the CRISPR/Cas9 system binds at a specific site of the target gene and cleaves with high efficiency. These sequence-specific nucleases cleave the target DNA which creates double-strand breaks (DSBs) at the gene of interest that stimulate the natural DNA repair machinery[38]. The DSB can be repaired by either error-prone non-homologous end-joining (NHEJ), or homologous recombination (HR) pathway (Figure 2)[39]. These advanced technologies have been progressing at an unprecedented pace and are being applied for editing TF genes.

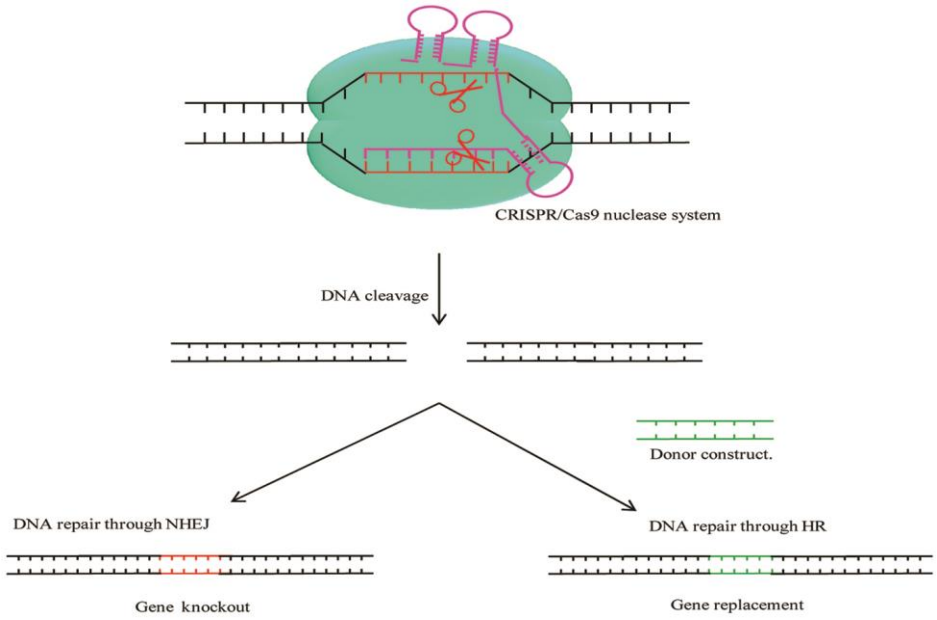

Figure 2. Genome editing using CRISPR/Cas9. ***a***, CRISPR/Cas9 nuclease system. ***b***, DNA cleavage at the targeted site through double-strand breaks (DSBs). DSB is repaired through either homologous recombination (HR) or nonhomologous end-joining (NHEJ) mechanism. NHEJ results in the insertion/deletion of targeted genes, whereas HR helps in gene replacement and gene tagging through insertion of donor construct.

Mutation in a single TF gene may not be sufficient to suppress phenotypic expression, as TFs work in a complex network. Thus the transcription factor decoy (TFD) approach is preferred nowadays. TFDs are double-stranded oligo-deoxynucleotides (ODNs), about 10–20 bp long with consensus binding sites for targeted TFs[40]. They compete with endogenous *cis*-elements for TF binding. Binding of targeted TFs to these alternate binding sites creates a *trans*-factor-free environment and attenuates the expression of disease[41]. Thereby, specific interference of negatively regulating TFs can be possible at pre-transcriptional level[42]. This strategy would be effective for targeting key negative regulators of plant defence.

On the other hand, post-transcriptional down regulation of TFs is possible by silencing gene expression via either RNA interference (RNAi) or peptide interference (PEPi) approach (Figure 3). In RNAi, expression of small complementary RNA blocks the messenger RNA (mRNA) to translate into protein either by mRNA degradation or through interrupting the binding of ribosomal subunits. Usually, small non-coding RNAs, i.e. siRNA or miRNA are employed for RNA inhibition and functional inactivation of the target gene. Exogenous application of siRNA is possible with certain limitations, whereas miRNA is totally endogenous. Hence, use of artificial miRNA (amiRNA) is the new alternative[43]. Artificial microRNAs (amiRNAs/amiRs) work like natural miRNA and are designed by replacing the miRNA-sequence in the duplex of hairpin stem-loop within amiRNA precursor with artificial sequence that is complementary to the target gene[44,45]. Evidence for exploiting amiRNA to target specific genes regulating biotic as well as abiotic stress tolerance has been documented[46–48]. However, very few reports are available showing knockdown of TF using amiRNA-based gene silencing technology. Chen *et al.*[49] used this technique to knockdown *AtMYB14* for enhancing cold tolerance. This shows great promise for targeting any undesirable plant TF that negatively regulates plant defence.

In spite of its wide application in plant science, RNAi has its own limitations, like off-target effects, unstable suppression of TFs etc[50–52]. To overcome these, peptide interference (PEPi) intervened by artificial small interfering peptides (a-siPEPs) is proposed for post-transcriptional control of TFs. Small interfering peptides (siPEPs) are a group of small proteins with approximately 100 residues, having unique structural organization, carrying only dimerization domain required for nonfunctional heterodimer formation with certain specific TFs. Unlike TFs, they lack functional domains essential for DNA binding and gene regulation[53–55]. Basically, they are generated either as genome-encoded proteins or as the splice variants of TF at the translational phase in plants during stress response, but transcriptionally remain inactive due to the absence of functional DNA-binding domain and regulatory domain. Finally, they suppress the activities of functional TF proteins by forming nonfunctional heterodimers. In this way, siPEPs establish a distinct self-regulatory network[55] and negatively regulate the function of targeted TFs at the protein level.

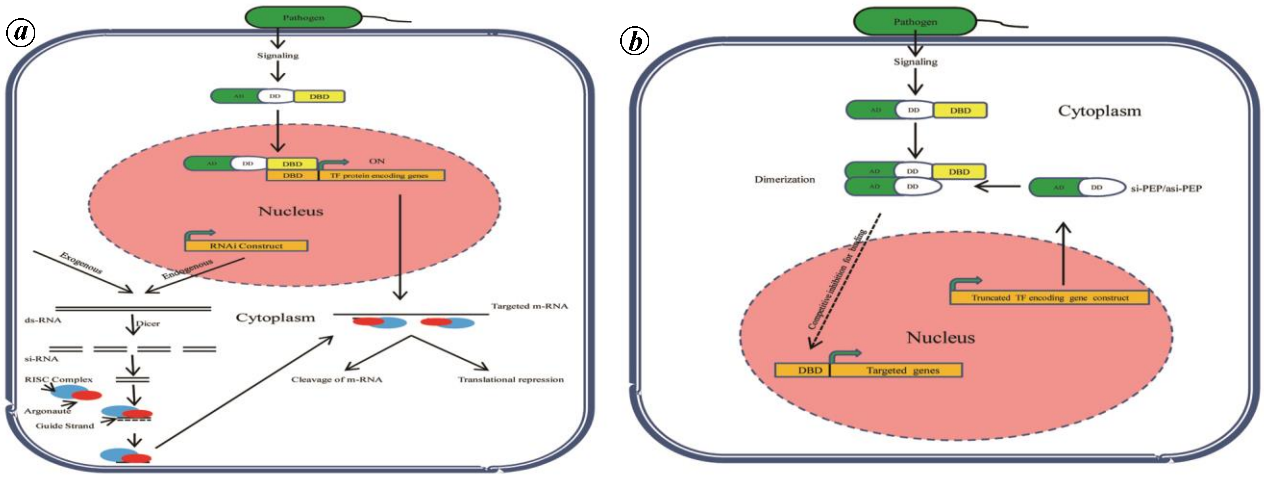

Figure 3. Post-transcriptional silencing of TFs. ***a***, RNA interference (RNAi); ***b***, Peptide interference (PEPi).

The a-siPEPs are a truncated form of TF proteins and comprise of a dimerization domain only. These are designed by engineering the TF gene and have structural resemblance to genomic siPEPs present in plants[56]. Transgenic plants overproducing such a-siPEPs have efficient protein knockout system for inactivating specific plant TFs necessary for growth and development. In this way, artificial peptide interference approach can be used to target TFs that negatively regulate plant defence.

Interfering negatively regulating TFs associated with plant defence have already been tested in various host-pathogen systems (Supplementary Table 3). As exemplified in rice, knockdown of *OsERF922* gene expression RNAi enhances host defence against blast pathogen[57]. During plant–pathogen interaction, expression of these TFs is induced to suppress the defence-related genes like PR-proteins, phytoalexins, etc. and behaves as a susceptible host factor favouring infection and multiplication of pathogen[58]. Therefore, suppression of these TFs would be novel for making a plant resistant.

Engineering metabolic pathway for plant defence by synthetic transcription factors: Plants synthesize an array of secondary metabolites as defence compounds, such as antimicrobials (phytoalexins, phytoanticipins) along with lignins and other cell-wall strengthening molecules, which together provide strong protection against diverse pests and pathogens. Based on structural chemistry, these molecules are categorized into different groups like alkaloids, flavonoids, phenylpropanoid, terpenoids, etc. Biosynthesis of these natural metabolites follows different complex but interrelated metabolic pathways. Biotechnological manipulation of these secondary metabolic pathways associated with plant defence is also novel for achieving host resistance. This is because genetic engineering-based approach is not only able to modulate the levels of natural plant defence compounds, but can also produce an array of entirely new metabolites by incorporating novel metabolic pathways[59].

So far, several TFs have been identified, characterized and overexpressed to manipulate the concentration of defence molecules in many plants (Supplementary Table 4). This can be successful only when the targeted single TF influences the entire defence metabolic pathway. However, the single TF gene targeting approach is often insufficient for metabolic engineering and the complexity of the regulatory network limits its application. Therefore, synthetic transcription factors (STFs) are an alternative tool for engineering plant metabolic pathways[60]. STFs are designed by the fusion of artificial DBDs with transcriptional activator or repressor domains and nuclear localization signals. Initially the zinc finger proteins (ZFPs) and transcription activator-like effectors (TALEs) proteins are exploited for constructing STFs. A typical, ZFP is composed of a tandem array of 3–6 identical ZF domains, and specifically binds to 9–18 bp targeted DNA binding site[61,62]. Whereas DBD of TALEs consists of multiple copies of tandemly arrayed 34-amino acid repeat sequences, each binding to every single nucleotide in the targeted DNA-binding site[63]. The sequence of each repeat is highly conserved with the exception of two amino acids at the 12th and 13th positions, called the repeat variable residue (RVD), that are essential for specific DNA binding of these repeats[64]. Synthetic ZF-TFs and TALE-TFs have been made by intermingling custom-designed DBDs to either the activator (VP16, VP64) or repressor (SID, KRAB, EAR, SRDX) domain[63]. They are useful for the regulation of desirable endogenous plant genes involved in various defence metabolic pathways. These synthetic TFs have an advantage over natural TFs in their binding specificity to targeted endogenous gene promoter. Besides this, synthetic TFs can be used for regulating defence genes in many plants by engineering domain specificity[63], and will be the potential tool

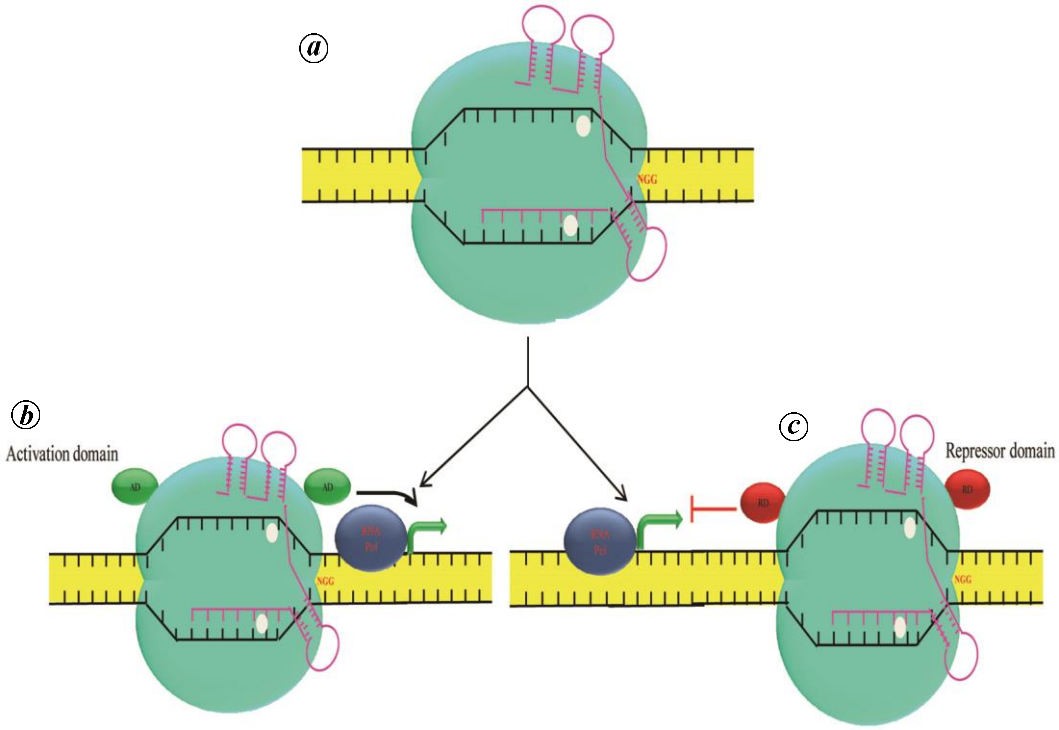

Figure 4. Regulation of gene expression using synthetic TFs. ***a***, CRISPR/Cas9 with deactivated catalytic domain (dead Cas9). ***b***, Transcriptional activation of targeted gene using dCas9 fused with activation domain (AD). ***c***, Transcriptional repression of targeted gene using dCas9 fused with repressor domain (RD) via CRISPR interference (CRISPRi); RNA polymerase (RNA pol).

for plant metabolic engineering against diverse pathogens.

Regulating genes with synthetic ZF-TFs and TALE-TFs are surpassed by the advent of CRISPR/Cas9 system as it is based on RNA : DNA interactions rather than protein : DNA interactions for ZF TFs and TALE-TFs[65]. For gene regulation, Cas9 is first catalytically inactivated by a single amino acid mutation/substitution (D10A and H840A) in the catalytic domain (HNH or RuvC) to generate 'dead' CAS9/'deactivated' CAS9 (dCAS9). This dCAS9 loses its endonuclease activity, but can still bind to the target specific DNA site. So dCas9 could be converted into STFs by fusing it to the transcriptional activation domain (VP64, p65) or repressor domain (KRAB, SRDX) for activation or repression of specifically targeted genes (Figure 4)[66]. These synthetic dCas9-TFs are also used to target transcriptional regulation in many plants for engineering metabolic pathway[67–69]. Therefore, STFs would be a potential tool for manipulating the biosynthesis of bioactive plant defence compounds against diverse pathogens. However, production of bioactive defence molecules in different plant systems is difficult due to their multicellularity, complex metabolic activity regulated by intertwined transcriptional networks. Hence, targeting a single key gene may not help to accumulate metabolites at the desired level. Therefore, STFs along with synthetic promoters can also be employed for targeting multiple plant defence genes. However, limitation in its specificity and wider applicability leads employment of alternate approaches like multiplexed transcriptional activation of genes using multiple guide RNA (sgRNA)-mediated dCas9-based system. The recruitment of such synthetic gene circuits within the plant system is unique for generating 'synthetic' bioactive plant defence molecules. It requires adequate production of dCas9 protein with sgRNA transcript within the cellular system, which can only be possible through simultaneous delivery of these components as a single module. These experimental constraints still persist in many plants with their slow transformation and regeneration; thus they become cumbersome to implement and to achieve desirable outcome.

Application and future prospects

The targeting TFs through various approaches provide 'desirable' broad spectrum defence. Among these approaches, activation of defence using microbial agents is the most widely used for disease management. However, exploration and exploitation of suitable microbial agents for different agroecological niches is difficult and also has bio-safety issues for use on a large scale. Sometimes the magnitude of defence induced by the microbial agents is very limited to provide complete protection. To tackle this, application of elicitor molecules is promoted in the field. However, temporary activation of defence limits its application in broad genetic base. Moreover, application

after visualization of disease symptoms, sometimes when inoculum density crosses the threshold level, makes it less effective. Hence, the target is shifted towards various endogenous modification strategies for obtaining permanent solution. Endogenous modification can be implemented in a transgenic and non-transgenic way through either introduction/activation of superior gene pool of TFs or deactivation of negatively regulated TFs. The overexpression of positively regulating TFs or interfering with the activity of negatively regulating TFs is a common strategy, but is mostly exploited on an experimental scale. This overexpression or down-regulation of TFs may also affect other physiological traits. For example, constitutive overexpression of defence regulating TFs may compensate plant growth and yield. The continuous supply of energy for defence may cut shot the energy flow for growth and yield which results in negative side effects like growth retardation and yield loss[1]. Therefore, such strategies are not introduced in the farmer's field. The possible way to resolve this problem is the use of alternatives like tissue-specific/time-specific synthetic promoters, or inducible promoters (i.e. non-constitutive). This will allow only tissue-specific and stage-specific defence gene expression. The switching on and off of defence genes is largely determined by stress signals. Thus, the supply of energy is auto-regulated based on physiological needs. This can only be achieved through transgenic approach. The generation of transgenics is a cumbersome and time-consuming process. Moreover, their acceptability is also limited in public domain. Hence, we have to opt for non-transgenic strategies.

Recent and rapid advancement in synthetic biology has provided scope of utilizing STFs for regulating plant defence. STFs are applicable in two ways, i.e. either through genome editing, or targeted regulation of defence related genes. Their functional application in different host–pathogen model system is the prime target. However, it is still in the nascent stage and needs a more authentic study to judge its function and commercial applications.

So far, studies on regulating biotic stress responses have been mainly localized on single TFs and their individual functional assay. However, TFs are always functioning in hubs with many regulatory protein components forming a dynamic network[17]. These networks are highly interconnected and regulated by different sensory transcriptional regulators that act as the key node for different signalling pathways and respond differentially to different stress conditions, depending upon the nature of stress. However, how a plant can sense diverse environmental signals and release cellular clues to channelize diverse upstream signalling pathways and ultimately pass on information to downstream transduction pathways remains to be explored[70]. Therefore, elucidation of crosstalk among these sensory transcription networks in different spatial and temporal scales is important to uncover the mechanism of trade-offs between biotic and abiotic stress tolerance in plant, even between different biotic stresses, viz. herbivore insects and biotrophs, necrotrophs and herbivores. Thus, when targeting TFs for plant resistance, it is also important to consider their role in plant growth and development, other than their function in defence against biotic or abiotic stress, or both[71]. Thus an understanding of the transcriptional network and interaction between TFs of the same family and that of other families within a plant system under different stress conditions will be helpful to modify plant immunity against both biotic and abiotic stress and reallocate resources from growth to defence against stress.

Conclusion

TFs are master regulators of both biotic and abiotic stress responses. A wealth of information on different TF families, viz. AP2/ERF, bZIP, MYB, MYC, WRKY and their differential regulatory role in biotic and abiotic stress tolerance is available. Some are identified as positive regulators, while others are negative regulators of plant defence. Moreover, some are specifically associated with immunity response against biotrophs; whereas others actively regulate defence against necrotrophs and herbivores. A few express simultaneously against both biotic and abiotic stress. Besides deciphering their mechanism, it is important to exploit TFs for targeting complex defence system and translate to a commercial scale. Different possible ways to target TFs have been highlighted considering their merits and demerits. The practical implications of these strategies need to be proved successfully in different crop species. Engineering multiple regulatory genes to offer resistance against multiple pathogens will also be a challenge. Our future efforts would be the identification of key TFs involved in abiotic and biotic signalling cascade crosstalk and establishing fine-tuning between them at specific temporal and spatial scales to avoid negative effects on growth and yield. Besides their advantages, biosafety and environmental safety issues need to be considered before commercial release. The impact of new strategies on the changing population dynamics of plant pathogens needs to be regularly evaluated. In conclusion, these strategies will revolutionize the field of plant science and will help to achieve a significant level of plant.

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