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Manipulating disease and pest resistance pathways in plants for enhanced crop improvement

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ABSTRACT

Plants are sessile organisms, therefore cannot escape challenges of their surrounding environment. The rich source of nutrients plant possesses attracts various organisms. Biotic stress results from array of organisms such as bacteria, fungi to various insects, pests and herbivores. Plants have evolved sophisticated mechanisms to protect themselves against invaders. In this review, we explore the plant surveillance system, different nodes in the defence pathways involved in plant protection and how it can be manipulated to get a resistant crop. Emerging technologies have provided us with a vast number of potential candidate genes from plants, pathogens and other organisms. We here, illustrate examples of technically useful solutions to make crops tolerant to pathogens and pests.

KEY WORDS: PLANT DEFENCE, BIOTIC STRESS, R GENE, DEFENCE SIGNALING TRANSDUCTION, NPR1, MAPK, GENETIC ENGINEERING

ABBREVIATIONS

MAMPs - Microbe Associated Molecular Patterns,SAR - Systemic Acquired Resistance, VOCS – Volatile Organic Compound, R gene – Resistance Gene, HR – Hypersensitive Reaction, ROS – Reactive Oxygen Species, MAPK – Mitogen Activated Protein Kinase, *avr* – Avirulence, ETI – Effector Triggered Immunity, NBS – Nuclear Binding Site, LRR – Leucine Rich Repeat , pv. – pathover, NO – Nitric Oxide, SA – Salicylic Acid, JA – Jasmonic Acid, ET- Ethylene, NPR1 – Non Expressor of PR Genes 1,PR – Pathogenesis Related, LTP –Lipid Transfer Protein, PPO – Polyphenol Oxidase, POD – Peroxidase, UV- Ultraviolet, HIPV – Herbivore Induced Plant Volatile, QTL – Qualitative Trait Loci, SIPK - Salicylic Acid Induced Protein Kinase, OS – Oral Secretion, FACs – Fatty Acid –Amino Acid Conjugates, WIPK – Wound Induced Protein Kinase, MEKK- Mitogen-Activated Protein Kinase Kinase, ISR – Induced Systemic Resistance, PRSV - Papaya Ring Spot Virus, ZFN – Zinc Finger Nucleases, TALENs – TAL Effector Nucleases, GM – Genetically Modified, RPP2 – Recognition of *peronospora parasitica* 2

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INTRODUCTION

Plants are nutrient rich organisms and therefore many invaders prey on their food reserve. Some of the invaders are significant threats to crop production, worldwide. In the process of co-evolution (Seidl and Thomma, 2017); plants, pathogens and insects have evolved various strategies to avoid each others' defence system. The goal of producing crops with durable and increased resistance to a broad spectrum of diseases and insects is therefore, a major focus in plant research.

In nature, plants are continuously challenged by different organisms, whereas, only few are successful in gaining entry into a prospective host. Plants have developed an elegant defence system with a wide variety of constitutive and inducible defences to protect themselves from damages of different biotic factors. Constitutive defences include many preformed barriers such as waxy epidermal cuticles, cell walls and bark (specialized morphological structures). Inducible defences include production of repellents, toxic chemicals, pathogen-degrading enzymes, anti-nutritional effects and deliberate cell suicide (Freeman and Beattie, 2008). Plants often do not produce toxic compounds or defence-related proteins until pathogens are detected due to the metabolic cost associated with the production and maintenance of such compounds. Plants have evolved to live in environments where they are very often exposed to different stress factors in combination. Plants have developed various mechanisms that allow them to detect precise environmental changes and respond to complex stress conditions, minimizing damage (Saskia and Jorunn, 2011).

NATURE OF ATTACKERS

Plant pathogens can be broadly divided into biotrops and necrotrophs. Bacteria and fungi can adapt to both lifestyles (Freeman and Beattie, 2008).Viruses are quintessential biotrophs, although they eventually kill the host cell. Insects, on other hand, cause damage by chewing and sucking. Plants respond to the insects by producing protease inhibitors and anti-feedants such as alkaloids [(Hanley et al., 2007; Jeffery and Jonathan, 2001). Nematodes can adapt to complex modes of parasitism by exhibiting variety of parasitic modes affecting the development responses of plants, causing galls, root knots or cysts (Jeffery and Jonathan, 2001; Davis et al., 2004; Roland and Maurice,2011) . Thus, plant immune system is highly polymorphic in their capacity to recognize and respond to different stress factors (Jeffery and Jonathan, 2001).

PLANT SURVEILLANCE SYSTEMS

Although plants lack immune system comparable to animals, they have developed sophisticated surveillance mechanisms, which can respond rapidly before harmed. These surveillance systems are linked to specific pre-programmed defence responses. Direct defences are mechanical protection on the surface of the plants which protects from all biotic factors (*e.g.*, hairs, trichomes, spines, thorns and thicker leaves) or toxic chemical production.

Basal resistance is the first line of pre-formed and inducible defences. It is also known as innate immunity (Freeman and Beattie, 2008; Owen and Zamir, 2010), and protect plants against entire groups of pathogens (Freeman and Beattie, 2008). Basal resistance is triggered when plants recognize microbe-associated molecular patterns (MAMPs). MAMPs include specific proteins, lipopolysaccharides, and components of cell wall commonly found in microbes. During evolution pathogens also have developed counter measures that are able to suppress basal resistance in certain plant species. If the basal defence is somehow suppressed, plants respond with hypersensitive response (HR) (Freeman and Beattie, 2008). In HR plants limit the pathogen's access to water and nutrients thereby sacrificing few cells in the infection site *i.e.* deliberate cell suicide (programmed cell death). HR is more pathogen specific than basal resistance. It is triggered in presence of disease-causing effector molecules. Once the hypersensitive response is triggered, plant tissues become highly resistant to a broad range of pathogens. This phenomenon is known as systemic acquired resistance (SAR) (Freeman and Beattie, 2008; Nelson et al., 2017), which represents readiness of plant metabolites to defend plants, in case of a heightened attack.

Mechanical damage caused by insects is not generally considered "true" plant disease although plants have developed surveillance systems designed to not only recognize insect pests, but also to respond with specific defence mechanisms. General wounding can be different from insect feeding in a way that elicitors are present in insect saliva. In response to insect chewing, plants release volatile organic compounds (VOCs), secondary metabolites and proteins that have toxic, repellent, and/or anti-nutritional effects on the herbivores (Freeman and Beattie, 2008; Saskia and Jorunn, 2011; Abdul Rashid War et al., 2012). Sometimes volatiles released by plants also attract beneficial predators (natural enemies) that prey on the destructive pests (Abdul Rashid War et al., 2012; Walling, 2000; Rashid and Chung, 2017). Plants become phenotypically plastic when induced defence is triggered as a result it decreases the chances of the attacking insects to adapt to the induced chemicals (Abdul Rashid War et al., 2012).

In addition, plants can defend themselves against viruses by a variety of mechanisms which include RNA silencing (Novina and Sharp, 2004, Csorba and Burgyan, 2016). Plants can recognize the foreign double stranded RNA or DNA, produced by viruses in the host cell during replication, and respond by digesting the genetic strands into non recognizable fragments and thereby stopping the infection. The interaction of plants with symbionts, pathogen, herbivores, and the natural enemies, both above and below the ground is the focus of a large amount of research effort and has great potential for utilization in crop protection.

With cultivation of huge areas of genetically identical crops, protection relies on a small number of inbred disease resistance genes per crop species and on the wide-spread application of pesticides. Unfortunately, an absolute control is very difficult to achieve through pesticides (Cesari, 2017), as pathogens can overcome disease resistance genes and/or become resistant to pesticides (Nelson et al., 2017; Zhonghua et al., 2005). Genetic manipulation can help solve the problem by inserting multiple genes as transgenes by careful selection from wild parent of the same plant species or from different plant species (Campbell et al., 2002). Therefore a search is on for genes that can confer a durable broad-spectrum resistance against biotic factors. To make it more environment friendly the gene product should be safe for all organisms and also reduce the need of harmful pesticides. However, the success so far achieved is very less. In majority of cases the tansgene results in unpredictable expression in different parts of the plants, this phenomenon is not due to the transgene itself, per se (Hammond-Kosack and Parker, 2003; Stuiverand Custers, 2001) . Therefore, optimization of transgene expression patterns needs close attention. Inducible expression of such gene is essential (Hammond-Kosack and Parker, 2003; Michelmore, 2003). A highly inducible promoter specific for defence gene expression can help the plant in directed recourse allocation by metabolic and transcriptional adaption during stress. Plant can optimize source sink relationship thus increasing yield or biological harvest index (Hammond-Kosack and Parker, 2003).

ENGINEERING PLANTS WITH INCREASED RESISTANCE AGAINST PATHOGEN AND INSECTS: TARGET GENES

(First generation strategies)

R gene

R genes (resistance genes) are important components of plant surveillance system. A diverse array of defence mechanism is triggered when R genes recognize pathogen or insects (Cesari, 2017). PR-gene induction, accumulation of inhibitory metabolites and oxidative burst response by production of reactive oxygen species, are some of the downstream responses triggered by R genes which lead to hypersensitive response (Owen and Zamir, 2010).

Pathogens possessing *avr* genes can overcome basal immunity of plants by blocking perception of PAMP or by inhibiting MAP kinase signaling cascade, which is known as effector- triggered susceptibility. In case of effector triggered immunity, the pathogen's effector molecules are recognized by R proteins either by direct or indirect interactions. Thus, enhancing the plant resistance and it is faster than PTI. To trigger ETI, R proteins must recognize specific avirulence proteins (Avr) in order to generate resistance. However, mutation in either *avr* gene or R gene can change the scene *i.e* it will result in compatibility and therefore loss of resistance. R genes encode proteins which have nuclear binding sites (NBS) and leucine rich repeat (LRR) domains (NBS-LRR

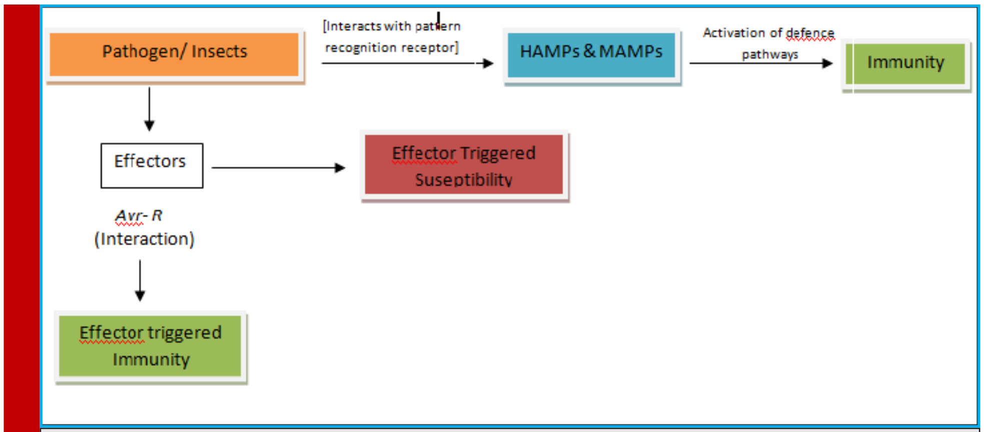

FIGURE 1. Shows pathogen triggered immunity when pathogen/herbivore associated molecular patterns are recognized by the cell receptors. However in presence of effectors, pathogens can surpass this immunity. In presence of R gene, effector triggered immunity induces defence response in plants.

proteins) (Cesari, 2017; Jeffery and Jonathan, 2001; Mari et al.,2013, Nelson et al., 2017).

Functional R genes conferring resistance against an array of different biotic factors such as bacteria, virus, fungus, nematodes and even insect pathogens have been isolated (Cesari, 2017, Zhao et al., 2005; Vossen et al., 2005; Reinink et al., 1989; Brotman et al., 2002). Even though the mode of action as well as the effector molecules of pathogens and insects are very different, R genes encode only a few classes of proteins. NBS –LRR class of proteins are the largest class of R gene which encodes 'nucleotide binding site with leucine rich repeat (Jeffery and Jonathan, 2001). It is reported that NB-LRR type R genes can confer resistance to multiple pathogens even though the pathogens belong to taxonomically distinct families (Mari et al., 2013). It is also termed as MDR or multiple disease resistance. In a maize recombinant inbred line (RIL) a QTL, *qMdr* have been identified for resistance to several diseases i.e, Norther blight, grey leaf spot and souther leaf blight. The molecular mechanism underlying the resistance is yet not known. In a research it is found that a gene, ZmCCoAOMT2, which encodes a caffeoyl-CoA O-methyltransferase is associated with conferring quantitative resistance to both southern leaf blight and gray leaf spot (Yang et al., 2017).

Bacterial effectors are delivered through type III secretion system, which can be up to 30 per strain, and by mimicking or inhibiting eukaryotic cellular functions colonization is achieved (Abramovitch et al. 2006). An example of a specific R-gene, *Rxo1* from maize conferred resistance to bacterial streak disease caused by *Xanthomonas oryzae* pv. Oryzicola(Zhao et al., 2005), when introduced in rice. In another instance, R gene *RCT1* from *Medicago truncatula* expressed in alfalfa conferred resistance to *Colletotrichum trifolii* (Yang et al., 2008), RPI-BLB2 from wild potato gave resistance to *Phytophtohora infestans* in day to day cultivated potato (Vossen et al., 2005). Some of the R gene work in pairs and are functional only when both genes are present (Mari et al.,2013). Some of the examples of such R gene pairs are *RPP2A/RPP2*(Sinapidou et al., 2004), *Pi5-1/Pi5-2* (Lee et al., 2009) *and Lr10/RGA2* (Loutre et al., 2009). Examples in wheat rust*, Sr31* from rye was effective against all *Pgt* races for many years until the appearance of Ug99 (Pretoriusetal.,2000).

Many single R genes responsible for resistance against insects are mapped in cereal crops, including wheat conferring resistance to Hessian fly (Hatchet et al., 1970). For decades, R genes have been used to control Hessian fly infestation in wheat. It is evident in support of gene-for- gene model in plant- insect interactions.

Some of the insect resistant R genes that are effective against aphids include: the lettuce *Nr* gene which gives resistance against aphid species *Nasanova ribisnigri* (Reinink et al., 1989), the *Vat* gene from melon confers resistance against the melon/cotton *Aphis gossypii* aphid (Brotman et al., 2002), in another instance, the *Sd1* gene gives resistance against *Dysaphis devecta* aphid in apples (Walling, 2000; Roche et al. 1996), the *RAP1* gene gives resistance against the Pea Aphid in *Medicago truncatula* (Stewart et al., 2009), and the *Mi-1* gene in tomatoes (Rossi et al., 1998) found to be responsible for resistance against different organisms, the potato aphid *Macrosiphum euphorbiae*, the root-knot nematodes *Meloidogyne* spp., and the whitefly *Bemisia tabaci* (Nombela et al. 2003). The diverse resistance conferred by the *Mi-1* gene makes it is a very useful tool for integrated pest management. While, *Bph14 confers* resistance to the rice brown planthopper, *Nilaparvata lugens* (Zhang et al., 2009). However, a constitutive expression of a R gene can have a negative impact in absence of attackers. Constitutive expression of R gene can be detrimental to plants and therefore needs to be expressed with inducible promoters (Belbahri et al., 2001; Takakura et al., 2004).

For decades, R genes have been used in conventional breeding programme (Balconi et al., 2012) ; however, the resistance is only against a strain of pathogen or a particular species of insect. Traditional breeding strategies most often use only one R gene at a time. Pyramiding multiple R genes can promise a long lasting resistance as the pathogen has to accumulate mutation in multiple *Avr* genes to escape resistance. Effective combinations of R and APR gene by pyramiding or stacking can be considered for effective rust resistance (Jeffrey et al., 2014). However, it is a lengthy process to introduce a R gene into an elite cultivar by conventional breeding. R-genes from unrelated plant species can be introduced through genetic engineering, which often remain functional in the new host plant (Collinge et al., 2008) . The limitation of this technology being that resistance is conferred only against a single pathogen similar to breeding (Balconi et al., 2012). Additionally, R-gene only confers resistance against pathogens that essentially act as a sink for the host plant's metabolism *i.e.* biotrophs.

Shuffling of multiple R genes can also be considered rather than only pyramiding. Plant pathogen *Cladosporium fulvum* elicitors are recognition by *Cf* genes in tomato which belongs to the Hcr9 gene clusters (Brande et al., 2004). Studies have shown that Hcr9s are composed of sequences that have been generated by sequence exchange between individual homologues, Intra and intergenic recombination, gene conversion, point mutation, duplication and translocation. Therefore, shuffling multiple R genes might increase recognition specificities and engineering R gene for novel disease resistance specificities in plants can be achieved (Cesar, 2017). For example, gene shuffling done in tomato *Cf4* and *Cf9* R

Table I. List of R gene against different pathogens

R gene	Source (Donor)	Examples of transgenic crop	Against Pathogen	References
Rox1	Maize	Rice	Xanthomonas oryzae pv. oryzicola	Zhao *et al.*, 2005
RCT1	Medicago truncatula	Alfalfa	Colletotrichum trifolii	Yang *et al.*, 2008
RPI-BLB2	Potato (Solanum bulbocastanum)	Potato	Phytophtohora infestans	Vossen *et al.*, 2005
Bs2	Pepper	Tomato	Xanthomonas campestris	Tai *et al.*, 1999
Rpg1	Barley	Barley	Stem rust	Brueggeman *et al.*, 2002
Ve1 and Ve2	Tomato	Potato	Verticillium spp.	Kawchuk *et al.*, 2001
RRS1-R	Arabidopsis	Arabidopsis	Ralstonia solanacearum	Deslandes *et al.*, 2002
Pi-d2	Rice	Rice	Chinese rice blast	Chen *et al.*, 2006
RPW8	Arabidopsis	Arabidopsis, tobacco	Broad spectum resistance against powdery mildew	Xiao *et al.*, 2003
Pto	Tomato	Tomato	Pseudomonas syringae	Frederick *et al.*, 1998

genes lead to the identification of sequences required for the Avr-dependent HR in tomato (Brande et al., 2001).

SIGNAL TRANSDUCTION NETWORK

Plants can sense changes in their environment through signaling pathways (Pankaj and Atle, 2013). When pathogen elicitors interact with host receptors, signal transduction cascades are likely to be activated including oxidative burst (ROS), calcium fluxes, ion channel fluxes, NO production (Bollwell et al., 1999) and various protein kinases. Subsequently, transcriptional and/or post transcriptional activation of transcription factors takes place which lead to the induction of defence gene.

Plant hormones which play important role in defence are SA, JA and ET. SA is primarily involved in the protective response against biotrophic and hemi-biotrophic pathogens and systemic acquired resistance (SAR) (Grant and lamb, 2006). Some mutants insensitive to SA shows enhanced susceptibility to biotrophic pathogens. Methyl salicylate is a mobile inducer of SAR and is induced when the plant is infected with a pathogen in tobacco plants (Park et al., 2007). After pathogen challenge the elevated level of SA increases the expression of PR genes, therefore increasing resistance. Whereas the level of JA and ET are elevated against necrotrophic pathogen and herbivorous insects (Park et al., 2007).

Most often the SA and JA/ET defence pathways are antagonistic, however reports of synergistic interaction also exist (Kunkel and Brooks, 2002; Mur et al., 2006; Schenk et al., 2000) . Specific biotic factors regulate the positive or negative cross talk between SA and JA/ET pathways (Adie et al., 2007). In nature it is not one factor that affects the plant but several attackers, here plants have to employ complex regulatory mechanisms to cope with the complex situation. The mechanism by which plant is able to prioritize the responses is not known.

Non expressor of *PR* genes 1 (NPR1) is one of the important components of SA signaling. NPR1 plays an important role in SA-JA interaction (Dong, 2004). Downstream of NPR1 are several WRKY transcription factors which is also important is SA-dependent defence response. WRKY70 maintains the balance between the SA and JA pathways (Li et al., 2004; Li et al., 2006). Another key component which is involved in mediating the antagonism between SA and JA signaling in *Arabi-*

Table II. List of R gene against different insects

R gene	Source (Donor)	Against Insect	Reference
Nr gene	lettuce	Aphid species *Nasanova ribisnigri*	Reinink *et al.*, 1989
melon *Vat* gene	Melon	Melon/cotton *Aphis gossypii* aphid	Brotman *et al.*, 2002
Sd1 gene	Apple	*Dysaphis devecta* aphid	Roche *et al.*, 1996; Walling, 2000
RAP1 gene	Pea	Pea Aphid in *Medicago truncatula*	Stewart *et al.*, 2009
Mi-1.2 gene	Tomato	Potato aphid *Macrosiphum euphorbiae*, the root-knot nematodes *Meloidogyne* spp., and the whitefly *Bemisia tabaci*	Rossi *et al.*, 1998

dopsis is mitogen activated protein kinases (Petersen et al., 2000). In the second generation strategies, these signaling nodes will be discussed. The goal of effective and sustainable disease resistance can be achieved by the knowledge of signal transduction pathways (David et al., 2010), as the increased understanding has made it clear that successful pathogen process through pathogenicity factors (effectors). The disease resistance gene are mostly downstream genes and often do not act as specific receptors produced by pathogens and insects. A complex signaling network is also established when herbivorous insects attack a plant. To identify new molecules important for fine tuning of plant defence signaling, there is a need of dynamic modeling and simulation of signal transduction pathways (Beckers and Spoel, 2006; Erb et al., 2009).

Various plant protectant and defence gene are activated by the primary and secondary signals. The defence gene products include glutathione S-transferases, peroxidases, cell wall proteins, proteinase inhibitors, hydrolytic enzymes (e.g., β-1,3-glucanases and chitinases), pathogenesis-related PR proteins (Balconi et al., 2012).

PR proteins

Other potential candidates for manipulation are pathogenesis related (PR) genes, which shows promising activities against biotic factors *i.e.* pathogens as well as insect pests. Pathogenesis related (PR) genes could increase the level of pre-existing barriers (Owen and Zamir, 2010; Hammond-Kosack and Parker, 2003). Naturally occurring PR proteins are constitutively expressed at low levels and are induced to high levels challenged by pathogens or application of either salicylic acid or jasmonic acid (Ferreira et al., 2007) . PR proteins include several groups of unrelated proteins. Seventeen classes of PR protein have been examined, and numbered chronologically in order of discovery *i.e.* PR-1 to PR-17 (Balconi et al., 2012). PR-2 (b -1,3-glucanases), PR-3, -4, -8 and -11 (chitinases) target the pathogen cell wall (Owen and Zamir, 2010; Honee, 1999), PR-1 and PR-5 (thaumatin-like proteins and osmotins) are termed as permatins as they target the membrane, PR-10 has weak ribonuclease activity therefore may target pathogen RNA or play a role in defence against viruses, PR-6 proteins (proteinase inhibitors) may target nematodes, whereas the PR-7 protein (an endoproteinase) may be involved in microbial cell wall dissolution (Jorda et al., 2000) . The PR-9 family may enhance resistance to multiple pathogens by catalyzing lignifications which helps in cell wall reinforcement (Passardi et al., 2004). Since PR-10 family has weak ribonuclease activity it can be used against viruses (Park et al., 2004), PR-12 (defensins), PR-13 (thionins) and PR-14 (lipid transfer proteins) predicts antibacterial and antifungal activities (Epple et al., 1997) , some proteins generating hydrogen peroxide and are toxic to pathogen and pest, PR-15 (oxalate oxidases) and PR-16 (oxalate oxidase-like proteins) belongs to this family (Hu et al., 2003). PR-17 (uncharacterized) is detected in infected tobacco, wheat and barley (Christensen et al., 2002).

Most investigated PR proteins are chitinases and β1–3 glucanases (Owen and Zamir, 2010). Over-expression of chitinase have been moderately successful against fungal pathogens. Studies have found chitinase have role in insect resistance as well. The combined expression of chitinases and β1–3 glucanases have proven to enhance resistance by synergistic effect (Anand et al., 2003; Jach et al., 1995; Jongedijk et al., 1995; Zhu et al., 1994). Chitinases originating from *Trichoderma harzianum* (biocontrol agent), exhibit higher anti-fungal activity (Dana et al., 2006; Baranski et al., 2008; Kumar et al., 2009). Ectopic expression of thionins and defensins has conferred broad spectrum disease resistance, though the resistance is at low level (Punja, 2001). For example radish defensin RS-AFP2 (Kostov et al., 2009) when over-expressed in tomato resulted in up to 90% reduction in disease against agriculturally important pathogens. Lipid transfer proteins (LTP) are one of the important PR proteins which act as a potential mobile signal for systemic acquired resistance (SAR) in plants (Maldonado et al., 2002). LTP activates SAR over-expression of LTP might result in deleterious effect (Walters, 2007) , so far no such effect is observed. A highly inducible promoter can be used to over-express this gene in order to achieve the goal of disease resistance.

The plant defensive metabolites are termed as secondary metabolites play an important role in plant defense against herbivore and other interspecies defense, thus increasing the fitness of the plant. They can be either constitutively stored (phytoanticipins) as inactive forms or induced in response to the insect or microbe attack (phytoalexins)(King *et al*., 2014). Herbivore induced plant volatiles (HIPVs) play very important role in defense by either attracting the natural enemies of the herbivores or by acting as feeding and/or oviposition deterrent (Rashid and Chung, 2017). HIPV are released by healthy plants as well, however a different blend of volatiles is produced in response to herbivory and is very specific for a particular insect-plant system (Liu et al., 2012). For example, plants tend to release volatile compounds in response to aphid –attack to attract parasitoid wasps. In corn, plants release terpenoids in response to aphid attack. Many other volatile compounds like MeSa, C_6 volatiles etc influence plant-insect, pest and pathogen interaction.

Metabolite engineering can play an important role in developing plant with insect resistance. Increasing the flux of defence related secondary metabolites by engineering the respective pathways can be of great importance in developing crops with insect resistance (Sanchez -Vallet et al., 2013). There are some reports of metabolic engineering of dhurrin, a cyanogenic glycoside in transgenic *A. thaliana* plants which, resulted in minor effects on the whole metabolome and transcriptome (Dudareva et al., 2013). Resistance to green peach aphid (*Myzus persicae*) feeding have been enhanced by metabolic engineering of raffinose in the phloem of *A. thaliana* (Jirschitzka *et al*., 2013). In another instance, manipulation of plant volatile emissions has enhanced the effectiveness of biological control agents. This can be used as a strategy to fight insect pests in an ecologically sound manner (Degenhardt *et al*., 2009).

SECOND GENERATION STRATEGIES

Master switch genes

Over-expression of a single defence-related gene is generally unable to provide high levels of resistance against a broad range of biotic factors like pathogen and herbivores. The knowledge of pathogen-induced signaling pathways in plants suggests that modifications of existing innate signaling pathways or expression of 'masterswitch' genes such as kinases and transcription factors (Owen and Zamir, 2010; Hammond-Kosack and Parker, 2003; Sarah and Paul 2005), which regulate a large number of defence genes could increase resistance against biotic factors (Owen and Zamir, 2010; Sarah and Paul 2005). The disadvantage encountered by this approach could be the harmful effect on plant development, due to potential yield loss which is common with over-expression of large number of genes at a time constitutively (Owen and Zamir, 2010). Therefore, the ideal candidates are the genes that activate partial pathways or augment pathways.

Transcription Factors

Transcriptome and QTL data analysis suggested transcription factors to be promising candidates for genetic engineering to increase disease resistance characteristics in plants (Sarah and Paul 2005) . They might behave as master switch gene by taking care of the expression of several genes in a single pathway. Therefore capable of making large changes in single trait causing very few disturbance on other traits (Doebley and Lukens, 1998). A good example is WRKY transcription factors (Owen and Zamir, 2010; Sarah and Paul 2005).

WRKY transcription factors are involved in SA- mediated defence pathways. Several WRKYs have the potential for increasing disease resistance, among them the most studied are *WRKY70* from *Arabidopsis* [50].Several other transcription factor families that have roles in plant defence could yield useful master switch genes like WRKY, ERF, TGA, MYB, Dof, GRAS, bHLH, GT1 and the Whirly factor Why1(Desvaux et al., 2004). The only limitation being, transcription factors mostly consist of large multigene families and identifying the best candidate can be difficult due to the functional redundancy (Eulgem et al., 2000). However, several of *Arabidopsis* WRKY has been identified have good functionality against pathogens (Sarah and Paul 2005).

MAP Kinase

Potential candidate master –switch genes which also play vital roles under different stress are protein kinases (Sarah and Paul 2005) . MAP kinase (MAPK) signaling is a necessary part of many defence-signalling pathways. When tobacco MAPK, SIPK is over-expressed it led to activation of defence responses and HR-like cell death showing the potential role of these genes (Zhang and Liu, 2001) . Enhanced resistance to virulent *P. syringae* and *Botrytis cinerea* was observed when MKK4a, MKK5a were over expressed transiently and MEKK1 was activated constitutively (Asai et al., 2002) . Other potential protein kinases are calcium dependent sensor proteins that changes Ca^{2+} defence response (Romeis et al., 2001) . In response to herbivore-induced cues such as insect oral secretions (OS) and oviposition fluid compounds, plants undergo a change in transcriptomes, proteomes, and metabolomes. The major components of the oral secretion of insects are fatty acid-amino acid conjugates (FACs) which activate the mitogen-activated protein kinase (MAPK) pathway. The MAPK pathway not only play an important role in signaling transduction in responses to a number of stresses including cold, heat, ROS, UV, drought, pathogen and insect attack but also regulate plant growth and development (Wu et al., 2007). On application of FACs in oral secretion of *M. sexta* leads to activation of several compounds/molecules of MAPKs, salicylic acid induced protein kinase (SIPK) and wound-induced protein kinase (WIPK), JA, SA and ethylene. In another case brown plant hopper *N. lugens induces* expression of putative *OmMKKI* (MAPK). Several FAC elicitors have been isolated from various lepidopteran species (Wu et al., 2007; von Dahl et al., 2007) .

NPR1

One of the most promising candidates of second generation strategy is *NPR1* (Cao et al., 1994). Pathogen or insect pest resistance can be achieved through signaling modification. The *Npr1* gene was discovered originally from various independent genetic screens. The *Arabidopsis* mutants *npr*-1 do not respond to inducers of systemic acquired resistance (SAR) such as salicylic acid (Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997) or lost the ability to accumulate PR transcripts and were also hypersensitive to biotrophic pathogens (Pieterse et al., 2004). NPR1 acts as a switch between the signaling pathways involving ethylene/jasmonic acid (ET/JA) (ISR) and salicylic pathway (SAR), therefore resistance to both necrotrophic and biotrophic pathogens depends on modulation of NPR1 gene (Li et al., 2004; Cao et al., 1994; Pieterse et al., 2004) . NPR1 is the key master switch as it constitutes a node which links SAR, ISR, SA, JA, ethylene, and also R gene-mediated resistance (Pieterse et al., 2004). The activation of NPR1 gene is through redox pathways by SA accumulation in the cytosol and then translocated to the nucleus, however without binding to DNA directly it acts through transcription factors, which in turn induces expression of several PR genes (Pieterse et al., 2004). *NPR1* is constitutively expressed at low levels, when challenged by pathogen or treated with SA, transcript accumulation increases up to two-fold. SA gives better defence against piercing and sucking insect pests than the chewing pests (Zhao et al., 2009).

SA-mediated expression of proteins by *NPR1* include the WRK70 transcription factors this lead to suppression of JA-dependent signaling events (Li et al., 2004; Ndamukong et al., 2007). However, nuclear localization of *NPR1is not required for direct regulation of* JA-pathways which indicates a dual function between the cytosolic and nuclear located *NPR1* (Glazebrook et al., 2003; Spoe et al., 2003; Yuan et al., 2007).

As both SA and JA dependant pathways are controlled by NPR1, it can be targeted to achieve broad spectrum disease resistance through genetic engineering. There are several instances where over-expression of NPR1 has resulted in resistance against both biotrophic (Cao et al., 1994; Lin et al., 2004). Necrotrophic (Lin et al., 2004; Makandar et al., 2006; Wally et al., 2009) pathogen in several plant species as well as against insect pest in tobacco plants. Over-expression of NPR1 resulted in quicker and higher intensity of PR proteins for longer duration. The function of NPR1 remained unchange when AtNPR1 was expressed in different crop like rice (Fitzgerald et al., 2004) , wheat (Makandar et al., 2006), carrot (Wally et al., 2009) tobacco (Meur et al., 2008) and tomato (Lin et al., 2004) indicating the conserved functionality of the signaling system as well as the NPR1 like proteins.

However, when AtNPR1 or the rice ortholog OsNH1 was expressed in transgenic rice, the constitutive expression of PR genes lead to stunted growth of plants and more light sensitivity apart from desired increase in disease resistance (Chern et al., 2005). Green tissue specific expression of AtNPR1 in rice reduced such developmen-

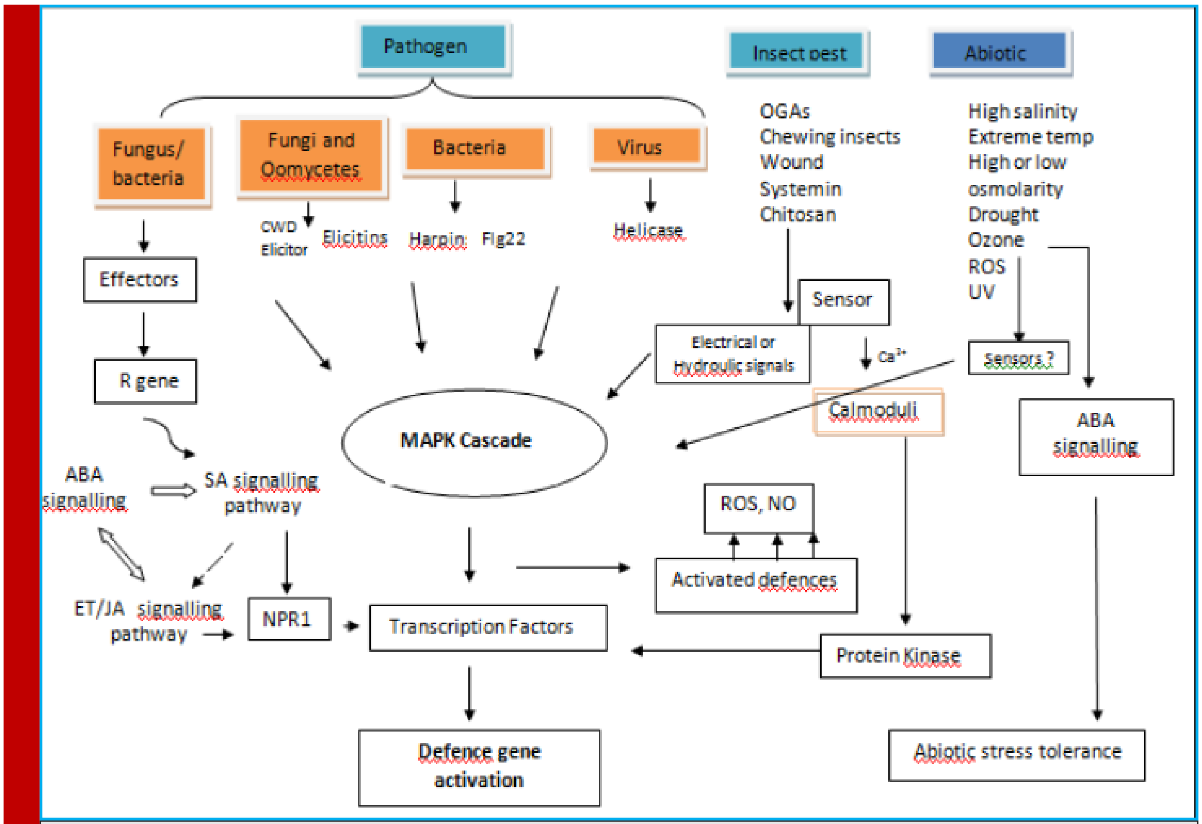

FIGURE 2. A summary of role of MAPK, NPR1 and transcription factors in plant defence.

tal abnormalities and conferred resistance to the sheath blight pathogen without compromising the growth and yield parameters (Molla et al., 2016)

MANIPULATION THE EXPRESSION OF TARGET GENES

Expressing *Avr* protein

De Wit (1992) proposed an interesting exploitation of R gene response, where a plant can be designed to express an active Avr protein under the control of a pathogen-responsive promoter for which the plant has a R gene. The induced Avr product would induce responses which would result in incompatible reaction to a wide range of pathogens (Dewit, 1992). The pathogen inducible promoter (*hsr203J*) in tobacco resulted in successful exploitation of HR elicitor cryptogein (Keller et al., 1999). The main benefit is resistance against a wide range of pathogens. However the real value of this strategy is yet to be exploited.

Synthetic modifications of PR proteins

To enhance the effectiveness of PR proteins, synthetic modifications such as linking a single chain antibody gene against a particular pathogen can be done (Peschen et al., 2004). The antibodies would then attach to the invading pathogen's cell wall and the antimicrobial proteins would effectively degrade the fungi. It has been demonstrated against *Fusarium graminearum*. It was highly effective against nine different species of the Fusarium genus, in *Arabidopsis*, however, not effective against unrelated pathogens (Peschen et al., 2004). This method has been also implemented in transgenic wheat, which reduced the disease symptoms against Fusarium head blight (Li et al., 2008).

Toxic gene products to engineer local cell death

One of the first strategies applied for increased disease resistance in plants was generation of an 'HR-like' local cell death artificially by expressing a toxic gene (Li et al., 2008). This strategy is only successful when 'HR' is restricted to infection sites otherwise uncontrolled cell death will occur even in uninfected tissues which is undesirable. Components of the pathogen can be expressed as toxic genes. But the promoters used so far have undesired background expression in uninfected tissues. Moreover, the toxicity level of the gene product needs to be studied well before the product is marketed.

RNAi

A useful tool inhibiting pathogen expression is through RNAi (Csorba and Burgyan, 2016; Novina and Sharp, 2004) technology. It inhibits the expression at both the transcriptional and post transcriptional levels in plants. RNAi has been exploited to develop many virus resistant plants (Fuentes et al.,2016). For example, papaya ringspot virus (PRSV) coat protein protected papaya in Hawaii has already been commercialized.

Stacking antimicrobial compounds

Expressing antimicrobial proteins, phytoalexins and enzymes in plant cell reinforcement or in the breakdown of pathogen infection structures has also been tried. The limitation of this strategy is resistance towards a specific pathogen. However to broaden the spectrum of resistance, stacking of antimicrobial peptides could be a reasonable approach (Van der Biezen, 2001).

Targeting inducible promoters

With the significant advances in sequencing technologies for transcriptome analysis, number of important crop genomes have been sequenced, which make it feasible for high throughput recognition of promoters and putative cis elements. Cis regulatory elements function as molecular switches in respose to various stress signals (Kazuko and Kazuo, 2005). Transcription factors interact with cis acting elements in the promoter region and forms a complex to initiate transcription thus can help in formation of initiation complex when activated and act as molecular switches to determine transcription initiation events. Therefore, it is important to determine the elements in the stress responsive promoters to understand the molecular switches of stress inducible genes. Apart from this, plant pathogen molecular interaction has shown that the promoter region also plays an important role in pathogen recognition (Patrick et al., 2009) . In gene for gene interaction pathogen effector interacts with the promoter region for activation of R gene. For example, some bacterial effectors like TAL effectors Avr BS3 and AVR Xa27 interact with the promoter region and activate the corresponding R genes (Patrick et al., 2009).

The current limitation of development of resistant transgenic crops using genetic transformation is unavailability of the right kind of promoter. Strong synthetic inducible promoters can be designed to address the issue of biotic stress. Promoters can be designed to not only recognize specific predators but also effector molecules from different pathogen and pests, thus giving a broad spectrum resistance against several biotic factors. It is also possible to use bidirectional promoters to activate two genes at the same time.

CONCLUSION

Durable pest and disease resistance so far has been achieved by traditional breeding and chemical applications. However, conventional breeding has prioritize quality parameters and agronomic adaptation over resistant breeding. Therefore, new improved genomic tools are required to empower the process of genetic analysis and crop improvement. High through put sequencing and complete genome sequencing of many crops allows understanding of many metabolic pathways and disease resistance mechanisms. Understanding of omics are shedding light on the different compounds associated with plant defense. Using new technologies, it might be possible to achieve more durable and long term resistance through various genetic approaches. The wide spread application of pesticides can also be reduced through this technology. There are several success stories of plant genetic engineering which include herbicide resistant for weed control and insect resistance for lepidopteran insect control. However, transgenic disease resistance crop and resistance against sap sucking insects represent a very small portion of transgenic crops. Also the scope is wide with the advancement of genome editing tools like CRISPR-Cas9 and new digital phenotyping technologies, to develop a more sustainable agriculture that involves adaptation to changing climates. The global food demand needs to be fulfilled and therefore, it is the need of the hour to combat yield losses caused by diseases and sap sucking insect pests on a global scale. Also, an increased and stable yield is required to address decreasing land availability issues. Engineering disease resistance with new tools available needs to be made a priority.

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