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Classical and Modern Strategies to Enhance Plant Disease Resistance Against Fungi-A Review

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Review Article

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ABSTRACT

Pathogenic fungi are associated with devastating plant diseases since centuries, causing huge epidemics in history. Plant pathogenic fungi have been managed by employing multiple strategies including chemical control, biological control, organic management etc. Despite, being a quick mean to provide an effective control, chemical management has hazardous effects on human health and environment. Fungal pathogens can be managed by using transgenic technology, molecular technology and other approaches aiming at genetic manipulation. Genetic engineering technology has been widely researched in recent era and many transgenic plants with remarkable resistance against potential fungal pathogens have been developed. Transgenic plants have the advantage of being environment friendly. Transgenic technology has aimed at engineering for the expression of many antifungal genes including pathogenesis related (PR) proteins, phytoalexins, hydrolytic enzymes, antimicrobial peptides and resistance (R) genes. The expression of these antifungal genes was successfully imparted into plants via, transgenic technology contributing to significant resistance against fungal pathogens. Another approach include RNA silencing "switching off" of the expression of specific genes by introducing double stranded RNA's is gaining huge importance since last decade. Many fungal genes encoding for pathogenicity factors have been sequenced successfully. The application of RNA silencing against fungal pathogens is still limited. In this review all strategies which have been employed so far to enhance resistance against fungi will be discussed briefly.

INTRODUCTION

Plant Pathogenic fungi are posing a continuous threat to multiple agricultural crops worldwide. Fungi are responsible for considerable amount of loss in major crops. These drastic pathogens are causing more than 70% of all major crop diseases and a substantial damage to crop production [1]. In past, conventional methods have been used to reduce crop loss from fungal infections in plants, including the enhancement of natural resistance in plants. Breeding programs have been extensively used for introducing qualitative or quantitative resistance against specific pathogens in different crops. Plants have naturally existing resistance (R) genes which act against avirulence (Avr) gene products produced by the pathogen during the event of invasion and can provide complete resistance against fungal pathogens. Due to this reason R genes have been used in conventional breeding programs enhancing resistance in previously susceptible varieties [2]. Breeding for race specific resistance by introduction of naturally occurring R genes has provided remarkable resistance against many fungal pathogens [3]. A number of resistant cultivars or varieties have been developed but these cultivars became obsolete in a very short time span due to evolution of new virulent and more vigorous races of pathogens having the capability to overcome resistance response. Plants can activate a variety of defense mechanisms as a response to pathogen attack at the site of infection which include programmed cell death (Hypersensitive response), tissue reinforcement and antibiotic production [4]. These responses trigger systemically acquired resistance in plants against multiple pathogens [5-6]. These defense responses are genetically programed, and activated only upon invasion of a plant by the pathogen [7]. The understanding of genetically controlled defense responses and the genetic basis of products (enzymes or proteins) being produced during defense actions has opened new vistas of molecular research on plant diseases. To achieve effective control over plant diseases is the prime interest of biotechnologists. The most significant contribution in this area is

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varietal development having transgenic resistance against fungi through gene isolation and genetic transformation technology. Genetic engineering has remarkable potential to overcome many limitations associated with conventional breeding programs and provide resistance or tolerance against devastating fungal pathogens [8] with least effect on soil microflora. This review will discuss classical and modern approaches being utilized to enhance plant resistance against fungi ranging from conventional breeding to transgenic plants expressing R-genes, antimicrobial genes, pathogenesis related proteins (PR), enhanced structural barriers, destruction of pathogen's virulence factors and RNAi mediated resistance (involving RNA classes from RNA family). The beneficial association of modern approaches and classical approaches to provide resistance in plants will strengthen sustainable agricultural practices.

Genetic Engineering for Resistance against Fungal Pathogens

Genetic engineering technology for resistance against fungi has not been widely adopted yet. But there are several advancements in this area showing positive results [9]. Many transgenic plants showing remarkable resistance against multiple fungi have been developed successfully. Keeping in view the mechanisms adopted by plants to confer resistance against fungal pathogens transgenic plants have been developed using following defense related molecules.

PR-proteins (Pathogenesis related Proteins):

Genes encoding for pathogenesis related (PR) proteins provide a promising source of resistance against fungal pathogens. There are several proteins in host plants which are activated upon fungal infection, these proteins have been characterized and used to provide significant resistance against fungi [10]. PR-proteins have a distinct role in natural defense system of a plant because they are activated during hypersensitive response (HR) and systemically acquired resistance (SAR). PR-proteins include osmotin or thaumatin like proteins (TLP) with antifungal activity that have been introduced into plants through genetic engineering techniques. PR-proteins comprise of 17 protein families ranging from PR1-PR17 [10] based on their structure, amino acid composition and biochemical response. PR-5 proteins specifically interact with plasma membrane of fungal cells and produce transmembrane pores and subsequent leakage of fungal cells [11]. Osmotin or thaumatin like proteins have also been found to have *in vitro* antifungal activity [12] against *Fusarium, Rhizoctonia, Botrytis* and *Sclerotinia* [13]. Other PR-proteins include thionins and lectins [14]. Two pathogenesis-related protein genes were incorporated in Chinese white poplar (*Populas tomentosa* Carr.) consisting of motherwort lipid-transfer protein (*LJAMP2*) and *Beauveria bassiana chitinase* (*Bbchit1*) exhibiting significant higher reduction in infection by *Alternaria alternata* [15]. A PR-5 family protein (ObTLP1) from *Oscimum basilicum* was functionally characterized, and its ectopic expression in transgenic *Arabidopsis* plants enhanced tolerance to *Botrytis cinerea* and *Sclerotinia sclerotiorum* infections [16].

Hydrolytic Enzymes

Fungal pathogens have cell wall made up of chitin and glucan molecules which is prune to degradation by chitinase and glucanase enzymes produced as a result of fungal infection. These enzymes degrade glucan and chitins causing disintegration of fungal cell walls. This phenomenon has been widely utilized in transgenic plants to induce overexpression of genes encoding lytic enzymes [17,18]. Chitinases and glucanases belonging to the group of PR-proteins have been widely overexpressed to provide antifungal activity *in vitro* [19,20]. Overexpression of these PR-proteins in plants is thought to cause lysis of fungal hyphae and inhibit fungal growth [21]. Since these enzymes are frequently expressed in resistant as well as susceptible tissues, hence their specific roles in non-transgenic plant resistance are difficult to prove.

Moreover, the expression of these enzymes can also be activated by stress, growth conditions i.e, plant senescence and environmental triggers [22]. Transgenic plants introduced with genes for chitinase production have been evaluated *in vivo* exhibiting reduced fungal growth or infection rate leading to disease resistance [23,24]. In rice and tobacco expression of chitinase genes plant resistance against fungi [25]. The synergic effect of chitinase and glucanase in transgenic tobacco, carrots and tomatoes prevented the development of many fungal pathogens [26,27]. It can be concluded that transgenic expression of more than one hydrolytic enzymes in combination can provide broader resistance as compared to expression of a single enzyme [24,28,29]. Rice RC_{24} chitinase gene was introduced into wheat to confer resistance against *Puccinia graminis* f. sp. *tritici* [30]. Transgenic expression of Rice chitinase class-I gene (RCH_{10}) in lilium increased its resistance against infection of *Botrytis cinerea* [31].

Phytoalexins

Phytoalexins are low molecular weight secondary metabolites possessing antimicrobial resistance against many fungal pathogens. Phytoalexins are produced as a result of pathogen's attack and they have been expressed transgenically to provide resistance against many fungal pathogens [32]. The synthesis of phytoalexins undergoes a complex biochemical pathway [33], including shikimic acid pathway. To achieve genetic manipulation of these pathways in order to suppress or enhance phytoalexin production is quite difficult. Similarly, as in the case of hydrolytic enzymes, it has always been difficult to precisely explain the involvement of phytoalexins in enhancing disease resistance against many fungal pathogens. An *Arabidopsis* mutant which was lacking the production of the indole-type phytoalexin camalexin exhibited susceptibility against infection by *Alternaria brassicicola* compared to *Botrytis cinerea* [34]. Transgenic plants with overexpression of genes encoding phytoalexins exhibitted great reduction in disease development. A phytoalexin resveratrol encoding gene PcRS was incorporated in *Arabidopsis* from *Polygonum*

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cuspidatum to enhance resistance against *Colletotrichum higginsianum* [35]. The understanding of complex biochemical pathways involved in the production of phytoalexins have enlightened new ways for genetic engineers to effectively utilize the expression of phytoalexins in genetic engineering for resistance response in plants.

R-Genes for Resistance

The initial understanding of plant resistance mechanism was developed by the hypothesis of Flor given in 1955 [36] who gave the gene for gene concept which stated that for every kind of resistance gene (R-gene) in plant there is a corresponding avirulence gene (Avr-gene) in the pathogen. The Avr genes by the pathogens trigger/activate the R-genes in plant and if the interaction between both these genes is incompatible it results into resistant response in plant system termed as effector triggered immunity (ETI). In case if the interaction is compatible it results in susceptible response termed as effector triggered susceptibility (ETS) [37]. It can be concluded that plants develop immunity against the pathogens by directing R-genes against specific targeted *Avr* genes. R-genes act as coding factors for transmembrane nucleotide binding peptides with leucine rich repeats (NB-LRR) which contribute to specific recongnition [38]. NB-LRR's recognize Avr gene products by the pathogens and trigger multiple responses such as accumulation of inhibitory metabolites, salicylic acid (SA) leading to systemically acquired resistance (SAR), PR induction and other compounds such as reactive oxygen species that ultimately result in hypersensitive response or programmed cell death [39,40].

Genetic engineering has made it possible to introduce R-genes from multiple plant species into new host plants as a mean to provide resistance. Extensive research has been conducted to introduce resistance in different plant species by exploiting the potential of R-genes through genetic engineering techniques. The discovery of these R-genes has proved a breakthrough in genetic engineering because after this a number of genes were cloned against many fungal pathogens, the first ever Avr gene was cloned about 30 years ago[41]. The first NB-LRR gene was identified in *Arabidopsis* [42]. Later on, many R-genes were identified mostly form *Arabidopsis* and tomato conferring excellent amount of resistance against many fungal pathogens [43,44]. After that R-genes were cloned form rice, tobacco, maize, flax and several other plant species. A gene Hm1 R-was cloned from maize encoding a NADPH dependent reductase having the potential to inhibit a toxin produced by *Cochliobolus carbonum* [45]. R-gene from *Medicago truncatula* was introduced into alfalfa providing resistance against *Colletotrichum trifolii* [46], Rpiblb1 from *Solanum bulbocastanum* was introgressed in cultivated potatoes that provided durable resistance against *Phytophthora infestans* associated with late blight of potatoes [47] and Rp1 gene conferring resistance against stem rust disease [48]. Despite many advantages there are certain limitations of breeding for R-gene resistance in plants because these genes may provide resistance to only a single pathogen and that pathogen may confer threat to the plant by evolving an alternate Avr gene affecting overall fitness of the plant [49]. Moreover, it is difficult to predict successful intergeneric transfer of R-genes in distant species because some events of transduction pathways may be specific to certain R-genes which are not present in distant species.

To overcome, this problem a combination of multiple R-genes can be introduced into several plant species such as antifungal proteins. A complete understanding of functional domains of R-genes is very important to utilize their potential in genetic engineering for resistance conferring broad spectrum resistance against fungal pathogens [50]. A two component strategy was proposed by De Wit in 1992 to engineer for broad spectrum resistance. This strategy was based on expression of R-gene only after the expression of Avr gene by the pathogen [51]. The Avr genes induce defense responses in plants, thereby activating resistance against many biotrophic pathogens. The resistance is only activated after the expression of Avr genes by the pathogens which are incompatible with R-genes of the plant, independent of race specificity. The main challenge is to find promotor region that will only be activated after pathogen infection. HR (Hypersensitive response) elicitor cryptogein was expressed successfully under the control of the pathogen-inducible promoter hsr203J in tobacco plants by utilizing De Wit's strategy [52]. This strategy is of great significance because it can provide resistance against a variety of fungal pathogens. The limitation of this strategy is that the promotor region should be completely inactive in the absence of pathogen [52]. R-genes that are able to recognize a multiple number of pathogen races there by conferring broad spectrum resistance are of special interest. A recessive gene mlo from barley presents a significant amount of non-race-specific durable resistance against powdery mildew [53]. Resistance breeding by utilizing the potential of R-genes against multiple isolates of a pathogen can prove to be a reliable strategy to enhance resistance against fungal pathogens.

RNA Silencing

RNA mediated gene silencing is a quite advanced approach for enhancing resistance against fungi. It is a tool for reverse genetics which is involved in switching of the expression of certain genes by targeting specific genes of fungi responsible for pathogenicity [54]. The expression of genes is silenced either at transcriptional or post-transcriptional level i.e., epigenetic modification at transcriptional level and degradation of specific nucleotide sequences of mRNA through introduction of dsRNA at post transcriptional level [55]. Plant pathogenic fungi develop direct connections with their host plants via a specialized structure known as haustorium which act as an interface for signal exchange as well as nutrient uptake [56]. It allows fungi to uptake dsRNA's or siRNA's during nutrient uptake from targeted host plant to activate RNA-mediated gene silencing. Gene silencing by introduction of dsRNA has been successfully employed against many fungal pathogens that include *Magnaporthe oryzae, Neurospora crassa, Venturia inaqualis* and *Aspergillus nidulans* [57-60]. Nakayashiki et al. silenced mpg1 gene and polyketide synthase genes [61]. Mpg1 gene is a hydrophobin gene act as a trigger for development of appressorium hence, plays an important role in pathogenicity [62]. These genes were successfully silenced by p-Silent-1 based vectors in upto 90% transformants. This vector was also success-

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fully employed in *Colletotrichum lagenarium* to silence GFP reporter proteins. This concept of RNA mediated gene silencing was recently observed in case of barley powdery mildew caused by *Blumeria graminis* [63].

CONCLUSION

Breeding for resistance has went through many scientific challenges and modifications till now. Currently, genetic engineering technology has been most widely and successfully implemented in enhancing resistance against numerous fungal diseases. Genetic engineering for genes related to defense including PR proteins, hydrolytic enzymes, antimicrobial peptides and phytoalexins have provided significant amount of resistance against fungal diseases. The combined expression of all defense related genes have served as a remarkable source of resistance. Transgenic technology integrated with classical breeding has revolutionized agricultural research and opened new vistas of research to enhance resistance and productivity of economically important agricultural crops.

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